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## CONTENTS

### REVIEW

  The Role of Organic Products in Implementing the State Policy of Healthy Nutrition in the Russian Federation .................................................................................. 4

- **Khramtsov A.G., Ryabtseva S.A., and Nesterenko P.G.**
  Paradigm of postgenomic conception on milk science LactOmics formation... 14

- **Panfilov V.A. and Andreev S.P.**
  Engineering of Complex Technological Systems in the Agroindustrial Complex .......................................................................................................................... 23

- **Tereshchuk L.V., Starovoytova K.V., and Ivashina O.A.**
  Practical Aspects of the Use of Emulsifiers in Manufacturing Emulsion Fat-and-Oil Products ................................................................................................. 30

### FOOD PRODUCTION TECHNOLOGY

- **Abhari Kh., Jafarpour D., and Shekarforoush S.S.**
  Effects of In-package Pasteurization on Preventing Spoilage in Emulsion Vacuum Packaged Sausages During Refrigerated Storage ........................................... 40

- **Kriger O.V., Kashirskih E.V., Babich O.O., and Noskova S.Yu.**
  Oat Protein Concentrate Production ............................................................................... 47

- **Izgarishev A.V., Izgarisheva N.V., and Ostroumov I.A.**
  Development and Study of Food Product with Anti-anemic Agent Based on Farm Animal Blood........................................................................................................ 56

- **Lisitsyn A.B., Semenova A.A., Kuznetsova T.G., Dydykin A.S., and Nasonova V.V.**
  Study of the Effect of Sex and Type of Muscles on the Development of Quality Defects in Turkey Meat after the Slaughter ........................................................................... 63

  Optimization of Prescription Composition of Jelly Masses Using the Scheffe’s Symplex Plan ........................................................................................................... 71

- **Mayurnikova L.A., Rudnev S.D., Davydenko N.I., Novoselov S.V., and Popova D.G.**
  Development of a Technical and Technological Solution for the Production of Carrot Nectar ........................................................................................................ 79

- **Nepovinnyk N.V., Klyukina O.N., Kodatskiy Yu.A., Ptichkina N.M., and Yeganehzad S.**
  Study of the Stability of Foam and Viscoelastic Properties of Marshmallow without Gelatin ........................................................................................................... 90

  Development of Integrated Technology and Assortment of Long-Life Rye-Wheat Bakery Products .......................................................................................... 99

- **Tien N.P., Siripongvutikorn S., and Usawakesmanee W.**
  Prototype of Vietnamese Tamarind Fish Sauce Fortified with Iron, Zinc and Vitamin A ................................................................................................................ 110

- **Timakova R.T., Tikhonov S.L., Tikhonova N.V., and Gorlov I.F.**
  Effect of Various Doses of Ionizing Radiation on the Safety of Meat Semi-Finished Products ........................................................................................................ 120

- **Usenko N.I., Khlestkina E.K., Asavasanti S., Gordeeva E.I., Yudina R.S., and Otmakhova Y.S.**
  Possibilities of Enriching Food Products with Anthocyanins by Using New Forms of Cereals ................................................................................................. 128
<table>
<thead>
<tr>
<th>Biotechnology</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyulkin S.V., Vafin R.R., Zagidullin L.R., Akhmetov T.M., Petrov A.N., and Diel F. Technological Properties of Milk of Cows with Different Genotypes of Kappa-Casein and Beta-Lactoglobulin ............................................................................ 154</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Processes, Equipment, and Apparatus for Food Production</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maytakov A.L., Yusupov Sh.T., Popov A.M., Kravchenko S.N., and Bakin I.A. Study of the Process of Concentration as a Factor of Product Quality Formation ........................................................................................................... 172</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standardization, Certification, Quality and Safety</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnilomedova N.V., Anikina N.S., and Gerzhikova V.G. Profile of Sugars in a Grape-Wine System as The Identifying Indicator of the Authenticity of Wine Products ......................................................................................... 191</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Food Hygiene</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aktaş Karaçelik A. and Sahin H. Determination of Enzyme Inhibition and Antioxidant Activity in Some Chestnut Honeys ................................................................................................................................. 210</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemistry and Ecology</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khvorova L.S., Lukin N.D., and Baranova L.V. Glucose Nucleation in the Presence of Surface Active Agents ........................................................................................................................................ 219</td>
<td></td>
</tr>
<tr>
<td>Krasnova T.A., Timoshchuk I.V., Gorelkina A.K., and Belyaeva O.V. Effect of Priority Drinking Water Contaminants on the Quality Indicators of Beverages during their Production and Storage ........................................................................ 230</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Information</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information for Authors ............................................................................................................... 242</td>
<td></td>
</tr>
</tbody>
</table>
ROLE OF ORGANIC PRODUCTS IN THE IMPLEMENTATION OF THE STATE POLICY OF HEALTHY NUTRITION IN THE RUSSIAN FEDERATION


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Abstract: The state policy of the Russian Federation in the field of the healthy nutrition of the population includes a set of measures aimed at meeting the needs of various groups of the population for healthy nutrition, taking into account their traditions, habits and economic situation. The complex system development of the consumer food market, in addition to the economic aspect, covers the issues of public health, demography, effective nature management, resource saving and ecology. The current scientific approaches in the field of healthy food production involve the addition of functional ingredients, biologically active substances or probiotic microorganisms capable of replenishing the deficiency of necessary substances to a base product. However, in addition to their high nutritional value, healthy food products must be absolutely safe and be of high quality, which is extremely difficult to realize in the present conditions of raw material production. The review provides a rationale that the organic products manufactured using traditional technologies without the addition of chemical fertilizers, pesticides, food additives, hormonal and other drugs, can be attributed to healthy food products. The development of the organic sector of food production at the state level will allow not only to implement the adopted normative acts within the framework of the state policy of healthy nutrition, but also to fundamentally improve the situation in other areas: economy, health and ecology.

Key words: Healthy nutrition, state policy, organic products, organic agriculture

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The concept of healthy nutrition is so extensive that its precise definition is much more difficult to find than the slogans of how important it is to eat healthy and diversified food. Popular magazines contain hundreds of articles about the health benefits of a particular food product. Scientific literature describes various nutrition theories, among which there is balanced rational and allopathic nutrition with the appropriate evidence base and distinctive features [1, 2]. The mass media broadcast about the harmful effects of some products, traditional for Russians, on the body and promote healthy nutrition with the use of biologically active supplements and doubtful non-conventional medicines.

What does the concept of "healthy food" used both by consumers and at the highest state level really include?

As a rule, healthy nutrition is understood as a diet that provides the growth, normal development and life activity of a human that contributes to health promotion and the prevention of diseases. The World Health Organization (WHO) has developed and implemented some countrywide integrated noncommunicable disease intervention (CINDI) programmes [3] and programmes to develop and implement the nutrition, infant feeding and food safety policy [4]. These programs contain a clear message that food products and the role they play in health
promotion should be regarded as an integral part of the "primary link of health" [3].

The CINDI Program Manual is a basic document for the design of national and regional nutrition programs. The basis of the development thereof is the scientifically based benefits of vegetable products that contain the biologically active substances (BAS) and vitamins that promote the normalization of blood cholesterol and blood sugar and prevent cancer, cardiovascular diseases, obesity and diabetes [1]. It is proposed to use the CINDI Manual as a basis for the development of regional programs and strategic plans for healthy nutrition, as well as for making the population to promote health. It differs from other documents in the field of nutrition by the fact that it considers not individual components and nutrients, but products in general, arranging them in the form of a pyramid with the use of the color scheme of traffic lights: green means "keep moving", orange – "caution", and red – "stop and think before you use". The essence of the document is twelve principles of healthy nutrition, each of which should be considered in the context of the others:

1. Eat a variety of food products, most of which are vegetable products.
2. Bread, flour products, cereals and potatoes should be included in the diet several times a day.
3. Eat a variety of vegetables and fruits several times a day (at least 400 grams a day), it is better to eat fresh ones and those grown in the locality.
4. In order to maintain the body weight within the recommended range (the body mass index of 20–25), moderate daily physical activity is necessary.
5. Control the intake of fat from food (no more than 30% of the daily caloric value) and replace animal fat with vegetable oil.
6. Replace fatty meat and meat products with beans, cereals, fish, poultry or lean meat.
7. Use low-fat milk and dairy products (kefir, yogurt, sour milk and cheese) with a low fat and salt content.
8. Choose the food products with a low sugar content and eat a moderate amount of sugar, limiting the amount of sweets and sugary drinks.
9. The total amount of salt in food should not exceed 6 g (one teaspoon) a day. Iodized salt should be used.
10. The total content of pure alcohol in alcoholic beverages (when consumed) should not exceed 20 g a day.
11. Cooking should ensure food safety. Steam cooking, microwave cooking, baking or boiling will help you to reduce the amount of fat, oil, salt and sugar used when cooking.
12. Encourage infants to breastfeed for only the first 6 months. Complementary food should be introduced gradually, in parallel with breastfeeding [2, 3].

Generalizing dozens of different concepts [1–3, 5, 6], the following most complete formulation can be provided: healthy food is the reception of a variety of balanced food products with the minimal human effect to provide the life, growth and development of a human and, combined with regular physical activity, health promotion and the prevention of diseases. The general recommendations for healthy nutrition developed by WHO are as follows:

– the control of body weight and caloric value of products;
– the restriction of consumption of fats, simple carbohydrates and salt;
– the increase in the proportion of fruits, vegetables, whole grains, legumes and nuts in the diet;
– the consumption of vitamins and the food products rich with micronutrients;
– the development of individual diets by dietitians [6].

However, there is no information in WHO documents on the nature of the origin of the recommended products. A person can adhere to all the principles of healthy nutrition recommended by WHO, but at the same time to replace animal fats with the vegetable ones derived from genetically modified plants, to eat fresh vegetables containing agrochemical residues, lean meat and skimmed dairy products with antibiotics several times a day. It is not known whether synthetic fertilizers, pesticides, growth stimulants and genetically modified organisms (GMOs) were used in their cultivation and production. There is a question in this context – will there be a real benefit from the use of such products?

The problem of healthy nutrition is considered at the highest level in a lot of countries of the world. The state policy of the Russian Federation in the field of healthy nutrition of the population means a set of measures aimed at providing the conditions that satisfy the needs of different groups of the population for healthy nutrition in accordance with the requirements of medical science, taking into account their traditions, habits and economic situation [7]. The Concept of the National Policy of Healthy Nutrition of the Population of the Russian Federation was for the first time formulated in 1998. An important aspect of the implementation of the Concept was the adoption of the programs aimed at improving the structure of food consumption of the population as well as the organization of health-improving food centers, including child and dietary nutrition centers, and the formation of a healthy lifestyle for the population [8].

Recognizing a sharp increase in mortality caused by noncommunicable diseases, including cardiovascular diseases, diabetes and certain types of cancer, WHO member states have proposed the formulation of the Global Strategy on Diet, Physical Activity and Health (hereinafter referred to as the Global Strategy). The global strategy, adopted in May 2004, includes:

– a need to ensure correct and balanced information – education, information and public awareness, including full marking;
– taking measures in the field of food and agriculture – promoting the manufacture and consumption of healthy products, the formation of agricultural and fiscal policies and the organization of food programs;
– a multisectoral policy to promote physical activity of citizens;
– attracting investment in supervision, scientific research and much more [6].

The national strategies developed on the basis thereof should include specific goals, objectives and
actions, taking into account the climatic, cultural and other characteristics of countries, as well as a diet and physical activity guide.

The Order of the Government of the Russian Federation "On the Fundamentals of the State Policy of the Russian Federation in the Field of Healthy Nutrition of the Population for the Period up to 2020" notes that, despite the positive trends in the nutrition of the population, the mortality caused by chronic diseases remains significantly higher than in most European countries. The nutrition of the majority of the adult population does not meet the principles of healthy nutrition because of the consumption of the food products that contain the large amounts of animal fat and simple carbohydrates, lack of vegetables and fruits, fish and seafood in the diet, which leads to an increase in overweight and obesity, the prevalence of which has increased from 19% to 23% for the last 8–9 years, increasing the risk of diabetes, cardiovascular and other diseases.

The Russia's state policy in the field of healthy nutrition is aimed at preserving and promoting the health of the population and the prevention of the diseases caused by poor and unbalanced nutrition. The main tasks are the expansion of domestic production of high-quality food raw materials and food products, including enriched and special ones; the development of innovative technologies and educational programs on healthy nutrition issues; the organization and provision of adequate nutrition for children, pregnant and lactating women and organized groups, as well as the monitoring of the nutritional status of the population [7, 8].

The key trends of implementation of the state policy in the field of healthy nutrition are:
- the development and adoption of the technical regulations that concern food products;
- the legislative consolidation of the manufacturer's responsibility for the manufacture of the food products that are falsified and do not conform to the specified requirements;
- the development of the national standards that ensure the compliance with the requirements of technical regulations for food products and food raw materials;
- improving the quality control mechanisms in relation to the food products and food raw materials produced on the territory of the Russian Federation and supplied from abroad;
- the priority development of fundamental studies in the field of modern biotechnological and nanotechnological methods for obtaining new sources of food and the medical and biological estimation of their quality and safety;
- an increase in the promotion of healthy nutrition of the population, including that with the use of the media [7].

In addition to the economic aspect, the integrated system development of the consumer food market covers the issues of public health, demography, effective use of natural resources, resource saving and the ecological component. Over the years, a number of documents have been approved at the highest state level aimed at providing adequate nutrition, preventing diseases, increasing the duration and improving the quality of living of the population, stimulating the development of manufacture and circulation of food products of appropriate quality in the food market, including the Food Security Doctrine [9] and the Strategy for Improving the Quality of Food Products in the Russian Federation until 2030 (hereinafter referred to as the Strategy) [10, 11].

The Food Security Doctrine has been approved by the Decree of the President of the Russian Federation as part of the Russian National Security Strategy and represents a set of official views on the goals, objectives and main trends of the state economic policy in the field of food security of the Russian Federation. Food security is understood as the most important component of the demographic policy and a necessary condition for the implementation of a strategic national priority – improving the quality of living of Russian citizens by guaranteeing the high standards of livelihood.

The strategic goal of food security is to provide Russians with safe agricultural products, fish and other products from aquatic biological resources and food. The guarantee of its achievement is the stability of domestic production of safe raw materials and products, its physical and economic accessibility to ensure the active and healthy lifestyle of every Russian citizen and the availability of the necessary reserves.

The implementation of the provisions of the Doctrine will ensure food security as an essential part of national security, predict and prevent emerging threats and risks for the country's economy, increase its stability, create conditions for the dynamic development of agro-industrial and fisheries complexes and improve the well-being of the population [9].

To form a healthy diet, the Doctrine proposes:
- to develop the fundamental and applied scientific research on the medical and biological estimation of the safety of new sources of food and ingredients, to introduce the innovative technologies that include bio- and nanotechnologies, organic food product and food raw material manufacturing technologies and to rev up the production of new enriched, dietary and functional foods;
- to develop the educational programs on healthy nutrition for the population as an essential component of a healthy lifestyle with the involvement of the media;
- to formulate the norms of social catering and measures to support it;
- to develop and implement a set of the measures aimed at reducing the consumption of alcohol and other alcohol-containing products [9].

The strategy is a basis for the formation of a national food quality management system aimed at providing the quality of food products as an essential component of health promotion and an increase in the duration and quality of living of the population, generating demand and supply and respecting for consumers' rights to such products.
The objectives of the Strategy are proposed in the following areas:
(1) The development of a regulatory framework in the field of food quality, including the legal aspects of compensatory mechanisms for protecting consumers' rights. The improvement of the state regulation, control (supervision) and application of administrative measures for non-compliance with the requirements to the quality of food products by the manufacturer;
(2) The improvement of the methodological basis for estimating the compliance of food quality indicators. Ensuring the monitoring thereof;
(3) The development and integration of a quality management system for food products. Creating a single information traceability system;
(4) The creation of incentive mechanisms for manufacturers to produce the foods that meet the quality criteria and healthy nutrition principles, as well as the products of the new generation with the specified quality characteristics;
(5) The recovery of the production of food ingredients in the Russian Federation;
(6) Updating the current normative levels of the content of food supplements, flavors, biologically active and potentially hazardous substances in food products;
(7) The priority development of scientific research in the field of nutrition of the population, the prevention of non-communicable diseases and the development of the production technologies aimed at improving the quality of food products;
(8) Promoting the principles of healthy nutrition [10].

The abovementioned documents are bound by one common goal – to ensure the health of their citizens, which is achieved, first of all, by providing the population with safe and balanced food products. The state took the necessary measures to identify unscrupulous manufacturers and to curb the manufacture of poor-quality and falsified food products. Through agitation and propagation, Russian citizens began to understand the importance of adequate nutrition and food safety for their own health.

In pursuance of the above documents, specialists conduct the research and design the new types of products, as well as enriched, special and functional products that meet the specified requirements including dietary products for curative and preventive nutrition. Such products have a balanced ratio of substances and components and scientifically grounded and confirmed properties that replenish a nutritional deficiency and prevent the risk of certain diseases. The developed methodologies and scientific approaches in the field of manufacturing healthy food products imply the introduction of functional ingredients, biologically active substances or probiotic microorganisms into the base product [12–17]. Thus, Sanitary Rules and Norms SanPiN 2.3.2.2804-10 that supplement SanPiN 2.3.2.1078-01 "Hygienic requirements for food safety and nutritional value" were used for many years to enrich consumer products for children over 3 years old and adults with essential substances. In accordance with the specified document, at least 15% and not more than 50% of the daily requirement for micro- or macronutrients, BAS, vitamins and minerals that enrich the product should be contained in one average daily portion of enriched food products. The compliance with this requirement in the production of enriched products ensured that they would help to prevent a deficiency in the substances needed for the body and at the same time be safe for human health. The enrichment of products with essential substances (vitamin substances, BAS, etc.) in low amounts (less than 15% of the daily requirement per serving) is ineffective and does not benefit from eating them regularly [18].

A food product can be converted into a functional one in two ways – by reducing the content of the ingredients harmful to health in the product and by enriching products with scarce micronutrients.

The manufacture of functional products with a low content of harmful components suggests a change in the formulation, mainly in reducing the total content of fats and sugar. The reduction of total of fats in a product significantly reduces its energy value (caloric value). At the same time, it is especially important to reduce the consumption of animal fats – the sources of cholesterol and saturated fatty acids, as well as hydrogenated fats – the sources of trans-isomeric fatty acids. Reducing the content or complete replacement of sugar also helps to reduce the caloric content of a product and reduce its glycemic index [12].

The enrichment of products with the additional useful substances is justified and effective only if certain principles of food enrichment that are based on the long-term results of nutritional research in our country and abroad are observed [13–20]. The development of such products is necessary and timely.

However, the authors believe that attention should also be paid to one of the main tasks of the state policy in the field of healthy nutrition – the development and introduction of innovative technologies in agriculture and food industry, including biotechnology and organic production technologies. In this context, the organic (BIO) products, manufactured using traditional technologies without the addition of chemical fertilizers, pesticides, food supplements, hormonal and other drugs, also have a beneficial effect on the human body and can be attributed to healthy food products [23–25].

In accordance with GOST R 56104-2014 "Organic foods. Terms and definitions", an organic food product is a product in the natural or processed form manufactured from the vegetable and animal raw materials grown in the areas for organic farming, as well as the forest, bee and fish products grown, manufactured, processed, certified, labeled, stored and sold in accordance with the rules of organic production regarded as the food for consumption in the processed or unprocessed form. In other words, the manufacture of organic products is nothing more than processing, by means of sparing methods, the raw materials obtained using the traditional method for cultivating the land, plant growing, cattle breeding, etc., applied by our ancestors-farmers a hundred or more years ago [26]. At present, such activity is called "organic agriculture".
This term implies a system of agricultural production that excludes the use of chemically synthesized mineral fertilizers, pesticides, growth regulators, feed supplements and GMOs, which is based on the use of special modern varieties of plants and animal breeds, crop rotations, green fertilizers, biological pest control methods, mechanical cultivation of soils, and also corresponds to the officially approved special norms [27].

The International Federation of Organic Agricultural Movement (IFOAM) has formulated four principles that are the basis of the concept of organic agriculture:

1. the principle of health – the support and improvement of the health of soil, plants, animals, human and the planet as a whole;
2. the principle of ecology – the internal management and the management taking into account the characteristics of the natural system and life cycles and the environment;
3. the principle of justice – ensuring general food security, the availability of high-quality products, decent conditions for animals, plants, people and their descendants;
4. the principle of care – protecting the health and well-being of the present and future generations and the environment.

The following elements are the features of organic agriculture technology:

- special tillage;
- the rejection of chemically synthesized fertilizers;
- crops are selected taking into account their biological characteristics and specific conditions;
- the pest, weed and disease control system is developed individually for each crop on a particular farm;
- competent crop rotation to preserve soil fertility [28].

Organic agriculture is developed in more than 179 countries of the world. The organic sector is currently the fastest growing food trade branch in the world. According to the data for 2015, 2.4 million farmers have reclaimed 50.9 million hectares of organic agricultural lands, 45% of which are in Oceania and 25% – in European countries. The global ecologic product market has grown more than 4.5 times over the past 15 years: in 2000, the global sales of organic food and beverages amounted to 18 billion US dollars, in 2010 this value reached 59 billion US dollars, and in 2015 – about 81.6 billion US dollars. According to the forecasts, the turnover of organic food can reach 250 billion US dollars by 2020. The leading markets of organic products are the USA, Germany, France and the United Kingdom. At the same time, the EU and the USA consume 96% of all eco-products [29]. The domestic production capacity of these countries has almost been exhausted, and the demand for this type of products is steadily growing. In this regard, the organization of organic production is most active in developing countries: there are 585,000 manufacturers in India, 203,602 in Ethiopia, and more than 200,000 in Mexico. Australia ranks first in the list of the countries with the highest organic land growth in 2015 [30] where 4,350 thousand hectares of land were certified as organic, the United States with 474,8 thousand hectares take the second place, and India with 460 thousand hectares – the third. Russia ranks seventh: in 2015, 139.3 thousand hectares were identified as organic lands, over 10 years – 382 thousand hectares that account for only 0.2% of the total number of agricultural lands. [31–33].

Despite an increase in the areas of organic lands, it should be noted that the organic sector of food production in Russia functioned spontaneously until mid-2015. The only document in the sphere of organic products was SanPin 2.3.2.1078-01 (Supplements and Revisions No. 8). The largest manufacturers of organic products were: Ecoproduct Corporation (the Moscow region), AgriVolga Agricultural Holding (the Yaroslavl region) and a number of farms in the Tula, Kaluga, Penza and other regions. They have been certified by European companies for compliance with the requirements of EU Regulations No. 834/2007 and No. 889/2008 on organic production and labeling of organic products [22, 31].

In the developed countries, the regulation of the consumer market by state structures is one of the most important tasks. In general, the market regulation mechanism, which is a set of constantly acting measures implemented along the entire path of goods movement from the manufacturer to the consumer, must, on the one hand, prevent the appearance of dangerous products in the market and ensure the reliability of information about goods, and on the other – minimize administrative barriers for manufacturers.

The technical regulation document system of Russia, as a member state of the EEU, includes technical regulations (TR CU) and interstate standards (GOST) for products and measurement methods, as a result of which voluntary compliance with TR CU requirements is ensured. Regulations impose stringent requirements not only for raw materials and finished products, but also for terminology, food supplements, packaging and labeling (including the requirements for names, composition and other information applied). All the products manufactured and sold on the territory of the Russian Federation are to comply with the requirements of TR CU for quality and safety, as well as for marking.

At present, the output of organic products for the consumer market of Russia is in accordance with the general requirements of TR CU for all food products – there are no individual regulations with regard to the organic origin and production method, is provided with declarations, and its voluntary certification is carried out by certification bodies on the basis of international standards due to the absence of a functioning national system for organic products certification in Russia.

The emergence of organic products in the Russian market, the growing interest of domestic producers in organic production and the accession of the Russian Federation to the WTO have intensified the work on national standardization in this segment, and also protected the interests of the country and our consumers when developing international standards. The Technical Committee 040 "Organic Products"
established in March 2009 on the basis of the National Consumer Protection Fund has initiated the development of a number of standards for organic products in order to standardize relevant terminology, production rules and certification procedure.

The national standard GOST R 56104-2014 "Organic foods. Terms and definitions" was approved and put into effect by the Order of the Federal Agency for Technical Regulation and Metrology of September 10, 2014, No. 1068-st. The standard establishes terms and definitions in the field of production, composition and properties of organic food products and their derivative products that meet the requirements of organic production. The standardized terms are recommended for use in all types of documentation and literature on organic food products in the field of organic production that are part of the scope of standardization work and/or use the results of this work [26].

The Order of the Federal Agency for Technical Regulation and Metrology No. 844-st of June 30, 2015 approved and put into effect the national standard GOST R 56508-2015 "Organic production. Regulations for production, storage, transportation" developed by the State Duma Committee on Agrarian Issues. The standard establishes the requirements for the manufacture, storage and transportation of organic products in the natural and processed form and includes the requirements for organic production (plant growing, cattle breeding, beekeeping and aquaculture facilities), as well as the rules for collecting, packaging, marking, transporting and storing organic products [34].

GOST R 57022-2016 "Organic production. The procedure of voluntary certification of organic production" was approved by Order No. 906-st of August 5, 2016. GOST R 57022 establishes a procedure for the voluntary certification of organic production for compliance with the requirements of GOST R 56508. It is intended for use by organic production certification bodies, legal entities or individual entrepreneurs who claim to receive an organic production conformity certificate or the holders of organic production conformity certificates [35].

At present, OOO Eco-Control and NP Ecological Union – the Leaf of Life marking – successfully operate in Russia [31].

Russia is ideal in many ways for the development of organic agriculture. From 1990 to the present time, more than 40 million hectares of arable agricultural lands and millions of hectares of other lands are empty, the intensity of agricultural production in Russia is low, the level of anthropogenic impact and pollution of vast areas is low, most farms do not introduce fertilizers at all, and growing genetically modified plants is prohibited by law. With the abundance and a relatively low cost of land, to be engaged in organic, i.e. extensive low-cost, agriculture can be very beneficial and useful [11, 28]. In Russia, one of the leading agrarian countries in the world, organic production is not developed enough: according to IFOAM, the percentage of organic agricultural products in the world market that accrues to Russia along with the CIS countries, is only 1.4% [31].

In the annual messages of the President of the Russian Federation [36, 37], V.V. Putin repeatedly drew the attention of the Federal Assembly to the need to develop agriculture, improve the quality indicators of production efficiency and introduce advanced agricultural technologies: "... Russia is able to become the largest world supplier of healthy, ecologically clean and high-quality food which the Western producers have long lost, especially given the fact that demand for such products in the world market is steadily growing ... We need technologies for manufacturing, storing and processing agricultural products, as well as a seed and breeding fund of our own...".

With regard to agriculture, the Federal Law "On the Development of Agriculture" [38] is currently in force, as well as the State Program of Agricultural Development and Regulation of Markets for Agricultural Products, Raw Materials and Food for 2013–2020 [39] and the Federal Science and Technological Program for the Development of Agriculture for 2017–2025 [25]. The strategy of scientific and technological development of the country [41] determines the priority trends for the next 10–15 years that will allow us to obtain scientific and scientific and technical results and create the technologies that are the basis for the innovative development of the domestic market of products and services, will ensure Russia's stable position in foreign markets. The implementation of the measures in these areas should provide a transition to highly productive and environmentally friendly agro- and aquaculture, the development and integration of the systems for the rational use of chemical and biological protection equipment for agricultural plants and animals, the storage and effective processing of agricultural products, the manufacture of safe and quality, as well as functional, food products [38–40, 42].

At a meeting with the representatives of business circles of the Yaroslavl region, held on April 25, 2017 in Rybinsk, President Putin noted that organic agriculture is a promising trend of development the nation's health depends on. At the same time, it is important to make organic products economically available [43], for which it is necessary to provide special conditions; it is important to speed up the adoption of the draft federal law regulating this sphere by the State Duma.

The draft federal law "On organic production and circulation" has been under discussion and revision for more than 5 years. The bill establishes a legal framework for regulating the relations in organic production and circulation and covers the relations that arise in the course of organic production and circulation. Its purpose is to develop the domestic market and meet the needs of Russians in organic products, as well as to increase the competition among manufacturers and increase exports. In addition to the principles of organic products and the voluntary compliance confirmation system, the bill provides for the creation of a unified state register of manufacturers and their products. The register is necessary for informing consumers and providing state support in
accordance with the Federal Law "On the Development of Agriculture" [38].

As a result of the adoption of the bill, legal conditions will be provided to develop organic agriculture in Russia, which will make it possible to become a full-fledged participant in the international market. According to some expert estimates, the Russian market of organic products will grow more than 10 times and will be commensurable with the leading countries of Europe within 5 years from the adoption of the bill [31, 33, 44, 45].

Organic agriculture is a production system that improves the ecosystem, preserves soil fertility, protects human health, and, taking into account the local conditions and relying on the ecological cycles, preserves biological diversity and does not use the components that can be harmful for human and the environment [46]. It combines traditional farming practices, innovative technologies and modern scientific and technological developments that have a beneficial effect on the environment and, while maintaining a close interrelation among all the life forms included in the system, maintain and ensure their favorable development [47]. The qualitatively organized organic agriculture and organic production will allow us not only to implement the bases of the state policy in the field of healthy nutrition and state programs, but also provide some other positive results at the local and federal levels [32, 48].

With regard to organic products, a mechanism for implementing the state policy in the field of healthy nutrition can be easily triggered with the help of technical regulation tools – the development and approval of the appropriate technical regulations, federal laws, national and interstate standards, as well as uniform rules for conformity assessment, supervision and control. The remarkable features of the resources of the Russian Federation for the development of organic agriculture and an increase in its role in the field of healthy nutrition of the population are [11, 28, 38, 49]:
- more than 40 million hectares of free agricultural land [50];
- the soil that has not been used for more than 20 years and that has been recovered naturally;
- 20% of the world's fresh water supply;
- the opportunity to generalize the available world experience;
- according to expert estimates, more than 30 thousand farmers (instead of 70 at the moment) can switch to organic farming [31];
- the grow of the interest of Russians in environmentally friendly products;
- the development of product export related to the stable demand of exporters from the developed countries (primarily Germany and the USA) [31, 33].

The analysis of the principles of organic agriculture and the features of processing the obtained raw materials standardized in GOST R 56508-2015 [34] allows us to predict a possible social effect from the organization of organic production in Russia, both at the nationwide and local levels, and estimate the role of organic products in the implementation of the state policy in the field of healthy nutrition in general [11, 49, 51–53]:

1) "Organic production should be located far from the sources of environmental pollution, industrial facilities and intensive agriculture areas" – the implementation of this principle contributes to the reconstruction of obsolete and abandoned production facilities, as well as the construction of new cities remote from large ones. This will entail the formation of new settlements and infrastructure in environmentally friendly areas, the development of rural areas, the improvement of living standards by ensuring the employment of the population, the enhancement of the skills thereof and, ultimately, the improvement of the demographic situation;

2) "Organic plant growing should use the soil cultivation methods aimed at preserving the soil natural composition, preventing the development of degradation processes and maintaining the biodiversity of ecosystems; maintaining and enhancing soil fertility and biological activity" – in this regard, it is required to develop new high-yielding varieties and hybrids of various crops with a high resistance to diverse climatic conditions of our country;

3) "Hydroponic production and the use of mineral nitrogen fertilizers, synthetic herbicides, fungicides, insecticides and other pesticides, growth regulators and synthetic dyes is not allowed." Organic plant growing is the guarantee of improving the ecological situation and implementing effective nature management, preserving the existing and involving new agricultural lands in production, and improving the cropping culture through the natural recovery of soil fertility;

4) "Animals for organic production must be born or incubated in the conditions of production facilities that meet the special requirements of organic cattle breeding" – the implementation of this principle can act as a fillip to the recovery and development of cattle breeding and poultry in the Russian Federation and an increase in the total livestock number;

5) "Pastures, grazing areas or paddocks for cattle run must be available for all animals. At least 50% of feed for herbivores should be produced using organic production methods in the same region" – the introduction of this principle will allow the development of animal feed production, the reduction of the volume of their imports and the maximal involvement of agricultural lands in production;

6) "The prevention of animal diseases in organic cattle breeding does not allow the use of chemically synthesized drugs or antibiotics, including those that are used for a preventive purpose, as well as the use of special substances and hormones to stimulate growth and control reproduction" – there is a need to develop and organize the production of modern domestic medicines for veterinary use and to stimulate the development of zoopharmacy and this sector of economy;

7) "Organic food and feed are manufactured from organic raw materials using mainly biological, mechanical and physical methods, except when the ingredient is not an organic product; the use of food
supplements is limited "- this principle contributes to the development of science and technology, the expansion of the volume and assortment of domestic ingredients and the appropriate food supplements, and the promotion of healthy food principles;

(8) "The final result of completion of the technological process of the organic method of production from organic raw materials is ready-to-eat organic products" – the partial or complete resolution of the problem of import substitution (cheese, vegetables, fruits, etc.). The fundamental requirements for organic products cause the expansion of traditional products, for example, national dairy products according to interstate or national standards.

The development of organic complexes requires highly skilled specialists of various profiles – from agronomists and veterinarians to milk and meat technologists – can motivate young professionals to work in their specialty in rural areas having a decent salary and housing – a base for family foundation and children birth. Herewith, an important role is played by the psychological adaptation of specialists to new living and working conditions.

The implementation of a system of measures to stimulate agricultural manufacturers, develop state instruments for the standardization of organic products and support consumer agricultural cooperation and small and medium business that provides services to agricultural manufacturers has the key value to the successful development of organic agriculture in Russia. Equally important is the state support in the field of training and retraining of personnel, information and methodological and scientific and methodological support of the agricultural manufacturers who are engaged in organic agricultural production or the persons who are planning to organize it.

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PARADIGM OF POSTGENOMIC CONCEPTION ON MILK SCIENCE LACTOMICS FORMATION

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Abstract: Based on perspectives of the philosophy for science of dairy and postgenomic ideas the historical aspects, an attempt has been made to formulate the paradigm of LactOmics as a logical generalization of LipidOmics, ProteOmics and GlycOmics. The depth of experience is maintained thousands years. First paper was published 400 years ago. Up-to-date concepts have been cluster formed. The technology platform of LactOmics is based on the bio- nano- electromembrane technique of the dairy industry modernization, as part of full import replacement with export orientation. This provides the national Food safety for the entire range of dairy products for the determined population groups.

Keywords: Dairy science, Raw milk clusters, LipidOmics, ProteOmics, GlycOmics, LactOmics


INTRODUCTION

Bearing in mind the innovative priorities to upgrade the milk industry [1–3] and in compliance with the terminology principles to be formed lately of postgenomic trends in biology science, it seems advisable to share insights on the diary science, on whole. The exclusive vision of the subject-matter related to major components (complex) of raw milk, that is carbohydrates (lactose and its derivatives); lipids (milk fat) and nitrogen-bearing components (casein and whey proteins) is clarified in our respectable journals [4–6].

Definitely, LactOmics includes three logistically independent but organically associated stages: Stage I – milk raw product obtaining; Stage II – milk raw product processing; Stage III – dairy products application.

First stage is traditionally the prerogative right of farming of various forms of ownership, volume and technologies from the specific owner of a cow (goat, sheep and other lactating female) to automated plants to produce marketable milk products for commercial purposes. This exceptionally labor-intensive industry, with its specifics, should be studied independently (separately) as part of LactOmics. This field has been under persistent and successful study by our respected colleagues investigators and practices in the field of established agricultural sub-sector (Agrarian and Industrial Complex) as the dairy husbandry. This is the source of raw material for our sector. That is why the dairy industry has always been objectively concerned in well-managed and developing production of marketable milk. In view of science, our task (as part of LactOmics) is to study the milk composition and properties with retaining its biocoenosis to the highest level prior to industrial processing. Criteria of interrelations with milk raw stock producers include the mutual understanding and mutually beneficial partnership within a single integrated system, for instance, a Ministry or an Association (cooperation). We would hope that the Russian Dairy Union (Russian Union of Dairy Producers) and SoyuzMoloko, as well as all trading levels and forms will come to agree to a common platform for purposes of diary science development in
Russia. In terms of terminology – dairy raw processing production.

Second stage is the industrial processing (fabrication) of the milk raw stock to obtain the desired (required) range of dairy products that is the independent sector (today it is the Ministry of Agriculture of Russia as part of Agrarian and Industrial Complex) and is based on scientific accomplishments in almost all fundamental and applied sciences existing in the world. This part of LactOmics is the subject of consideration in this article as the constituent part of the investment portfolio accumulated for over 50 years of practical and academic activity. It is formalized as the dairy product technology in terms of science, human resourcing and information support [8].

Third stage is the use of products (dairy and milk-containing) produced by the industrial processing of milk raw stock, that is classified as commercial, public and home food, and in terms of science, it should be reviewed culture of consumption of dairy and milk-containing products, feed materials, pharmaceutical and veterinary means, as well as possible service semi-finished products. This stage of LactOmics, the same as the first stage, requires a separate (independent) study in terms of scientific and practical practice with integration of experience gained. For example, modern design and service are not involved in the dairy business at all and most often, the advertising is not positive.

Consideration of the milk raw stock lactOmics would be desirable to start with the tractate of Fabrizio Bertolletti [9], the inquisitive Italian monk who divided the milk into three components back in 1615 (the time of the great Leonardo da Vinci) as follow: Butyrum (fat); Serum (whey) and Caseus (protein). At our justifiable request and during the personal meeting, Saverio Mannino, the respected Professor of the Milan University, the well-known nanotechnologist, made photo-copies of pages of this unique scientific work (kept in the manuscript department of the University of Florence), with the fragment shown in Fig. 2 just for reference.

It is notable that only three components are identified in milk, two of which turned out to be the base for industrial and home production of milk products as the sweet butter – Butyrum and cheese (cottage cheese) – Caseus. By the way, F. Bertolletti used to evaporate (condense) the Serum (milk whey – curd) just being concerned to obtain the "manna" (pasty mass) that became the harbinger of lactose (milk sugar). In the "milk tree" by Wygand (cited as per I.G. Zadow) [10], as shown in Fig. 3, dozens of milk components can be identified as well as dairy products.

As per modern concepts [11], about 2000 components have been identified in milk, which include more than 100,000 molecular structures, which is impressive and requires a constant study of this unique product provided to the humans by nature ("amazing food" by our compatriot I. P. Pavlov, the Acad., Nobel Laureate) for the purpose of its rational (effective) use. This postulate is the purpose and content of the proposed science of LactOmics. At the same time, it is necessary to pay attention to the ethical component of LactOmics – milk fabrication is "blessed by the Almighty", and the cow-nurse in all nations is "poetized".

For example, the hymn of the Sumerians, that given below solely for the respect to the postulate of evidence of LactOmics life affirmation:

“Our force is cows!  
Cows feed us!  
We hope for cows!  
We win thanks to our cows!”

A cow is idealised and idolize (India and other countries) by a range of ethnic groups. To note, as a food product, the milk (as the animals are domesticated) by F. Engels ("Dialectic of nature"), is at least treated as the meat food and associated with formation of the homo sapiens of today. The motto – "Back to apes with no milk" was claimed at the 6th International Conference of Milk Whey (USA, 2011) [12] by health care professionals.

Let’s enlarge upon the second stage of LactOmics – industrial fabrication of milk raw stock.

First, it should be stated nowadays in compliance with LactOmics postulates that milk and all milk raw materials (cream, skim milk, buttermilk, whey, ultrafiltrates and casein-free phase) are the most complex and naturally synthesized – and thus, of specific value – bio-technologically pure ecosystem (BTES). This statement is claimed by our colleagues-live stock breeders [13] and fully corresponds to the science gradation in Category "Live Systems". It is methodologically justified in the system analysis, for example, in the unique works by the Academician of the Russian Academy of Sciences V.A. Panfilov [14, 15].

![Fig. 2. Fragment from F. Bertolletti work (1619).](image)
Second, based on BTES postulates, all the milk raw stock components should be considered as natural CLUSTERS – lipid (fat), nitrogen-containing (proteins), carbohydrate (lactose), mineral (salts), BAS and water. The Figure 4 shows the milk LactOmics clusters in the integrated illustrations exclusively for the visual verification of BTES.

It should be noted that each cluster is polymorphic, is of interest by itself and is considered in detail in our articles: fats (LipiOmics) [5] proteins (ProteOmics, PeptidOmics) [6], lactose (GlycOmics) [4]. At that, the size of certain clusters is idealized in terms of nanotechnology concepts – lactose anomers are shown in Fig. 5; mineral salts; water – they are heterogenetic for the molecular sieve effect-based separation (membrane technology) and subjectively (as "live systems") correspond to biotechnology postulates (bio-membrane technology).

Breakthrough researches by I. T. Smykov [16] conducted on the national level obviously verify the statements above, while the synthesis of nantubes...
of whey proteins (fat substitutes) [17] makes them "reassuring" as part of LactOmics science (Fig. 6).

As it was repeated again and again [3, 8], the classic example of bio-, nanotechnology principle implementation is the controlled hydrolysis of lactose (milk sugar) to glucose monosaccharides and galactose (Fig. 7), protein cleavage to peptides and amino acids (Fig. 8), as well as the controlled lactose transformation to lactulose (Fig. 9) and other derivatives (over 100 titles).

Third, the range of LactOmics diary products formed traditionally is great and may satisfy needs of any consumer, from birth to extreme ages "in sickness and in health". The list of cheese only includes over 10000 items [18]. The scientific support for dairy product technology is justified by our outstanding predecessors (I dare to mention only a few fellow citizens): by basics – D.I. Mendeleev, I.P. Pavlov, I.I. Mechnikov, N.V. Vereshchagin, N.V. Parashchuk, G.A. Kuk, G.S. Inikhov, Ya.S. Zaykovsky, S.A. Korolev, N.N. Lipatov, P.F. Dyachenko; by separate product line groups: butter making industry – M.M. Kazansky, G.V. Tverdokhlyob, V.I. Sirik; cheese making industry – A.I. Chebotaryov, P.F. Krasheninin, A.M. Shalygina; canned milk – M.S. Kovalenko, V.V. Strakhov, N.V. and L.V. Chekulaeva. The laid basis for LactOmics has been actively and purposefully developed by creative teams, among which the schools by the Academician of the Russian Academy of Sciences V.D. Kharitonov (All-Russia Dairy Research Institute, Russia) are recognized at the national level – whole milk products and canned milk; schools by Professor F.A. Vyshemirsky (All-Russia R&D Institute of Butter and Cheese Industry, Russia) – butter industry; schools by Professor L.A. Ostroumov (Kemerovo Technological Institute of Food Industry (University), Russia); by the Academician of the RAS Yu.Ya. Sviridenko (All-Russia R&D Institute of Butter and Cheese Making, Russia), by the Prof. A.A. Mayorov (Siberian Scientific Research Institute of Cheesemaking Industry, Russia), – cheesemaking; as well as the scientific school 7510.2010.4, now NCFU (North Caucasus Federal University – co-supervisors: the Acad. of the Russian Academy of Sciences A.G. Khramtsov and Prof. I.A. Evdokimov) – secondary milk raw processing. It should be emphasized that lots of creative teams, outstanding researchers and practitioners in this industry, research institutes, universities and commercial organizations are involved in scientific researches in the field of dairy science, including dairy technology. The work they are engaged in is noble and demanded. The scope of the publication does not allow mentioning them. In addition to hundreds of national research organizations and companies, the International Dairy Federation (IDF) has been operating for more than 100 years that represents LactOmics as the integrated science in a worthy manner.

Fig. 4. Clusters of milk raw stock LactOmics: (a) water, (b) lactose isomer (milk sugar), (c) protein (whey protein globule), (d) lipid (milk fat), (e) protein (casein micelle), and (g) atomic formula of β-glycosidic complex.
**Fig. 5.** Mutarotation of lactose anomers.

**Fig. 6.** Formation of nanotube structure of whey protein globules.

**Fig. 7.** Lactose hydrolysis (a classic example of nanotechnology).

**Fig. 8.** Transformation of lactose into lactulose with the scaling cut by neuronal network method.
Conceptual part of **Technological Platform** [19–21] of LactOmics, in terms of dairy product technology and its implementation and, in our opinion, it should lean on three pillars:

- the full use of the milk raw stock components with modification thereof, standardization (regulation), structuring and enriching (filling mixtures);
- the extraction of single components or groups of components producing the commercial product;
- the fabrication (synthesis) of derivative components of milk raw stock producing the commercial product, including pharmaceuticals and veterinary drugs.

The theoretical justification of each LactOmics thesis of dairy science on whole and the technology of dairy products in particular, practical implementation thereof require fundamental approaches to be developed, as A.M. Osintsev [22] attempted under the guidance of the Professor L.A. Ostroumov. However, it's not the end. The milk tree continuously grows and crops.

The conceptual part implementation of LactOmics of dairy product technology by the product line groups as above allows creating an alternative approach to the procedure of industrial processing of raw milk, under conditions of economic reforms and globalization of the dairy market, based on principles of non-waste and robotic technology in the complete and even captive manufacture with no draining wastes. A vast and continuously expanding range of dairy and milk-containing products, beverages, feed, pharmaceutical and veterinary agents, as well as commercial semi-finished products is worth to be listed in the Guinness Book of World Records.

The Technological platform (TP) of industrial processing (fabrication) of the milk raw stock, based on LactOmics principles, should be started from its conditioning phase. This problem is addressed as system issues in trade journals [23, 24] and requires specific development for widespread scaling in the industry [25–27]. The essential condition for the TP implementation is harmonization of biotechnological impact on the raw material system minimizing intramolecular alterations and retaining the system integrity, so called "steamed milk", as the high level of LactOmics in the biocenosys of the Earth.

Implementation of advanced innovative (high) bionanoelectro-membrane technologies [28–30] in line with the doctrine that we suggest [31] and upgrading logistics [32] will afford to ensure Food Safety in the Russian Federation [34], within the new technological wave [33] in terms of irreplaceable food of functional purpose, that is, milk and dairy products.

The logistic scheme of LactOmics market concept as terms is shown in Fig. 10 in respect to diary product technology.

The scheme above previously adapted to consumer's milk on international level, and to milk whey and secondary milk raw materials on national level reveals the entire complexity of the hierarchy of interrelations of a dairy enterprise in the cycle of turnover. The infinity of LactOmics on the Earth may be figuratively depicted (Fig. 11) against the galaxy as the family of lactose derivatives.
Development and implementation of specific solutions to milk raw stock processing

- Raw material monitoring;
- Range of products;
- Quality;
- Packaging;
- Promotion;
- Commodity market (wholesale and retail);
- Competition (perfect);
- Prices (rational);
- Profit (worthy);
- Feedback;
- Forecast.

Assessment of initial situation [management (professional), marketing (consumer and social, best effective)]

Search and solutions to sales problems of food made of milk raw stock [food; feed; veterinary and pharmaceutical agents; commercial purposes] – opportunities of entities and consumer preferences

Purpose tree to process milk raw stock [entity's capacities – economic, engineering]

Purpose tree to consume products of milk raw stock [local, regional, federal (national), international levels]

Life cycle of products of milk raw stock [short-term; long-term; forecasting] (profit earning due to meeting demands of consumers). Culture of diary product consumption

**Fig. 10.** Structure of LACTOMICS market concept in terms of technology.

**Fig. 11.** Infinity of LactOmics in galaxy.
In conclusion, emphasizing once again that this article is prepared to raise an issue for discussion and possible development of LactOmics provisions at all three interrelated levels, and I would hope that the dairy business in Russia will always stay the forefront in the great dairy Empire.

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ENGINEERING OF COMPLEX TECHNOLOGICAL SYSTEMS IN THE AGROINDUSTRIAL COMPLEX

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\textbf{Abstract:} The article is devoted to some aspects of the engineering of complex technological systems designed in the agroindustrial complex. The focus is on solving the problem of Russia's food safety by creating the industrial agro-complexes that implement the technologies for agricultural production and the technology for its storage and processing. The range of issues under discussion includes the structural complication of agro-industrial complex technologies with their simultaneous simplification due to the stabilization of the leading processes of converting agricultural resources into food products. Particular attention is paid to the fluctuations in the processes of "large" technological systems that make up a system complex, under the influence of internal and external disturbing factors. These fluctuations are considered as a necessary condition for the development of complex technological systems. The information about the conditions of self-organization of system technological complexes and their main characteristics has been generalized: interoperability, nonlinearity and instability. The principles of designing such complexes in the agroindustrial complex and the dialectics of mutual reinforcement of the technologies combined into a complex have been outlined. The necessity of the industrialization of agricultural technologies as the dialectical inevitability of the creation of industrial agrocomplexes that implement a new stage of the innovative revolution in the agroindustrial complex has been proved. The main provisions of the paradigm for the development of agro-industrial complex technologies have been formulated. A conclusion has been made about the first steps in the implementation of the engineering of complex technological systems in research organizations and universities.

\textbf{Keywords:} Food safety; the mutual adaptation of agro-industrial complex technologies; system technological complex; the synergetics of complex technological systems; the interoperability, nonlinearity and instability of technological complexes; the self-organization of a technological complex; the mutual reinforcement of combined technologies; the industrialization of agricultural technologies; the main provisions of the paradigm for the development of agro-industrial complex technologies.

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\section*{INTRODUCTION}

Russia is a great power that seeks to pursue its independent foreign policy, therefore, the issues of food safety and the reduction of dependence on imported supplies also refer to the strategic interests of the country.

Solving the problem of providing food safety is only possible by a significant increase in agricultural food production, while ensuring a more efficient use thereof, that is, reducing losses and increasing food production from a unit of raw materials.

Back in May 2009, Prime Minister of the Russian Federation V.V. Putin set such a task before the domestic agroindustrial complex. V.V. Putin also outlined the ways to achieve the task – the technological and technical re-equipping of the country's agro-industrial complex, which will allow to intensify all kinds of production in the agro-industrial complex, that is, to increase production not only due to the extensive growth of production capacities, but also due to the use of the fundamentally new technologies and machines that guarantee the efficiency and profitability of production, the high quality and competitiveness of products in the consumer market both within the country and abroad.

Until now, the food production (the food industry) and the production of raw materials for it (agriculture) function independently of each other in the domestic agro-industrial complex.

At the same time, a lot of technological problems of food production have always been solved, as a rule, due to the "adaptation" of agricultural products to the machines and devices of food production by selecting part of the raw materials suitable by conditions from the total amount of raw materials.

The time has come to mean not the selection of part of the manufactured agricultural products by the "adaptation" of agricultural production to food...
technologies, but their manufacture with the pre-determined parameters and allowances for them. The narrower allowances of the parameters for the inputs and outputs of the technological processes of food production with a high technological discipline will provide not only the necessary product quality, but also the stability of both individual processes and the process stream as a whole, and, consequently, the efficiency and profitability of both food and agricultural productions.

The current methods for stabilizing the production processes of food production through a rapid response to external disturbances have largely exhausted themselves, and the machine technologies of continuous productions in the form of mechanized and automated production lines created in the 30–80s of the twentieth century are, in essence, the first and so far the only generation of such technologies.

The creation of lines of the second and the next generations is due to the development of self-regulation systems, which is extremely difficult in itself. Due to the fact that the deviation of the process parameter from the nominal value can depend on the power of an external action at various rates, there is the task to take into account the dynamic factor of a process, so that even with an insignificant amount of the deviation that has emerged, to develop a control action with the necessary anticipation avoiding any excessive increase in the deviation even with a powerful disturbing effect. In other words, a very complex automation system is needed, which significantly reduces the reliability of a process stream.

The real way is to create a system complex by including the technological system of the appropriate agricultural production therein, that is, a transition to agrarian-food technologies of food products. This means that instead of solving the issues of creating the most complex technical means of control and management of technological processes for the existing food technologies, we should supply raw materials with the already known stable parameters to the input of these technologies, highly simplifying the entire further process of food production with the specified consumer properties [1].

Thus, the processes of agricultural production become the external supplement of food technologies in the creation of agrarian and food technologies.

The structural complication of a technology by no means excludes its functional simplicity, since there is a flexible and ambiguous link between the processes of complication and simplification. One of the consequences of this link is the mandatory simplification of technology due to the stabilization of all the leading processes. It is about simplifying production processes, since an increase in the structural complexity of food technologies is compensated by the significant simplification of the links between the leading processes due to the stabilization of the parameters of the initial processes. At the same time, the increasing volumes of information and the possibilities for unifying processes and aggregating machines, devices and bioreactors are used more efficiently.

The creation of agrarian and food technologies is the next stage of the natural development of the technological base of agrarian and food industries, a transition from the old technological basis of the "industrial era" to a qualitatively new basis of technologies in the form of the unified computer-integrated food productions.

The article aims at revealing and analyzing the engineering features of designing a systemic agro-industrial complex that arise under the influence of various system-forming factors when the processing and manufacturing technologies of the agro-industrial complex are combined into a single whole.

**SYSTEM TECHNOLOGICAL COMPLEX**

The system complex – an agrarian-food technology is a set of technological systems. Its generalized appearance, reflecting the most important features of its complex structure, can be represented in the form of a block diagram (Figure 1):
- the technological system (TS) of the assembly (synthesis) of agricultural products;
- the technological system (TS) of the disassembly (analysis) of agricultural products into anatomical parts;
- the technological system (TS) of the assembly (synthesis) of food products from these parts.

These three types of "large" technological systems interperse with technological storage systems. Such a complex technological system (system complex) is the result of convergence, connection and compression of agrarian, processing and food technologies in time and space. At the same time, various biological, biochemical, chemical, physical-chemical and physical processes, previously so remote from each other in time and space, and therefore weakly interacting with each other, sometimes not at all dependent on each other, now converge and are "compressed" taking into account rather close allowances for the values of the input and output parameters of all the leading processes that they begin to directly affect each other.

The figure also implies the concept of a "filter" of the system complex as a whole, as well as of each separate technological system, which is understood as the control and limitation of fluctuations of the input parameters of the leading processes in quality and quantity. And the process of effective functioning of a complex technological system is determined by the abilities of this filter, including the protection of the system and its constituent processes from disturbing external effects.

What distinguishes technological complexes, these complex technological systems, from "large" technological systems? First of all, the nature of internal links, the level of interaction, the mutual effect of the elements of the system (process operations), their interconsistency, interdependence and the ultimate goal. In the technological complexes of the agro-industrial complex, these properties assume decisive importance. It is complexes that are a new step in the development of systems of processes.
Fig. 1. Variant of the system complex “Agrarian-food technology”.

A technological system complex combines a large number of heterogeneous "large" systems – autonomous agricultural production, storage and processing technologies – into one. Such complexes have completely new properties, which are characteristic neither for the technologies of plant and animal production, nor for numerous technologies for its processing.

The system agrocomplex is created to obtain a significant increase in food production efficiency. But not every holding that connects an agricultural technology (for example, wheat production) and processing technologies (for example, flour, bread and pasta production) can make up a system technological complex. Only a set of those technologies, which themselves consist of a large number of heterogeneous and complex parts – subsystems that are closely interrelated, saturated with machines, apparatus, bioreactors, automation, information and computing systems and are well controlled – has the properties of system agrocomplexes. These agro-complexes are a natural, but qualitatively new stage in the development of technological systems, a stage that is directly connected with the innovation revolution in the agro-industrial complex. In such complex and responsible business as the creation of complexes of such a scale as "Agrarian-food technology", a rigorous engineering base is needed.

Complex technological systems are a newer, higher level of development of productive forces in comparison with the previous "large" technological systems in plant growing and cattle breeding, as well as classical production lines for the production of flour, cereals, bread, milk, meat, canned goods, etc.

The creation of the system complex "Agrarian-food technology" will allow to solve in full or in part the main technical contradiction of any process stream: "efficiency – quality." A condition for the organization of such a complex is the creation of large agro-industrial enterprises on a new technological and technical basis, the effect of which can be represented in the form of:

- an increase in labor efficiency;
- expanding the targeting of agricultural production;
- strengthening the technological properties of agricultural raw materials;
- ensuring the lifetime formation of food quality;
- implementing the traceability of food safety;
- approximating processing and food enterprises to the facilities of production of agricultural raw materials;
- developing the cooperative forms of labor organization;
- an increase in the technological discipline in agricultural production, the processing and food industries;
- creating highly automated and robotic food productions at processing and food enterprises;
- developing resource-saving and environmentally friendly processes for the entire process chain.

The creation of system technological complexes is, of course, a matter of future, but not of so distant as it might seem. In 50 years, it will be difficult to find the workers who manufacture agricultural products using the technologies of the beginning of the 21st century and the workers of processing and food enterprises engaged in workshops of a modern kind. Today, it is necessary to understand the mechanism of the emergence of very complex holistic formations (technological complexes) from "large" technological systems that have unfamiliar properties, features and regularities, which will have to be encountered in their organization, functioning and development. And we need to prepare for this right now. The fact is that the dialectics of the further development of man-made integrated systems leads to their complication, but simplifies the operation process and increases the efficiency of such systems.

The role of highly organized complex technological systems in the country's economy will undoubtedly increase, and their number will multiply. There will be new industrial, transport, mining, metallurgical and food complexes. When creating the complex "Agrarian-food technology", special attention should be paid to the possibilities, conditions and prospect for
the further development of such a complex technological system, since the features of the seventh technological order, the roots of which are in the sixth technological order, are already visible on the horizon.

SYSTEM TECHNOLOGICAL COMPLEX: SYNERGETICS AND ENGINEERING

The approach to the creation of exceptionally complex systems begins to form the strategy of research frontiers. This relatively new scientific trend was called "Synergetics." There is no doubt that synergetics will allow us to take a fresh look at modern agro-industrial technologies and accelerate their qualitative transformation [2, 3, 4].

The origins of synergetics can be seen in the basics of cybernetics, the development of which in the Soviet Union dates back to the 50–60's of the 20th century [5]. In elaboration of separate provisions of cybernetics, the general system theory was developed in the 70's [6]. And in the 80's and 90's there is the promising field of scientific research on the basis of the synthesis of cybernetics and the system theory – the theory of self-organization of very complex systems – synergetics.

The principles of cybernetics feedbacks work for the creation of processes of technologies of various industries and automated object management systems. Synergetics is just what reveals the mechanisms of development and self-organization of complex systems in general. And this is extremely important in the period of rapid development of the material and technical component of civilization, including the objects of the agro-industrial complex of Russia.

Analyzing the stages and trends of the historical development of cybernetics, the general system theory and synergetics, it can be concluded that in the second half of the 20th century a new, previously unknown approach of scientific and engineering research is being formed, due to the increasing complexity of anthropogenic systems of processes. This approach is manifested in the form of an "enzyme" or "catalyst", which does not replace the basic scientific and engineering disciplines, but stimulates the development of knowledge within their framework [7].

Synergetics develops certain concepts that were introduced by cybernetics and the general system theory. First of all, it refers to the concept of feedback, moreover, not only the negative feedback responsible for the process of maintaining the function of an object, its homeostasis, but also the positive feedback responsible for the process of forced development of an object. Therefore, synergetics can be considered as an instrument of our penetration into the future of technologies of the agro-industrial complex.

The term "synergetics" means a joint action, emphasizing the coherence of the links between the parts, reflected in the functioning of the system of processes as a whole. For example, the system complex "Agrarian-food technology" consists of 5–6 "large" systems (technologies) and is a set of 20–30 or more subsystems. The complex contains from 75 to 100 or more elements – the leading technological operations in machines, apparatus, bioreactors and devices, connected by hundreds of links (material, energy and information) with certain allowances regulated by technological instructions.

To be an object of synergetics, such a complex must have the characteristics of a self-organizing system: interoperability, nonlinearity and instability.

Interoperability is the ability of the system to constantly exchange material, energy and information with the environment and to have both the sources of raw materials and energy resources, as well as the information from outside, and waste in the form of products, material wastes, energy and information dissipation. Interoperability is a necessary condition for the existence of relatively stable technological processes in the leading parameters.

In open technological systems, the crucial role is played by random factors, fluctuation processes. This concerns not only the agricultural part of a system technological complex, in view of natural and climatic features, but also its processing part. It refers to the failures regarding the requirements of instructions in terms of the quality and quantity of the material resources supplied at all stages of a process stream, power supply failures and management failures. The fluctuation can be so intensive that it leads to the output of defective products after ploughing through the elements and links of the system as a wave. But on the other hand fluctuation processes are necessary, since they stimulate development processes. Moreover, in the conditions far from the states of processes with the maximum information entropy, weak fluctuations can have a strong impact on a technological system, destroying the existing structure and contributing to its radical qualitative change. In particular, this concerns the downward accuracy of dispensers, since this is necessarily followed by significant (depending on the formulation) changes in the physical and mechanical properties of the treated media, and, as a result, the failures of the leading processes, which requires the revision (development) of technical support of technologies.

Nonlinearity is the conceptual basis of synergetics. Whenever the pattern of a complex object can be described by a system of equations, these equations turn out to be nonlinear. Nonlinear equations can have several qualitatively different solutions. This implies the physical meaning of nonlinearity: a set of trends of development of the system described by this non-linear model corresponds to a set of solutions of a non-linear equation.

A set of processes of the system complex "Agrarian-food technology" is nonlinear, since at different times and under different external effects, its pattern is determined by various laws. This is expressed, in particular, in a variety of technical solutions to a particular technological problem.

The most important conclusion on the organization of their scientific and technical prediction and management follows from this behavioral peculiarity of non-linear systems of the agro-industrial complex.

Instability can be defined as the state of an open system, when the macroscopic parameters of its elements (technological operations), links and performance change. It should be noted that the system
complex, which is in an unstable state, is sensitive to external effects consistent with its own properties. Therefore, such fluctuations in the environment are not "noise", but a driving factor in the development of its elements, links and structure. In this case, external effects that are small but consistent with the internal state of a complex may prove to be more effective than large ones. This is what the mechanism of resonant excitation consists in, which leads to the appearance of branch points for the further development of a complex. These bifurcation points can bring a system both to a stable state (which is of interest for the future functioning of the system in the same capacity) and to an unstable state (which is of interest from the point of view of its transition to a new quality state) [8].

Therefore, synergetics prompts us to realize the concept of the development of very complex technological objects in the agroindustrial complex as open nonlinear systems that, in an unstable state, are carriers of the diverse forms of their future organization.

SYSTEM TECHNOLOGICAL COMPLEX: ISSUES OF SELF-ORGANIZATION

Nonlinear systems, being unstable and open by themselves, create and maintain fluctuations in the environment. In such conditions, the relations of the positive feedback between the system and the environment can develop, i.e. the system affects the environment in such a way that some conditions are created in the environment that cause changes in the environment itself. The consequences of such interaction of an open system and its environment can be the most unexpected. And we can see these phenomena in the technologies of the agroindustrial complex.

The self-organization of a technological complex takes place only in the case of the predominance of the positive feedbacks that act in an open system over negative feedbacks. And synergetics directs the engineer to reveal the mechanisms of self-organization of such exceptionally complex systems created by man and functioning with his participation.

Dozens and hundreds of elements of a system complex, i.e. technological processes in equipment, are endowed with such properties as stability and variability, technological reliability and unreliability, linearity and nonlinearity, controllability and uncontrollability, sensitivity and insensitivity to extrasystemic and inrasystemic effects, etc. All this set of properties of elements leads under certain conditions to complete unpredictability in the pattern of a complex system as a whole. And the pattern of the same system can be absolutely predictable and natural when the external conditions change.

In general, a transition (with the participation of man) of an open, unstable nonlinear system from less to more complex and ordered forms of its organization is meant by self-organization. At the same time, the development of such systems proceeds with an increase in complexity and order with the simultaneous simplification of the operation process. One of the trends of development of complex systems is the creation of low-sensitivity processes in a process stream [1].

SYSTEM TECHNOLOGICAL COMPLEX: MUTUAL REINFORCEMENT OF COMBINED TECHNOLOGIES

The experience of developing system complexes in other areas of the national economy makes it possible to formulate the following basic principles for the design of such complexes in the agro-industrial complex:

– the rational choice of technologies that provides the fulfillment of all the tasks by a system complex as a whole;
– the careful integration of technologies into a single, well-functioning process stream;
– the overall, scrupulous autonomous verification of technological reliability of each component of a complex;
– the quantitative assessment of the level of stability of each of the integrated technologies as a subsystem of a complex;
– the quantitative assessment of the level of integrity (level of organization) of a system complex as a whole and determining its efficiency in solving the set tasks.

It is necessary to note the culmination of this paradigm in the development of technologies of the agro-industrial complex — the circumstances under which the integrity of an industrial system complex is formed, providing the integrated technologies of agricultural production, storage technologies, processing technologies and food processing technologies with super abilities and super efficiency. It refers to the system-forming factors that can be different in terms of technological solutions in each of the technologies that make up a complex. For example, in one technology, this is the synchronization of the functioning of processes, in another, this is the narrow specialization of processes, in the third, it is the high stability of the outputs of processes, and so on. Thus, the basis of the additional effect of the functioning of each unit (a technology within a system complex) is its system-forming factor. And the effect of a system complex as a whole is determined by the level of implementation of these system-forming factors in separate technologies that make up a complex. And if in one of the technologies the system-forming factor is realized below the design abilities, the "junctions" between technologies, their links and the chain of processes will be broken and the effect of a complex will decrease or even fall to an unacceptably low value.

Thus, the mutual reinforcement of the technologies integrated into a complex is the source of the effect of complex systems.

SYSTEM TECHNOLOGICAL COMPLEX: INDUSTRIALIZATION OF AGRICULTURAL TECHNOLOGIES

The processing part of the system complex "Agrarian-food technology" has already been quite strictly organized into a process stream and operates at works, factoaries, mills and other large food enterprises with a minimum of external disturbing factors and a high level of mechanization and automation, unlike the part of the production complex that manufactures vegetable and animal raw materials. For this reason, it
is absolutely necessary to organize agricultural production on an industrial basis. In agriculture, the regularities that describe the structure, functioning and development of technological processes are much more complex than the regularities of technological processes at processing and food enterprises and are primarily of a probabilistic nature. This is due to the features of the main means of production – the ground with its productive capacity that highly changes depending on weather conditions. Plants and animals also have their own biological characteristics of growth and development. Therefore, the solution of the problem of manufacturing agricultural products stable in quality and quantity should be sought in the creation of fundamentally new technologies of an industrial type [10], which in turn requires the creation of new high-yield plant crops and new highly productive animal breeds.

The basis of industrial technologies of crop production should be the organization of the harmonious system of soil cultivation, fertilization, precision sowing, the mechanized care of crops, fighting diseases and pests, harvesting and storage. At the same time, precision seeding is only possible after special treatment: sorting, grinding, calibrating and pelleting with giving them a spherical shape. Precision seeding, in terms of agrotechnical parameters of seeding, is just what the agrarian-food technology of food products from plant raw materials, which has the close allowances of parameters of all the leading production processes, up to the process of packaging the finished products, should begin with.

The basis of industrial technologies of livestock production should be the organization of automated livestock management processes so that man is engaged not in managing animals, but in maintaining automated production systems. This technology leads to the concept of "farm – plant", when agricultural labor acquires the features of factory labor and livestock production is conducted in a single production – technological cycle.

Thus, the industrialization of fields and farms should make some fundamental quality changes in the technology of agricultural production.

How, then, should agricultural production technologies obtain an industrial image? The answer to this question is given by Leonid Pogorely, Academician of the Russian Academy of Agricultural Sciences, Director of the Ukrainian Research Institute for Forecasts and Testing of Machinery and Technologies for Agricultural Production in 1991–2003 [9].

At the end of the 20th century, important initial prerequisites for the design of agro-bioplants and agro-zoofarms were obtained in our country.

In field husbandry, such a path is possible if you withdraw from the traditional tractor pulling concept. This concept of the function of a mobile energy device must be radically changed: working power machines must be converted into the carriers and transmitters of energy to the working bodies and machines that move along a special track or artificial paths. One of the trends in the development of the field husbandry model is the creation of bridge mobile systems. It is nothing more than a mobile agricultural plant.

The factory trends in the development of the agrarian part of the industrial system complex "Agro-food technology" are also characteristic of industrial livestock production. Cattle breeding complexes can become plants and factories due to the introduction of the advanced technologies based on the integrated mechanization and automation of line production methods and modern management systems. The similarity of this concept of organization of an agricultural enterprise with the concept of an industrial processing plant is ensured by the high concentration of production with its narrow specialization.

The accuracy, stability and technological reliability of agricultural production processes can be provided at agricultural factory-type enterprises. It is these processes that can provide the quality of links along the entire technological chain of the system complex "Agro-food technology", which will enable processing and food enterprises to use the rotary technology and rotary machinery [1].

**SYSTEM TECHNOLOGICAL COMPLEX: THE MAIN PROVISIONS OF THE DEVELOPMENT PARADIGM**

The methodological crisis in the engineering of the agro-industrial complex of Russia can be overcome if the ideology of creating industrial system complexes, leading to an excessive positive effect, is a new paradigm in food production. This is the essence and inevitability of the dialectical development of anthropogenic systems. The main provisions of the paradigm are as follows.

**The first provision** is a change in the thinking style of the scientist and engineer. The old deterministic physicochemical pattern of separate technological processes is replaced with a new stochastic pattern, since all the technologies as the complex systems of processes function according to the laws of the probability theory, mathematical statistics and information theory.

**The second provision** is the comprehension of the need for analysis and synthesis. At the present time, it seems that differentiation processes in science prevail over synthesis processes. But this impression is related to the class of the problems being solved. The synthesis procedure already requires the account of external disturbing factors and the account of the mutual effect of the leading processes in technology. This leads to a change in the mathematical apparatus and the construction of a stochastic model of the object.

**The third provision** is the consideration of technologies for the production and processing of agricultural products as systems of processes. Such technologies as the objects of research and optimization should meet the following requirements:

– there should be a system-forming factor that determines the interconnection of subsystems in a system and has a synergistic effect;

– a quantitative measure should be established that determines the level of organization (integrity) of a system;

– a system should be part (subsystem) of the system if is enclosed in (supersystem).
The fourth provision is the construction of a graphical model of cross-cutting technology as a complex system of processes. The procedure for constructing the model of such an object, as the technology of a system complex, consists in the sequential use of analysis and synthesis. The complete graphic model of a system complex is synthesized from subsystems. The outputs of subsystems are the points of quality control of the technology of this complex.

The fifth provision is the diagnosis of a technological system in terms of its performance. It is necessary to perform two diagnostics: before and after the appropriate intervention in a particular technology. The comparison of the quantitative results of these two diagnostics is what will show the depth of elaboration of R&D.

The sixth provision is the actual development of the technological system based on the results of the first diagnostics, which results, for example, in the mutual adaptation of the technological properties of the initial agricultural raw materials to the processes of its transformation, on the one hand, and the mechanisms of processes in the machines, apparatus and bioreactors of processing industries to the technological properties of raw materials, from the other hand.

The seventh provision is the search for the most effective methods for supplying energy to the processes of production, storage and processing of agricultural raw materials into food products. It refers, first of all, to the wave actions on the processed media: the variable electromagnetic field of ultrahigh and low frequencies, magnetic fields, light pulses, pulsating electric fields, infrared and ultraviolet radiation, cavitation, ultrasound, the electrochemical and laser action. Widespread prospects are seen in a combination of these wave processes with the traditional methods for supplying energy to the processed media.

CONCLUSION

We should learn to look 40–50 years ahead. If we turn back and look at the 50-year old technology and machinery of agriculture and processing enterprises (these are the 60s of the 20th century) and compare them with the modern technologies and machinery in the agro-industrial complex, then we could note the same technological shift by looking at the current technologies of the agro-industrial complex from the 60–70s of the 21st century. Therefore, a backward look from the present to the past and from the future to the present is highly advisable. It stimulates scientists and engineers to predict and create the inordinary innovative technologies and equipment of the agro-industrial complex.

In this connection it is necessary:
– to analyze the modern technologies of the agro-industrial complex of Russia and their technical support from the point of view of the possibility and expediency of creation in the long term of system technological complexes of industrial production and processing of agricultural production into food products;
– to develop the long-term programs of search, fundamental, applied and experimental design works with state budgetary financing for the cross-cutting technologies of a system complex for the period up to the second half of the 21st century and beyond;
– to organize the training of scientific and engineering personnel by combining agricultural and food higher educational institutions or establish agrarian and food technology faculties in agricultural higher educational institutions.

REFERENCES


PRACTICAL ASPECTS OF THE USE OF EMULSIFIERS IN MANUFACTURING EMULSION FAT-AND-OIL PRODUCTS

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Abstract: Expanding the scope of margarine products requires the search for new technological solutions when creating stable food emulsions for various specified purposes: hard, soft and liquid margarines with various fat content for bakery, for use in confectionery production, for frying in public catering networks and as spreads for direct consumption. The article systematizes the materials on the surfactants used in the technology of food emulsion products, in particular, margarines and spreads. The analysis of emulsifiers and their mixtures used in manufacturing emulsion products with various fat content has been carried out. The results of the studies of the composition and effect of individual and complex emulsifiers on the properties of the direct and reverse type of the obtained emulsions have been summarized. The article considers the theoretical issues and practical aspects of creation of food emulsion products with the use of various emulsifiers. A step-by-step system for choosing the optimal mixture of emulsifiers for a particular product based on the hydrophilic-lipophilic balance of surfactants has been provided. A number of examples of complex emulsifiers for emulsion products for various specified purposes have been presented. It has been shown that a change in the amount and type of an emulsifier makes it possible to produce various emulsions with the required structural and rheological characteristics and with the specified composition and properties. The aspects of manufacturing margarine products with various fat content have been presented.

Keywords: Food emulsifiers, emulsion products, margarine, spread, mono- and diglycerides of fatty acids, hydrophilic-lipophilic balance, iodine index


Of a variety of fat-and-oil products, a group of solid emulsion fat-and-oil products can be emphasized, including margarines, vegetable-cream and vegetable-fat spreads. These products were initially developed as an alternative to butter, however, their scope of application has significantly expanded at this stage of development of the food industry. It should be noted that the structure of consumption of solid fat-and-oil products has recently changed with a decrease in the proportion of consumed butter, margarines and spreads as edible products. The reason for these changes is due to a more attentive attitude of the population towards health and the fulfillment of the recommendations of the health authorities to reduce the consumption of fats, in particular, saturated fats. Despite this fact, the consumption of solid fat-and-oil products on the whole continues to grow. To a certain degree, this is due to the fact that the scope of margarines and spreads is not limited to direct consumption as a sandwich type product. Margarine products are widely used in public catering and in the HoReCa sector, as well as in the confectionery, bakery, canning and other food industries.

According to the Russian technical regulations (TR TS 029/2011) margarine is an emulsion fat-and-oil product with a fat content of at least 20%. The formulation of margarine can include both natural and modified vegetable oils, water, milk and its derivative products. The mass fraction of fat in margarines varies within quite a wide range, usually from 60 to 82%.

Spreads – a relatively new group of emulsion fat products with a mass fraction of fat of 39% and the melting point of the fat phase not higher than 36°C – are the most similar to butter in properties. Spreads, in comparison with margarines, have a more plastic consistency. Cream-vegetable and vegetable-cream spreads are made of natural and modified vegetable oils, milk fat, cream and butter. Vegetable-fat spreads are obtained from natural or modified vegetable oils with or without the addition of food supplements and other ingredients that make it possible to obtain a stable emulsion [10].
Being homogeneous in appearance, emulsions consist of two liquids that are practically insoluble in each other. The complete or partial insolubility of the disperse phase in the dispersion medium is a necessary condition for the formation of an emulsion. In fact, all emulsions contain water as one of the phases. The other phase is organic, non-polar, and it is conventionally called "oil."

Food emulsions, which include margarines and spreads, are disperse systems formed by two mutually insoluble liquids (oil and water), one of which is dispersed in the other in the form of tiny spherical droplets. The substance of drops is considered a disperse, discrete or internal phase. The substance that constitutes the surrounding liquid is called a dispersive, continuous or external medium.

As a rule, both the aqueous and the oil phase in emulsions are complex systems and are characterized by a multicomponent structure. Thus, the oil phase, or fatty base, in a margarine emulsion is a mixture of transesterified triacylglycerols different in origin and the melting point of hydrogenated fats (soybean, sunflower, rapeseed, etc.) and liquid vegetable oils (sunflower, soybean, etc.). The aqueous phase, which determines organoleptic properties in margarines, includes flavoring agents, preservatives, citric or lactic acid to enhance microbiological resistance, thickeners, structure-forming agents (especially for low-calorie margarines), antioxidants and dyes.

There are two types of emulsions: oil-in-water (or first-kind) and water-in-oil (or second-kind). In oil-in-water emulsions, the oil phase is dispersed and the aqueous phase remains continuous. They are denoted as O-W. For example, milk, cream and mayonnaise refer to oil-in-water emulsions. In water-in-oil emulsions, on the contrary, the aqueous phase is dispersed and the fat base remains continuous. They are denoted as W-O. Margarines are one of the examples of water-in-oil emulsions. Some conditions can lead to an inversion, when there is a transition of one type of emulsion to another. For example, the prolonged mechanical treatment of an emulsion can lead to the coalescence of disperse phase droplets, and the liquid of the dispersion medium is crushed into droplets and dispersed in a newly formed dispersion medium by itself [9].

In addition, emulsions are classified according to the concentration of the disperse phase. In dilute emulsions, the proportion of the disperse phase is up to 0.1%. They belong to highly-dispersive emulsions, the droplets of the disperse phase in these emulsions have a spherical shape and their diameter is about 100 nm. In concentrated emulsions, the amount of the disperse phase is from 0.1 to 74. In the case when the concentration of the disperse phase is from 25 to 50% (low-calorie margarines, spreads and mayonnaises), its viscosity does not practically differ from the viscosity of the dispersion medium, therefore, in order to increase the aggregative stability of such emulsions and imparting certain rheological properties, the substances that increase viscosity (the so-called "thickeners") are added to the dispersion medium.

In emulsions with the specified concentration, the maximum content of spherical undeformed droplets is possible. In highly concentrated emulsions, the concentration of the disperse phase is not below 74%. They usually have deformed droplets, in which case the disperse phase therein can often turn into thin layers. In some cases, with a high degree of polydispersity in highly concentrated emulsions, the spherical shape of particles remains unchanged. Due to polydispersity, small droplets fill the spaces between large spherical particles. It is possible to create mixed emulsions [10].

In food production, concentrated and highly concentrated emulsions that need an increase in the aggregative stability for a long time are as a rule obtained. This is achieved by adding special emulsifying agents which are various natural or synthetic compounds. Most often, these are the substances that are soluble in one of the phases. Insoluble solids are also used in a finely disperse form. The first group of emulsifying substances is more extensive and is more often used in practice, surfactants hold a unique position therein [1, 2, 3].

The process of formation of an emulsion and its end-use properties depend on such factors as the surface tension of the phases and the interfacial tension of the heterogeneous system. Surface tension is the most important parameter that determines the stability of an emulsion. The decrease in surface tension provides an increase in the stability of the system [6].

Surface energy can be reduced with the preservation of the interfacial surface only by reducing the interfacial tension value. The ability of substances to change the surface tension of the solvent during dissolution is called surface activity. Ana the substances that are adsorbed at the interface between the two phases and decrease surface tension are called surfactants.

It is known that the decrease in interfacial tension is a consequence of a special surface phenomenon called adsorption. During adsorption, the emulsifier should easily transfer from the volume to the surface layer and, moreover, retain and concentrate in the surface layer. Low molecular substances called diphilic or amphiphilic ones that consist of various functional parts usually have such properties. The irreversible character of adsorption causes the high stability of emulsions.

The emulsifying ability of a surfactant is specified by the presence of hydrophobic and hydrophilic functional groups.

The hydrophilic part of an emulsifier molecule is usually hydroxyl, carboxyl, phosphatide, nitrogen, ester and other groups that have a significant dipole moment and are capable of forming hydrogen bonds with water molecules.

The hydrophobic part in a surfactant molecule is a hydrocarbon radical which does not usually contain the atoms capable of forming hydrogen bonds and is insoluble in water. Most often, these are the hydrocarbon radicals of fatty acids, usually palmitic C_{16}, stearic C_{18}, or other saturated high molecular weight (long-chain) fatty acids. Medium-chain fatty acids...
Emulsifiers such as lauric C_{12:0} and myristic C_{14:0} are undesirable in the composition of emulsifiers due to their low resistance to hydrolysis, which can lead to an unpleasant foreign taste of the finished product. The presence of unsaturated fatty acids (oleic C_{18:1}, linoleic C_{18:2} and linolenic C_{18:3}) in an emulsifier is also undesirable since they are easily oxidized.

The common emulsifiers, the proportion of which in the total consumption of food emulsifiers exceeds 70%, are monoacylglycerols. Phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylethanolamine, phosphatidic acids, etc.) also have good emulsifying properties due to their diphilic molecules. Emulsifying properties are also characteristic of proteins the molecules of which include hydrophobic and hydrophilic fragments.

Obtaining an oil-in-water or water-in-oil emulsion depends on the type of emulsifier and a method for its addition. When obtaining an oil-in-water emulsion, the oil phase is introduced into the aqueous phase in small portions. The emulsifier is dissolved in the aqueous or in the oil phase before application. Water-in-oil emulsions are obtained by adding the aqueous phase to the oil solution of the emulsifier. This condition is only feasible when adding a small amount of the disperse phase, otherwise the phases can be reversed and the emulsion can be stratified [5].

The hydrophilic-lipophilic balance number (HLB number) of an emulsifier can be a measure of its affinity with oil or water. The term "hydrophilic-lipophilic balance" was first proposed by Clayton and related to the ratio of the hydrophilic and hydrophobic (lipophilic) parts of a molecule of a diphilic surfactant. The hydrophilic properties are determined by the interaction of the polar group of an emulsifier with water, and the lipophilic properties are determined by the interaction of a non-polar surfactant fragment with oil. Since the determination of the HLB number is a simpler method than the relatively accurate determination of the geometric and surfactant distribution factors in the miscible phases, the HLB number is currently an important factor in choosing an emulsifying system for a certain type of emulsion. Table 1 shows the dependence of the type of emulsion being formed on the proportion of hydrophilic groups in an emulsifier molecule.

An important factor to determine the type of emulsion being formed is the solubility or dispersibility of an emulsifier in the fat or aqueous phase. According to the Bancroft rule, fat-soluble, (or dispersible in fat) emulsifiers, the HLB values of which range from 2 to 6, form water-in-oil emulsions. Water-soluble (or water dispersible) emulsifiers, having high HLB values – from 11.0 and above, form oil-in-water emulsions.

To manufacture fat-and-oil products, both separate emulsifiers (monoglycerides and polyglycerol and ricinoleic acid esters (E 476), distilled monoglycerides (E 471) and lecithins (E 322)) and their complexes (E 471 : E 322 and E 471 : E 476) are used. The use of these emulsifiers makes it possible to form the mixtures of immiscible phases – oil and water – and keep them uniform. An important property that differs emulsifiers from the other classes of food supplements is surface activity that can be developed in different ways. The most demanded functions of surfactants are: emulsification which allows us to obtain qualitative, stable emulsions and aerating and stabilizing whipped systems by interacting with other food substances, such as proteins and carbohydrates. In addition, surfactants make it possible to intensify the process of oil and fat crystallization being crystallization centers and controlling fat particle agglomeration. The emulsifying compositions that comprise phospholipids contribute to an increase in the thermal stability of fat-and-oil products for baking and frying, preventing splashing. Having antioxidant properties, phospholipids also provide the high resistance of fat-and-oil products during storage. In addition to the main purpose – to stabilize an emulsion, emulsifiers help to improve the plasticity of margarines and spreads, and in the production of margarines for bakery they provide some specific properties of products, for example, they increase crumb porosity and the volume of the finished product.

The features of the food system being created and the technological tasks set determine the choice of a specific emulsifier. Preference should be given to an emulsifying supplement the technological functions of which will provide the best technological effect with the minimal risk of its application.

The domestic industry produces a wide range of mono- and diglycerides that differ from each other in a number of indicators, the main of which are the melting point and the iodine index that characterize an unsaturation degree.

The technology of manufacturing emulsion fat-and-oil products, including low-fat products, includes obtaining a highly-dispersive water-in-oil emulsion by emulsifying a mixture of vegetable oils and fats with dairy and other raw materials. In this case, the product must have a uniform, plastic and dense consistency, a clear, pronounced flavor or the flavor of the used filler. The presence of free vegetable oil or water in a product worsens not only its consumer characteristics, but also reduces the microbiological resistance of a product during storage.

The fat base of fat-and-oil emulsion products (as well as spreads and margarines) is a multicomponent mixture of natural or modified fats and oils with various physicochemical properties: the content of solid triglycerides, the melting point and hardness. It is these factors that determine the structural and rheological characteristics of the finished product. Margarine is considered a water-in-oil emulsion because of the predominance of the fat phase, the mass fraction of which is on average from 60 to 82%. In fact, it is not so much an emulsion as a dispersion of water droplets in a semi-solid fat-and-oil phase containing liquid oil and fat crystals [6]. The margarine emulsion production process requires considerable energy to reduce the size of disperse phase droplets in order to increase the interface between the two phases: aqueous and fat.

32
A margarine emulsion is left in the liquid state for a short time to be treated using full-time margarine production lines only at the stages before entering the cooler (votator or combinator) where the fat base is simultaneously crystallized and emulsified. A margarine emulsion does not require high resistance to coalescence since water droplets are fixed in a semi-solid fat phase upon cooling. The size of water phase droplets affects the organoleptic and microbiological indicators. Thus, a finely-dispersed emulsion (the droplet size is 2–4 μm) promotes the inhibition of mold growth. It should be borne in mind that the presence of larger droplets (more than 10 μm) improves the perception of a product taste. The uniformity of droplet size distribution is also affected by the nature of the emulsifier used. Its role is to reduce the interfacial tension between the fat and aqueous phases, which usually leads to a decrease in the size of water droplets, as well as a more uniform distribution of droplets in size. For this purpose, lipophilic emulsifiers are usually used: distilled monoglycerides containing high-molecular fatty acids (C_{16}:0–C_{18}:0) in combination with refined soya lecithin.

Depending on the purpose, margarines are divided into solid, soft and liquid ones. Solid margarines are used in bakery, culinary and confectionery production, in puff pastry production and for making creams, fillings, soufflé, sweets, etc. Soft margarines are mainly used in home cooking and in public catering. Liquid ones – for baking, in home cooking, for making bakery and confectionery products, as well as for frying in fast food stores.

Standard solid margarines containing more than 80% of fat are relatively stable under normal conditions and keep their shape at a temperature of 20 +/- 2°C; to produce them, the minimum dosage of lecithin and / or mono- and diglycerides is required in addition to the number of milk proteins usually specified in the formulation.

In the production of special margarine, i.e. for making cakes or for frying, and especially in manufacturing products with a low fat content, the emulsifiers should be chosen that meet the requirements of this scope in terms of the functional properties of fat products [2].

Soft liquid margarines and bar margarines with a low fat content, having a soft plastic consistency at a temperature of 10 +/- 2°C, are usually produced using the same fat base as standard consumer margarine. As a rule, other used ingredients also coincide with the standard margarine formulation, except for some cases.

**Table 1.** Dependence of the type of emulsion on the structure of an emulsifier

<table>
<thead>
<tr>
<th>Type of emulsion</th>
<th>water/oil→oil/water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of hydrophilic groups in the emulsifier, %</td>
<td>10</td>
</tr>
<tr>
<td>HLB</td>
<td>2</td>
</tr>
</tbody>
</table>

**Liquid margarine** is an emulsion product with a liquid consistency that keeps its uniformity at the temperatures specified for specific liquid margarine. As a rule, it is used for frying in public catering establishments. In this case, it is especially important that it does not splash. Splashing is caused by the fact that when melting margarine, the emulsion is destroyed, the water drops merge and, under the action of gravity, form an aqueous film covered with molten fat. When the boiling point is reached, the increased vapor pressure breaks through the fat layer, which makes the water phase to splash, sometimes with an explosion.

To prevent splashing, it is desirable to gradually evaporate water from small droplets with the formation of a thin golden brown sediment that does not adhere to the frying surface. An important part therein is played by the composition of margarine and the treatment methods used.

The typical formulation of liquid margarine contains about 82% of the fat phase based on soy or sunflower oil. At high pH values (about 6), the presence of salt and milk has a positive effect, while sugars and starches increase the splashing tendency. The correct choice of the type of emulsifier and its dosage can significantly improve the functional properties of frying margarine, while the emulsifiers have two functions. The first of them is an emulsifying one; thus, the use of citric acid ester and monoglycerides in combination with soy lecithin provides a stable aqueous dispersion with moderate splashing during frying. There are also some other emulsifiers that individually or together with lecithin help to prevent water phase droplets from merging during frying. The second function of an emulsifier is to prevent the oil separation during storage and to obtain a homogeneous product with a low viscosity.

In the case if it is not frying margarine, only distilled monoglycerides can be added. The aqueous phase of liquid margarine contains a certain proportion of dry skim milk and salt, as well as potassium sorbate as a preservative. The flavor supplements are added into both phases.

Particularly great is the role of emulsifiers in the production of vegetable-cream and vegetable-fat spreads. The choice of the type and dosage of an emulsifier in each specific case depends on the factors that include: the ratio of vegetable oil and milk fat, the total amount of fat in the product; the presence of an emulsifier in the used milk fat substitute; the features of technology and equipment [2].

Low-fat spreads are the only growing sector in the gradually declining spread market. With the exception of spreads with a very low fat content, they are water-in-oil emulsions, the mass fraction of fat therein is from 39 to 72%. In the production of low-fat spreads, it is necessary to balance the stability of an emulsion and the mouthfeel that depends both on the composition of the product and on the mode of production. Since the disperse (aqueous) phase may exceed by volume the continuous (fat) phase in this group of fat-and-oil...
products, the problems with product stability, its melting characteristics and taste perception are possible [2].

In high-fat spreads, milk proteins improve the mouthfeel and taste of the product, and also act as hydrophilic stabilizers for oil-in-water emulsions. However, their use in low-fat products, along with the need for significant energy costs, can cause phase reversal.

These problems can be overcome with the correct choice of a combination of formulation components and treatment methods. The important factors in the development of formulations are the melting characteristics of the fat mixture, type and dosage of an emulsifier and the addition of such thickeners as gelatin, sodium alginate, pectin and carageenan to the aqueous phase. It is possible to use small amounts of milk whey proteins, which improves the perception of taste, and also reduces the pH value of the aqueous phase, since milk whey proteins, unlike casein, do not precipitate at low pH values. The increase in acidity, as is known, contributes to an increase in shelf life. The treatment time, i.e., the rate of emulsion output from the coolant or its productivity, and the temperature of the product at the outlet also have a significant effect on the stability of spread.

Spreads that are oil-in-water emulsions have some advantages over water-in-oil spreads:
- the structure of the product does not depend on the type of fat used;
- any fat content from 39 to 50% or more is possible;
- the high dosages of whey protein are possible;
- such products are more economical and easier to manufacture;
- the taste and aroma are easier to perceive [14].

The main disadvantage of such products is an insufficiently low pH value, which leads to the need for UHT treatment and, if possible, aseptic packaging to provide a shelf life comparable to that of traditional spreads.

Oil-in-water spreads are still not widely used in the fat-and-oil product market. This is most likely due to the problem of microbiological spoilage, which can be solved by increasing the dosages of preservatives. Unlike water-in-oil emulsions, emulsifiers with high hydrophilic-lipophilic balance (HLB) values can be used in such systems to stabilize an emulsion with a continuous aqueous phase.

As a result of the predominant hydrophilicity of short-chain emulsifiers with less than 8 carbon atoms, they are well soluble in the aqueous phase and do not concentrate in the surface layer. Conversely, the long-chain emulsifiers with a carbon chain length more than 18 and the predominant lipophilic properties dissolve well in the oil phase and do not create the surface layer of an emulsifier either. For a good emulsifying effect, a relative balance of the hydrophilic and lipophilic properties is necessary, with some imbalance in favor of the nonpolar or polar parts depending on the type of emulsion.

The system for selecting an emulsifier based on HLB includes three steps:
- the determination of the optimal HLB value for the planned product;
- the determination of the best types of emulsifiers;
- the final HLB adjustment.

As for this method, the emulsifiers and mixtures thereof with the HLB values beyond the specified range can be discarded to reduce the number of trials using the trial-and-error method. The determination of the best HLB value includes the following steps:
1. Choosing a suitable pair of emulsifiers (one is lipophilic, the other is hydrophilic) with the known values; for example, mono- and diglycerides with the HLB value of 2.8 are lipophilic surfactants, and polysorbate 60 with the HLB value of 14.9 is a hydrophilic surfactant.
2. Preparing a series of experimental emulsions with the selected emulsifiers, which are mixed in such a way as to obtain various HLB values beginning with a completely lipophilic and ending with a completely hydrophilic substance. For the two emulsifiers selected at the first stage, the range of HLB values will be from 2.8 to 14.9. The mixture of emulsifiers should be used in an excessive amount or be about 10–12% of the fat content in the final product.
3. Estimating the obtained series of mixtures of emulsifiers using the appropriate methods for estimating functional efficiency based on the requirements for the product. Using one or more mixtures of emulsifiers, better emulsions will be obtained than with the others, but if all the mixtures prove to be good, a series of tests with a lower dosage of an emulsifier mixture should be repeated. If all the mixtures give bad results, it is necessary to increase the dosage and repeat the series of experiments.
4. The last stage of testing a mixture of emulsifiers should, with an accuracy of 2 units, determine the interval of hydrophilic-lipophilic balance that will be the best for this final product. If necessary, a more accurate HLB value can be determined using the following series of tests with the HLB values falling within this interval.

The appropriate chemical type of surfactants is as important as an HLB value. Once the HLB value is fixed, it is necessary to determine whether any other emulsifier mixture will work better and whether it will be more efficient or more economical with the same HLB value. The purpose of these tests is to select several pairs of emulsifiers that cover a fairly wide variety of chemical compounds. The estimates of the functional efficiency of these mixtures are the basis for choosing the ideal mixture of emulsifiers for this application.

As noted above, the concept of hydrophilic-lipophilic balance makes it possible to obtain a lot of useful information, as well as to calculate the HLB values for the mixtures of emulsifiers and to compile the tables of the experimentally obtained HLB values. For example, Table 2 shows the HLB values of individual surfactants and their mixtures.

At the same time, the HLB method is not the only condition for selecting surfactants, since other important factors, including molecular weight,
temperature changes and dissolution conditions, are not taken into account. To determine the value of HLB for some common food emulsifiers is rather difficult (for example, for phospholipids), and no information on the crystallization properties of monoglycerides and their derivatives can be obtained therefrom. Nevertheless, the concept of hydrophilic-lipophilic balance has proved to be a useful tool for the formation of a general idea of the likely properties of emulsifiers and their mixtures [14].

In addition to the hydrophilic-lipophilic balance, it is necessary to consider a lot of factors when choosing emulsifiers for fat products, including both the expected properties of the final product and the features of its manufacturing process. Of great importance are the type and properties of an emulsion system, the possible interaction of an emulsifier with other formulation ingredients, as well as its effect on the organoleptic properties of the product, including taste, aroma and sensory mouthfeel when the product is used for food. The technological parameters of mixing, homogenization, whipping and transferring by pumping also affect the choice of an emulsifier. Distilled monoglycerides of fatty acids (E 471), with a mono-ester content of at least 90%, are widely used for manufacturing a large assortment of margarine products. These emulsifiers differ from each other in the type of a fatty acid in the composition of a monoglyceride molecule. It can be stearic C18 : 0, oleic C18 : 1 and other fatty acids. The monoglycerides of different fatty acids differ in the iodine index, melting point and other physicochemical indicators. The iodine index of an emulsifier depends on the degree of saturation of fatty acids in the composition of a monoglyceride molecule. The monoglycerides of unsaturated fatty acids have a higher iodine index, while they have a lower melting point and stronger lipophilic properties. Sometimes the mixtures of monoglycerides and diglycerides are used to produce margarines and spreads. It should be noted that in this case, in order to obtain the desired stability of an emulsion, an increase in the dose of an emulsifier is necessary, in comparison with the use of only distilled monoglycerides.

The physicochemical indicators of various emulsifiers are normalized by the supplier, depending on the tasks that a particular supplement is to fulfill. Below, as an example, are the results of the study of composition and technological properties of two samples of distilled monoglycerides of different types.

The content of solid glycerides in the first sample of an emulsifier and its melting point are shown in Table 4 and in Fig. 1, the fatty acid composition is shown in Fig. 2.

The predominant fatty acids in this emulsifier are palmitic and stearic fatty acids. The analysis of the data obtained suggests that this emulsifier is more suitable for use in the production of emulsions with a fat content of 50–60%, since the iodine index (60 mg J/100 g) indicates a high degree of its unsaturation.

### Table 2. Values of HLB for some emulsifying systems [10]

<table>
<thead>
<tr>
<th>Emulsifier</th>
<th>HLB value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono and diglycerides</td>
<td></td>
</tr>
<tr>
<td>not less than 40% α-monoglyceride</td>
<td>2.8</td>
</tr>
<tr>
<td>at least 52% of α-monoglyceride</td>
<td>3.5</td>
</tr>
<tr>
<td>not less than 90% of α-monoglyceride</td>
<td>4.3</td>
</tr>
<tr>
<td>Esters of propylene glycol</td>
<td></td>
</tr>
<tr>
<td>mono- and diesters of propylene glycol</td>
<td>3.4</td>
</tr>
<tr>
<td>mono- and diesters of propylene glycol and mono- and diglycerides</td>
<td>3.5</td>
</tr>
<tr>
<td>Esters of sorbitan</td>
<td></td>
</tr>
<tr>
<td>sorbitan monostearate</td>
<td>4.7</td>
</tr>
<tr>
<td>sorbitan tristearate</td>
<td>2.1</td>
</tr>
<tr>
<td>Polyxyethylene sorbitans</td>
<td></td>
</tr>
<tr>
<td>polysorbate 60</td>
<td>14.9</td>
</tr>
<tr>
<td>polysorbate 65</td>
<td>10.5</td>
</tr>
<tr>
<td>polysorbate 80</td>
<td>15.0</td>
</tr>
<tr>
<td>Esters of polyglycerol</td>
<td></td>
</tr>
<tr>
<td>triglycerin monoshortening</td>
<td>6.0</td>
</tr>
<tr>
<td>triglycerine monostearate</td>
<td>6.2</td>
</tr>
<tr>
<td>hexaglycerol distearate</td>
<td>8.5</td>
</tr>
<tr>
<td>Lactylated esters</td>
<td></td>
</tr>
<tr>
<td>lactylated mono- and diglycerides</td>
<td>2.6</td>
</tr>
<tr>
<td>Lecithin</td>
<td></td>
</tr>
<tr>
<td>standard liquid</td>
<td>3.5</td>
</tr>
<tr>
<td>degreased, 22% phosphatydicholine</td>
<td>4.5</td>
</tr>
<tr>
<td>degreased, 45% phosphatydicholine</td>
<td>6.5</td>
</tr>
</tbody>
</table>

### Table 3. Physicochemical indicators of an emulsifier (Sample 1)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Characteristics of the test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Cream granules</td>
</tr>
<tr>
<td>Content of monoglycerides, %</td>
<td>93</td>
</tr>
<tr>
<td>Content of free glycerin, %</td>
<td>0.8</td>
</tr>
<tr>
<td>Content of free fatty acids, %</td>
<td>0.7</td>
</tr>
<tr>
<td>Iodine index, mg J/100 g</td>
<td>60</td>
</tr>
<tr>
<td>Melting point, ºC</td>
<td>50</td>
</tr>
</tbody>
</table>

### Fig. 1. Emulsifier melting curve (Sample 1).

### Table 4. Content of solid glycerides in the emulsifier (Sample 1)

<table>
<thead>
<tr>
<th>Content of solid glycerides, %</th>
<th>Melting point, ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>5ºC</td>
<td>10ºC</td>
</tr>
<tr>
<td>89.0</td>
<td>87.12</td>
</tr>
</tbody>
</table>
Fig. 2. Chromatogram of methyl esters of fatty acids of the emulsifier.

Table 5. Physicochemical parameters of the emulsifier (Sample 2)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Characteristics of the test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Cream granules</td>
</tr>
<tr>
<td>Content of monoglycerides, %</td>
<td>92</td>
</tr>
<tr>
<td>Content of free glycerin, %</td>
<td>0.7</td>
</tr>
<tr>
<td>Content of free fatty acids, %</td>
<td>0.9</td>
</tr>
<tr>
<td>Iodine index, J₂/100 g</td>
<td>2</td>
</tr>
<tr>
<td>Melting point, °C</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 3. Melting curve for the distilled monoglycerides (Sample 2).

Table 6. Content of solid glycerides in the emulsifier (Sample 2)

<table>
<thead>
<tr>
<th>Content of solid glycerides, %</th>
<th>Melting point, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>40</td>
</tr>
<tr>
<td>10°C</td>
<td>47</td>
</tr>
<tr>
<td>20°C</td>
<td>54</td>
</tr>
<tr>
<td>30°C</td>
<td>61</td>
</tr>
<tr>
<td>35°C</td>
<td>67</td>
</tr>
</tbody>
</table>

Table 5 presents the physicochemical parameters of the second emulsifier.

Table 6 and Fig. 3 present the content of solid glycerides and the melting point in the second emulsifier sample.

The second sample of an emulsifier is also distilled monoglycerides. However, it is characterized by a low iodine index and a higher melting point. Such an emulsifier can be recommended to produce margarine emulsions with a fat content of 72–82%. Since not so much the emulsifying ability is marked in the emulsifiers of this type as the ability to form a stable crystal lattice is, and thus to prevent the leakage of liquid vegetable oil.

To produce **high-fat margarines and spreads**, it is recommended to use the distilled monoglycerides of fatty acids of palm and other oils with a mono-ester content of 90%, with an iodine index of up to 2 mg J₂/100 g and a melting point within the range of 65–72°C. These emulsifiers are easily dosed and dissolved in oil, hydrogenated fat or any other form of fat. With a properly formed fat base, the introduction of 0.1% of this emulsifier and 0.2% of lecithin into the formulation is enough to produce high-fat margarines and spreads packed in packs or plastic cups.

In the production of spreads using the lines for pasteurizing an emulsion, it is possible to recommend the use of a complex emulsifier, which is a mixture of monoglycerides E471 and special lecithin. The presence of lecithin in a complex emulsifier makes it possible to increase the spreadability and plasticity of the final product.

To produce margarines and spreads with a mass fraction of fat of 60% or less, monoglycerides with a higher iodine index of 45 to 60 g J₂/100 g are used. We recommend using distilled monoglycerides (E 471) with an iodine index of 60 g J₂/100 g and a melting point of 57°C in a dosage of 0.2 to 0.4% in combination with 0.2% of lecithin.

The recommended dosage of an emulsifier may also depend on the type of equipment used to make margarine or spread. When using standard high-volume production lines, such as Kemtek, Johnson and Schroeder, it is enough to add from 0.2 to 0.3% of the emulsifier E 471 to the formulation. If no qualitative emulsification or homogenization of an emulsion is provided on the line before cooling, the amount of an emulsifier should be increased to 0.4%.
It should also be borne in mind that the introduction of milk proteins in the formulation of margarine is a destabilizing factor [8, 11]. Therefore, the amount of an emulsifier can also be higher for milk margarines and especially spreads.

This issue is very important in the development of spreads with the addition of a large amount of dried milk [7, 11]. In manufacturing such combined products using milk lines, the amount of distilled monoglycerides (E 471) has to be often increased to 0.4–0.5%.

In the case when the water content in the formulation of margarines and spreads is still higher, mono- and diglycerides with an iodine index of 80 mg I₂/100 g and a melting point of 50°C or lower should be used. Lecithins are not used in such margarines, as this can cause phase reversal and emulsion stratification. When obtaining margarines with a 50% fat content using standard lines, it is enough to use (E 471) with an iodine index from 80 to 105 g I₂/100 g. It should be borne in mind that margarines and spreads with such a fat content cannot be packaged in packs. This is due to a number of physical laws, and it is impossible to obtain a quality product with such a fat content packaged in packs by replacing an emulsifier.

In the production of margarines with a fat content of 40%, the amount of the aqueous phase is higher than that of the fat phase and monoglycerides alone are not enough to obtain a water-in-oil emulsion with sufficient plasticity. To produce margarines with a fat content of 40%, it is recommended to use the combination of monoglycerides and the esters of polyglycerol and ricinoleic acid (E 476). The emulsifier E 476 works well in the case of the combined introduction thereof with monoglycerides, the introduction thereof in the formulation alone does not give the desired result. Distilled monoglycerides (E 471) in the amount of 0.4–0.5% in combination with 0.1–0.2% of polyglycerol polyricinoleate (E 476) are usually used to produce 40% fat margarine. The introduction of this emulsifier into margarine makes it possible to obtain a stable emulsion that cannot be destroyed even if heated for long.

The production of margarines with a fat content of 20% is possible when an emulsifier for the fat phase and a stabilizer for water-milk phase are combined in the formulation. Distilled monoglycerides in the amount of 0.5–0.6% and polyglycerol polyricinoleate at a dosage of 0.3–0.4% are used as emulsifiers, just as in the case of 40% fat margarines. As a stabilizer for the aqueous phase, it is recommended to use pectin. The use of a stabilizer also allows us to make margarines more flavorful. This is especially important for margarines with a mass fraction of fat of 20 to 40% with a small amount of dried milk (0.1–0.2%) in the formulation. For non-milk margarines, the introduction of pectins into the formulation allows us to make the taste of the aromatizing agent in the aqueous phase more pronounced.

In the production of margarine for frying with a salt content of 0.3% and higher, it is enough to use 0.1–0.2% of distilled monoglycerides and 0.2% of lecithin in the formulation. It is also recommended to use an emulsifier that is a mixture of monoglycerides E471 and special lecithin E322. The use of this complex emulsifier in the amount of 0.3–0.4% in the margarine formulation allows, when frying, for the formation of fine-mesh foam on the frying surface, which does not allow margarine to splash and improves the color of the crust of the product being fried.

In the production of margarine for frying with a fat content of 60%, it is necessary to introduce the stronger emulsifier E479 into the formulation, which is thermally oxidized soybean oil in combination with mono- and diglycerides. It is capable of reducing the splashing tendency of margarine during frying, even with a large amount of water in the margarine formulation.

To produce liquid margarines in a customer-size package for household use, the combination of emulsifiers E479 (a mixture of monoglycerides and triglycerides) in the amount of 0.5–2.0% and E492 (sorbitan tristearate) at a dosage of 0.1% are used. The use of these emulsifiers makes it possible to obtain a stable liquid water-in-oil emulsion and prevent the release of liquid oil from a margarine emulsion during storage.

Sorbitan tristearate (E492) is also recommended for use in all types of margarines to prevent margarine defects – mealiness and crumbiness [9]. This is especially important when working with fatty acids of the same type in the fat base: sunflower oil and hydrogenated sunflower fat.

If margarine is used for baking whipped sponge cakes or for making confectionary creams [13], it is necessary to introduce a special emulsifier into the formulation to grip when whipping and to keep air in the mass of dough or cream. To this end, it is necessary to introduce, along with distilled monoglycerides (E471) at a dosage of 0.2–0.3%, an emulsifier in the margarine formulation – the esters of polyglycerol and fatty acids (E475) in the amount of 0.3–0.5%. The use of these two emulsifiers in the margarine formulation makes it possible to obtain uniform porosity in the baked products, to keep fat during baking, not to let it out, and there is also an increase in the volume of the finished products. The confectionery creams, cooked using such margarines, have a large volume and a high thermal stability. It should also be noted that in the production of margarines for such purposes, it is advisable to introduce 5–15% of coconut or palm kernel oil into the fat base.

To prepare margarine for puff pastry to retain plasticity during dough making, without being torn and keeping the same thickness of the layer when being rolled (at the same time it should not melt and impregnate the dough layers), it is advisable to use complex emulsifiers that are a mixture of monoglycerides (E 471) and polyglycerol esters (E 475) to control margarine crystallization processes at all the stages of its production and use in the amount of 0.8–1.0% and to additionally apply 0.6–0.8% of lecithin [13]. Table 7 presents some variants of emulsifiers and their compositions for margarines and spreads.
Table 7. Emulsifying systems recommended for margarines and spreads

<table>
<thead>
<tr>
<th>Status</th>
<th>Description</th>
<th>Scope</th>
<th>Permitted application rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 471</td>
<td>Distilled monoglycerides of fatty acids</td>
<td>Margarines with a different fat content, for a low fat content in combination with polyglycerol polyricinoleate</td>
<td>Not limited</td>
</tr>
<tr>
<td>E 471</td>
<td>Distilled monoglycerides of fatty acids and special lecithin</td>
<td>Margarines for frying</td>
<td>Not limited</td>
</tr>
<tr>
<td>E 476</td>
<td>Esters of polyglycerol and recinic acid</td>
<td>Margarines and spreads with a fat content below 41% in combination with DMH</td>
<td>0.4 (7.5 mg per kg of body weight)</td>
</tr>
<tr>
<td>E 475</td>
<td>Esters of polyglycerol and fatty acids</td>
<td>Margarines for whipped products</td>
<td>1.3 (25 mg per kg of body weight)</td>
</tr>
<tr>
<td>E 492</td>
<td>Sorbitan tristearate</td>
<td>Liquid margarines, elimination of “mealiness” in margarines</td>
<td>1.3 (25 mg per kg of body weight)</td>
</tr>
<tr>
<td>E 479b</td>
<td>Composition of mono- and diglycerides with thermally oxidized soybean oil.</td>
<td>Margarines, 60% fat, for frying</td>
<td>1.4 (30 mg per kg of body weight)</td>
</tr>
<tr>
<td>E 471</td>
<td>Mixture of mono- and triglycerides</td>
<td>Elimination of the release of liquid oil from margarine.</td>
<td>1.3 (25 mg per kg of body weight)</td>
</tr>
<tr>
<td>E 472c</td>
<td>Mixture of monoglycerides and citric acid esters of monoglycerides</td>
<td>Margarines for frying</td>
<td>Not limited</td>
</tr>
</tbody>
</table>

Table 8. Technological recommendations for the doses of the added emulsifiers

<table>
<thead>
<tr>
<th>Type of margarine</th>
<th>Emulsifier</th>
<th>Dose, % of the total weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table margarine</td>
<td>Fractionated lecithin/hydrolysed lecithin and monoglycerides (1 : 1-2)</td>
<td>0.25–0.4</td>
</tr>
<tr>
<td>with a low salt content (0.15%) with a high salt content (0.3%)</td>
<td>Standard lecithin and monoglycerides (1 : 1–2) (E 322/E 471)</td>
<td></td>
</tr>
<tr>
<td>Margarine with a low fat content</td>
<td>Fractionated lecithin and monoglycerides (1 : 3) (E 322/E 471)</td>
<td>0.5–0.7</td>
</tr>
<tr>
<td>Liquid margarine</td>
<td>Standard lecithin and monoglycerides (1 : 2) (E 322/E 471)</td>
<td>2</td>
</tr>
<tr>
<td>Margarine for puff products</td>
<td>Hydrolyzed lecithin and monoglycerides (1 : 1–2) (E 322/E 471)</td>
<td>0.7</td>
</tr>
<tr>
<td>Cream margarine</td>
<td>Hydrolyzed lecithin and monoglycerides (1 : 3) (E 322/E 471)</td>
<td>1.0–1.5</td>
</tr>
</tbody>
</table>

In addition to its technological functions, an emulsifier can increase the nutritional value of the emulsion product being created. A complex food emulsifier may contain phospholipids that have good emulsifying properties and are also physiologically valuable ingredients with, among other things, antioxidant activity. When this emulsifier was added to the spread and margarine formulations, there was an increase in the oxidative stability of the products.

The type of lecithin used basically depends on the salt content in margarine. For household margarines and industrial types for baking and making puff pastry, both standard and modified lecithins are suitable. Since salt is the additional stabilizer in classic margarines, the system becomes less stable in its absence. Therefore, it is recommended to use fractionated lecithin for salt-free and low-salted margarines which is enriched with phosphatidylcholine to provide a further increase in the stability of a margarine emulsion. At present, there is a stable trend of transition from standard lecithins to enzymatically hydrolyzed ones. They are well suited for the emulsions with a relatively high water content. In addition, they are more convenient to use than standard lecithins.

The lecithins used in the margarine formulation for frying improve its characteristics, in particular, heat resistance. The addition of lecithin prevents margarine from darkening during reheating and prevents splashing during frying. The margarine components prone to sedimentation, for example, milk casein, are covered with a thin layer of lecithin, which prevents their burning. In addition, the margarines that contain lecithin do not foam under heat treatment.

Adding lecithin to low-fat margarines improves their taste. In this case, in combination with monoglycerides, it forms a fine emulsion, which has a beneficial effect on the increase in shelf life.

The use of modified lecithins in margarine allows the product to resist heating to 280°C without splashing and darkening. The most optimal ratio of lecithin and mono- and diglycerides is 1 : 1; 1 : 2; 1 : 3 and 2 : 3.

Lecithin is a fat-soluble component, and therefore is added into the fat phase. There are a number of rules that should be followed when adding lecithin to oil (Table 8). The oil must be heated to a temperature of 55°C. Lecithin is added to oil gradually with constant stirring. When monoglycerides are used in the production of margarines, they must be dissolved in the oil phase together with lecithin.

It is very important that the mixture of emulsifiers is constantly mechanically treated over the whole technological process, the temperature should not be raised to more than 60°C.
Thus, based on the foregoing, it can be concluded that when selecting a particular emulsifier, all the features of the food emulsion it is intended for should be taken into account. Preference should be given to the food supplement a set of technological functions of which provides the maximum technological effect while preserving the nutritional value and organoleptic properties of the final product. In the final product, the emulsifiers should provide the stability of the water-in-oil emulsion due to their effective impact on the process of fat dispersion in the plasma, promote the formation of a homogeneous, plastic consistency of the product resistant to freezing-thawing due to the formation of structural bonds in the product that are easily restored after destruction. In addition, the emulsifiers in the composition of margarines and spreads control the processes of fat crystallization, i.e. affect the modification of the polymorphic form, the size and growth rate of fat crystals thereby providing the improvement of the creamy taste and other properties. The correct choice of an emulsifier makes it possible to increase the hardness and thermal stability of the product by taking part in the formation of its structure and also to prevent the release of moisture and liquid fat in the product monolith during long-term storage [11].

The most effective to provide the stability of an emulsion are the synergistic systems that consist of several emulsifiers. Despite the fact that the development of completely new emulsifiers is labor-intensive and is related to the need for long-term testing, the variety of factors that determine the composition and properties of emulsion products indicates that when creating new types of fat-and-oil products, the selection of optimal emulsifying systems, including those that consist of several emulsifiers, will be the subject of intensive scientific research and technological developments in future [14, 15].

REFERENCES


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EFFECTS OF IN-PACKAGE PASTEURIZATION ON PREVENTING SPOILAGE IN EMULSION VACUUM PACKAGED SAUSAGES DURING REFRIGERATED STORAGE

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Abstract: Lactic acid bacteria (LAB) dominate the spoilage populations of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures. An experimental investigation was carried out to evaluate the effectiveness of two in-package pasteurization treatments to prevent microbial spoilage in emulsion vacuum packaged sausages during refrigerated storage. D-values at 60 and 68°C for two isolated dominant LAB (Lactobacillus sakei and Lactobacillus plantarum) were determined in vitro and thermal treatments aimed to achieve a 4 log reduction in LAB. Sixty three sausage packs were divided into 3 groups: untreated packs (Control), treated at 60°C for 120 s (PAST 1) and treated at 68°C for 12 s (PAST 2). Microbial composition, pH values and sensorial changes were monitored in two-week intervals. In-package pasteurization resulted in an immediate 3.5–4.2 log CFU/g reduction in the population of LAB in PAST 1 and PAST 2 and remained at an acceptable level to the end of the experiment. On the contrary, during 84 days of cold storage, the LAB count increased significantly in the control samples and reached 9 log CFU/g. The control samples were also considered as unacceptable spoiled products after 28 days by sensorial aspects. All the pasteurized treatments also resulted resulted a significant (p < 0.05) reduction of psychrotrophic and the total mesophilic bacteria compared to the control ones. The data obtained showed that in both pasteurized groups none of the sensorial parameters were rated higher than the consumer-rejection threshold within 84 days of the study. No significant (p > 0.05) differences were observed in the number of spores, yeasts and molds between the pasteurized and control samples. It has been concluded that in-package pasteurization is an effective method without undesirable effects to prevent the spoilage caused by LAB growth and extend the emulsion vacuum packaged sausages shelf life to more than 3 months.

Keywords: In-package pasteurization, LAB, spoilage, shelf life

INTRODUCTION

It is considered that vacuum packaging combined with thermal treatment and refrigerated storage promotes extending the shelf life of meat products by reducing microbial growth. Vacuum packaging can inhibit the growth of the food-borne pathogens and spoilage bacteria frequently present in meat due to the restriction of the typical spoilage bacteria growth such as Pseudomonas in the absence of oxygen [1, 2]. Consequently, the environment conditions shift the bacterial flora to anaerobic psychrotrophic microorganisms dominated mainly by Lactic Acid Bacteria (LAB) which recontaminates the cooked products during handling and slicing prior to packaging. The spoilage caused by LAB metabolic activity appears in the form of sourness, off-flavors, off-odors, milky exudates, slime production, the swelling of package through gas production and discoloration and shortens shelf-life [2–4]. The characteristics of these bacteria is that they complicate the control of the proliferation of LAB during refrigerated storage, as they are psychrotrophic microaerophilic substances that tolerate nitrite and salt [4, 5]. Different approaches have been used to promote a reduction in the risk of post processing contamination, including chemical treatments, irradiation and thermal processes such as post-package pasteurization by submerging in hot water [5, 6]. In-package pasteurization is not a new approach; however, most of the early literature is used as a means of controlling Listeria monocytogenes in fully cooked meat products [6]. To the best of our knowledge, there is lack of investigation on the impact of in-package pasteurization of sausages on controlling spoilage microorganisms, primarily LAB and limited to Franz et al. [7] who applied in-package pasteurization processes for Vienna sausages and reported increasing in shelf-life from 7 days for non-pasteurized samples to 67, 99 and 119 days for the samples of three pasteurization treatments at 8°C storage.

This investigation was therefore undertaken to verify the impact of in-package pasteurization at 60 and 68°C in preventing microbial spoilage focused on LAB and extending emulsion vacuum packaged sausages shelf life during refrigerated storage (4°C).
**STUDY OBJECTS AND METHODS**

To study the impact of in-package pasteurization in preventing microbial spoilage and extending the shelf life of emulsion vacuum packaged sausages, the experiments were prepared as described below.

**Isolation, characterization and identification of strains.** Vacuum-packaged emulsion sausages were obtained from Demes Meat processing Company, Shiraz, Iran. Ten grams of samples were suspended in 90 ml sterile normal saline (0.9% w/v) and treated for 2 min in a stomacher. Then, the appropriately diluted samples were cultured on MRS (de Man Rogosa Sharp) agar (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h under anaerobic conditions using Anaerocult A gas packs (Merck, Darmstadt, Germany). From each plate of MRS agar, the colonies of catalase negative, gram positive rods or cocci were supposed to be LAB. were picked up and maintained on MRS agar at 4°C. The biochemical characterization of isolates was determined using the method described by Jafarpour et al. (Table 1) [8].

*Listococcus sakei* and *Listococcus plantarum* were determined as the dominant bacterial groups associated with the spoilage in refrigerated vacuum-packaged emulsion sausages according to biochemical tests and the previous study performed with vacuum-packaged emulsion sausages [8]. The long-term storage of isolates was done at −80°C in MRS broth with 30% glycerol.

**Preparation of bacterial cultures.** Each strain was cultured separately in MRS broth at 37°C for 24 h aerobically to reach the early logarithmic growth stage (O.D. 550 nm ca 0.3–0.5). The inoculum density was adjusted to 1 × 10⁷ CFU/ml using a ringer solution (Oxoid). The cells were centrifuged (14000 rpm for 5 min) and washed twice with 0.1 mol/l phosphate buffer. The cells were resuspended in ringer solution and vortexed for 1 min to disrupt cell clumps.

**Calculation of D- and z-values.** The cell suspensions (1 × 10⁷ CFU/ml determined on MRS agar using the plate-count method) were sealed in glass capillary tubes and heat inactivated using the previously described methods [7, 9]. Five replicate capillary tubes were heated for each of six time intervals (ranging from 0 to 125 s) at temperatures of 55, 58 or 61°C in a thermostat controlled circulating water bath. A set of five capillary tubes not subjected to heat treatment was considered as the control. The survived bacteria at each heating temperature and heating interval were enumerated on MRS agar. All the experiments were carried out in triplicate. The D-values expressed in seconds were determined from the linear regression analysis by means of Microsoft Excel 2010 software. The z-values were determined by computing the linear regression of the mean log D-values versus their corresponding heating temperatures by means [7].

**Thermal treatment/ microbiological analysis.** The thermal treatment aimed to achieve a 4 log reduction of the more heat (*Listococcus sakei*) based on the D-value results obtained in Table 2. These target temperatures were also set in order to correspond to the in-package pasteurization of this type of sausage and package, since they were low/short enough not to detrimentally affect packaging material or product quality and cause undesirable changes in sausage appearance (obtained in pilot studies).

<table>
<thead>
<tr>
<th>Table 1. Characteristics and identification of the LAB groups isolated</th>
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<tbody>
<tr>
<td>Identification</td>
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<tr>
<td>Cell morphology</td>
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<tr>
<td>Colony characteristics of agar media</td>
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<tr>
<td>Growth characteristics in liquid media</td>
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<tr>
<td>Gram reaction</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>CO2 from glucose</td>
</tr>
<tr>
<td>Production of lactic acid</td>
</tr>
<tr>
<td>Production of dextran from sucrose</td>
</tr>
<tr>
<td>Production of H2O2</td>
</tr>
<tr>
<td>Production of H2S</td>
</tr>
<tr>
<td>Growth at pH of 3.9</td>
</tr>
<tr>
<td>Growth at pH of 4.5</td>
</tr>
<tr>
<td>Growth at pH of 6.4</td>
</tr>
<tr>
<td>Acid produced from Galactose</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Imulin</td>
</tr>
<tr>
<td>Maltose</td>
</tr>
<tr>
<td>Mannitol</td>
</tr>
<tr>
<td>Rhamnose</td>
</tr>
</tbody>
</table>

The vacuum-packaged emulsion sausages were manufactured according to a standard procedure at Demes Meat processing Company, Shiraz, Iran. The emulsion sausages contained meat and binding component emulsion, 2.2% (w/w) sodium chloride, 150–200 ppm ascorbic acid and less than 100 ppm of residual nitrite which was filled into artificial casings. Eight slices were packaged into 400 g and 17 × 12 × 3 cm³ packs.

The packs from the production line were divided into 3 groups: untreated packs (Control), the packs which were subjected to in-package pasteurization at 60°C for 120 s (PAST 1) and the packs which were subjected to in-package pasteurization at 68°C for 12 s (PAST 2). The vacuum-packaged samples were simultaneously submerged into a water bath (Precision, model 186, Precision Scientific Incorporated, Chicago, USA). The sausage core temperatures were monitored as meat industry standard approach by inserting thermocouples into the core of a sausage located in the middle of a pack for each replication and then sealed.
To reduce the time reaching to treatment temperatures, the water bath temperature was set at higher values than those mentioned in PAST 1 and 2 (71°C). Once the core reached the target temperatures, the samples were held for 120 s in PAST 1 group and 12 s for PAST 2, then removed from the water bath and rapidly cooled by immersing in ice-water slurry for 60 s to prevent further cell death, then stored in a refrigerator at 4°C until further analysis. Both the pasteurized and non-pasteurized samples were tested on day 0 and in 2 weeks intervals on day 14, 28, 42, 56, 70 and 84 for microbial and sensorial analysis. On every sampling day, the packages were opened aseptically and 10 grams of sample was homogenized with 90 ml sterile 0.1% peptone water by placing meat into a sterile bag and stomached for 2 min (Seward Stomacher 400 Circulator, Seward Inc., Norfolk, U.K.). The homogenates were then serially diluted and appropriate serial dilutions were poured plated in duplicate. The following microbial parameters were determined: The total mesophilic bacteria plated on PCA agar plates (Merck, Germany) and incubated at 37°C for 48 h, Lactic Acid Bacteria (LAB) grown MRS agar (Merck 5413, Darmstadt, Germany) and incubated anaerobically using an anaerobic gas pack system (Anaerocult A, Merck, Germany) at 30°C for 48 h, Enterobacteria were enumerated on MacConkey agar (Merck 5465, Darmstadt, Germany) after incubation at 37°C for 24 h.

The psychrotrophic bacteria were enumerated using plate count agar (PCA) (Merck, Germany) after 10 days stored in 7°C. The spores were counted on (PCA) (Merck, Germany) after heat treatment at 80°C for 10 min and incubated at 37°C for 48 h.

The yeasts and molds were used cultured on Sabaraud Dextrose agar (SDA) enriched with an antibiotic to inhibit bacterial growth using a spread-plate technique and incubated at 25°C for 5 days [10].

**pH measurement.** The pH of the samples was determined by blending 25 g of the product with 225 ml of Ringer's solution, for 2 min, in a homogenizer (DI18B, Germany). The pH values were measured using a standardized electrode attached to a digital pH meter (CG824, Germany).

**Sensory analysis.** The sensory study, which was performed on each sampling day, evaluated the following parameters: the presence of gas, slime production (as a milky exudate), the presence of sour odor and yellow color. The sensory analysis was carried out according to the methodology described by Diez et al. [11]. A trained panel (a minimum of 5 panelists) scored the samples using a nine-point scale, on which one corresponded to the complete absence and nine to the maximum intensity of each parameter. A value of over 4 for all of the parameters under consideration was taken as the consumer rejection threshold.

**Statistical analysis.** In order to determine the difference among the treatments, an Analysis of Variance (ANOVA) was used and when differences were detected, a Duncan’s multiple comparison test was used to differentiate the treatment means. The analysis was carried out using SPSS (version 19, SPSS Inc) at a significance level of 0.05.

### RESULTS AND DISCUSSION

**D- and z-values for lactic acid bacteria.** Table 2 presents the D-values of *Lactobacillus sakei* and *Lactobacillus plantarum* at 55, 58 and 61°C. The z-values were measured at 8 and 7.68°C for *Lactobacillus sakei* and *Lactobacillus plantarum*, respectively, by computing the linear regression of the mean log D-values [7]. D<sub>60</sub> and D<sub>68</sub> for *Lactobacillus sakei* (the target LAB) calculated 30 and 3 s, respectively, considering D and z-values. The results from the heat resistance pattern of two main LAB in this study clarified that they are not able to tolerate the cooking process of 70 min to reach an internal of 72°C, therefore the recontamination after cooking was recognized as the source of spoilage LAB.

It is well known that common spoilage which appears as the souring, sliming and swelling of the pack and mostly occurs before the sell-by date is highly associated with lactic acid bacteria. This type of spoilage has become a major problem of the meat industry in recent years because the undesirable sensorial effect causes rejection by consumers [4, 12]. Several studies suggested recontamination after the heating process during slicing, chilling and packaging as the main causes of spoilage. Spoilage strains originating from raw materials may spread to slicing room via air, workers and equipment [13, 14].

Therefore, in the current study we aimed to extend vacuum packaged sausage shelf life and delay the spoilage time by the in-package pasteurization of a product. To achieve this aim, heating the final packaged product for a 4 log reduction in the LAB population was used as an approach.

**Microbiological analysis.** Fig. 1 illustrates the LAB count in PAST 1 and PAST 2. The results demonstrated an obvious decline (p < 0.05) on the first sampling day after heat treatment (day 14), on the contrary, there was a significant (p < 0.05) increase in the LAB population of the control group. During 84 days of cold storage, the LAB count increased significantly in the control samples and reached to 9.2 log CFU/g. On day 70, a significant (p < 0.05) decrease was recorded in the LAB population of the control samples which were ascended to 8.3 log CFU/g at the end of the experiment. The heat treatment led to a significant decrease in both PAST 1 and PAST 2 for the whole period of the experiment. Although there was a slow growth of LAB in the heat treated samples, particularly on day 28, the count remained at an acceptable level to the end of the experiment. The LAB count in the PAST 2 group was significantly (p < 0.05) lower compared to the PAST 1 group. At the end of the

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**Table 2. D-values for two major spoilage lactic acid bacteria in emulsion-type vacuum package sausages at 55, 58 and 61°C**

<table>
<thead>
<tr>
<th>Heating Temperature, (°C)</th>
<th>D-values (s)</th>
<th>D-values (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Lactobacillus sakei</em></td>
<td><em>Lactobacillus plantarum</em></td>
</tr>
<tr>
<td>55</td>
<td>51</td>
<td>42.37</td>
</tr>
<tr>
<td>58</td>
<td>39</td>
<td>18.17</td>
</tr>
<tr>
<td>61</td>
<td>24</td>
<td>14.13</td>
</tr>
</tbody>
</table>
trials the count of LAB, in the control group, PAST 1 and PAST 2 reached 8.3, 2.3 and 1.3 log CFU/g, respectively.

Throughout 84 days of the experiment, the population of LAB increased sharply in the control samples. Korkeala et al. [14] indicated that there was a strong correlation between the LAB count and the pH value of the product. They mentioned LAB produced lactic and acetic acid during logarithmic growth and especially at the stationary phase. They also demonstrated that whenever the counts of LAB reach $5 \times 10^7$ CFU/g, the lactic acid concentration increases sharply.

Fig. 1. Mean values (n = 3) for the lactic acid bacteria (LAB) counts of the control group (▲), PAST 1 (●) and PAST 2 (□) during 84 days of refrigerated storage. The bars present standard deviation values.

Fig. 2. Mean values (n = 3) for the total mesophilic counts of the control group (▲), PAST 1 (●) and PAST 2 (□) during 84 days of refrigerated storage. The bars represent standard deviation values.

Fig. 3. Mean values (n = 3) for the psychrotrophic bacteria counts of the control group (▲), PAST 1 (●) and PAST 2 (□) during 84 days of refrigerated storage. The bars present standard deviation values.

Fig. 2 shows the total mesophilic population for the control and pasteurized samples. The initial bacterial counts for the control samples were 3 log CFU/g and increased up to 7.36 log CFU/g during the first 2 weeks of storage and remained around 8 log CFU/g over time. The results showed the in-package pasteurization of vacuum packed sausages in both groups with a significant ($p < 0.05$) increase in the inhibition of the total mesophilic count compared to the control samples throughout the storage period.

When comparing the heat treated samples in PAST 1 and PAST 2, it was seen that, although the inhibitory effect of heating mesophilic bacteria was similar in the PAST 1 and PAST 2 samples, from day 14 on, the population of mesophilic bacteria in the PAST 2 samples remained lower ($p < 0.05$) than in the samples pasteurized at 60°C (PAST 1). Comparing the population of the total mesophilic bacteria in this experiment with the LAB count signifies that LAB might constitute the predominant portion of mesophilic bacteria (Fig. 1 and 2).

Fig. 3 illustrates the number of psychrotrophic bacteria. The initial count of psychrotrophic bacteria was 4.2 log CFU/g on day 0. An immediate significant ($p < 0.05$) increase in psychrotrophic population of the control samples was observed on day 14. On the contrary, the numbers of psychrotrophic bacteria decreased from 4 log CFU/g to 2.5 log CFU/g in both pasteurized treated groups after heating. The results showed a significant increase in the psychrotrophic counts of the control samples during 84 days of cold storage which reached 9.2 log CFU/g on day 28 and stabilized between 8 and 9 log CFU/g over time. Although there were no significant ($p > 0.05$) differences in the population of psychrotrophic bacteria between the PAST 1 and PAST 2 samples up to day 14, from day 28, the number of these bacteria was significantly ($p < 0.05$) higher in the PAST 1 samples compared to PAST 2. According to Fig. 1 and Fig. 3, the psychrotrophic bacteria growth pattern is similar to the LAB growth curves. These results show that the counted psychrotrophic bacteria in Fig. 3 were probably the spoiling LAB which grew on MRS agar due to their ability to grow at temperatures below 10°C. Although the presence of nitrite inhibits most psychrophils from contaminating products after they are cooked, this is not the case with psychrotrophic lactic acid bacteria, whose growth continues and eventually causes spoilage [7, 15, 16]. Additionally, in the anaerobe conditions and in the presence of salt and nitrite, most psychrotrophic bacteria such as *Pseudomonas spp.* are suppressed and the bacterial flora is gradually selected toward by LAB [16–18].

Figure 4 represents an enterobacteria count in the samples. The number of enterobacteria was 1 log CFU/g in all the groups at the beginning of the study. The enterobacterial number decreased in the PAST 1 and PAST 2 groups after heat treatment and remained at low levels to the end of the experiment. The enterobacteria showed a slight grow in the control group compared to the pasteurized samples. There were no significant differences in the enterobacterial count between PAST 1
and PAST 2. The inability of Enterobacteriaceae and yeasts to dominate the spoilage ecology of non-pasteurized and pasteurized sausages in this study also agrees with the previous studies of vacuum-packaged emulsion-type sausages [16, 19, 20]. This inability was contributed to the inhibition of Enterobacteriaceae and yeast by producing microbial lactic acid and a pH decrease in the product during the growth of lactic acid bacteria, as well as inefficient substrate utilization [20–22]. According to Fig. 4, although the preservation methods in the cooked vacuum packaged sausages inhibit an enterobacterial growth, the effect of in-package pasteurization on suppressing these bacteria in the PAST 1 and PAST 2 samples is considerable.

Fig. 5 presents the result of spore counts. The numbers of spores in all the experimental groups were around 2 log CFU/g on day 0. No significant changes (p > 0.05) in the spore growth pattern of the experimental groups were seen during 84 days of cold storage and the values were stabilized over time. No significant differences (p > 0.05) were seen in the spore numbers between the control and pasteurized samples. However, it was reported in the previous study [16] that in-package pasteurization may increase the risk of clostridium prevalence; no significant spore growth in the current study was seen in the pasteurized samples compared to the control ones.

According to the results, there were no yeasts and molds detected in any group. No significant differences (p > 0.05) in the yeasts and mold count were observed between the groups over the whole storage period. As it was expected, the hurdle concepts used in products such as cooking, an anaerobe condition, chemical preservation and the presence of competitive bacterial flora prevent a yeasts and molds growth and no notable differences were observed between the control and pasteurized samples.

**pH measurement.** The initial pH of vacuum packaged sausages in this study was approximately 6.7. The pH value showed a significant drop (p < 0.05) in the control samples during 84 days of storage and reached the lowest level of 4.7 on day 84. No significant (p > 0.05) change in pH of the PAST 1 and PAST 2 samples was seen during the experimental period. pH values in the PAST 1 and PAST 2 samples were around 6.5 on day 84 which was similar to day 0. There were no significant differences (p > 0.05) in pH values between PAST 1 and PAST 2 over time. Throughout 84 days of storage, the decrease in pH in the control samples was relative to the LAB growth (Fig. 6).

Several studies described that the samples are considered spoiled when pH falls below 5.8 to 5.9 and there is special organoleptic spoilage when pH values reach 4.6 to 5.5 [14, 15]. The same was in the current study, from day 28, that pH reached 5.5 and the LAB population counted to $10^8$ CFU/g, the typical signs of spoilage were observed. As it is shown in Table 3, the panelists rated the control samples as unacceptable spoiled products from day 28 considering the presence of slime, sour odor and the production of gas, Korkeala et al. [14] also indicated that when the LAB population reached $1.4 \times 10^7$ CFU/g, the samples could be considered unacceptable for human consumption from organoleptic aspects. On the contrary, the pH value in the PAST 1 and PAST 2 samples had no significant changes during the storage period and remained above 6 throughout the experiment. The obtained results in pH values are well correlated to the LAB population and sensorial analysis which demonstrated in both pasteurized groups that none of the sensorial parameters were rated higher than the consumer-rejection threshold of 4 throughout 84 days of the study. The low LAB counts in the pasteurized samples proved during 84 days of cold storage that in-package pasteurization is an applicable approach in LAB growth control.

Fig. 4. Mean values (n = 3) for the enterobacterial counts of the control group (△), PAST 1 (○) and PAST2 (●) during 84 days of refrigerated storage. The bars present standard deviation values.

Fig. 5. Mean values (n = 3) for the spore counts of the control group (△), PAST 1 (○) and PAST2 (●) during 84 days of refrigerated storage. The bars present standard deviation values.

Fig. 6. Mean values (n = 3) for pH of the control (△), PAST 1 (○) and PAST2 (●) during 84 days of refrigerated storage. The bars present standard deviation values.
Table 3. Mean ± SEM of the sensory evaluation of samples during the storage for up to 3 months

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PAST 1</th>
<th>PAST 2</th>
<th>PAST 1</th>
<th>PAST 2</th>
<th>PAST 2</th>
<th>PAST 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14</td>
<td>28</td>
<td>42</td>
<td>56</td>
<td>70</td>
<td>84</td>
</tr>
<tr>
<td>Yellow color</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.67 ± 0.47 Ab</td>
<td>2.00 ± 0.47 Ab</td>
<td>3.33 ± 0.67 Ac</td>
<td>4.00 ± 0.00 Ac</td>
<td>4.33 ± 0.47 Ad</td>
<td>4.67 ± 0.47 Ad</td>
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<tr>
<td>PAST 1</td>
<td>1.00 ± 0.00 Ac</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>2.00 ± 0.00 Ab</td>
<td>2.00 ± 0.00 Ab</td>
</tr>
<tr>
<td>PAST 2</td>
<td>1.00 ± 0.00 Ac</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.67 ± 0.47 Ab</td>
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<tr>
<td>Gas production</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>1.00 ± 0.00 Ab</td>
<td>2.33 ± 0.67 Ab</td>
<td>4.33 ± 0.67 Ab</td>
<td>5.33 ± 0.67 Ad</td>
<td>5.67 ± 0.94 Ad</td>
<td>6.67 ± 0.67 Ac</td>
<td>6.67 ± 0.82 Ac</td>
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<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>2.00 ± 0.00 Ab</td>
<td>2.00 ± 0.00 Ab</td>
<td>2.33 ± 0.67 Bc</td>
</tr>
<tr>
<td>PAST 2</td>
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<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.67 ± 0.67 Ab</td>
<td>1.67 ± 0.47 Ab</td>
</tr>
<tr>
<td>Sour odor</td>
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<tr>
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<td>4.33 ± 0.47 Ac</td>
<td>6.00 ± 0.00 Ac</td>
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<td>8.67 ± 0.47 Ac</td>
<td>9.00 ± 0.00 Ac</td>
</tr>
<tr>
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<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.67 ± 0.47 Ab</td>
<td>2.33 ± 0.47 Bc</td>
<td>2.67 ± 0.82 Bc</td>
</tr>
<tr>
<td>PAST 2</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.67 ± 0.67 Ab</td>
<td>1.67 ± 0.47 Ab</td>
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<tr>
<td>Presence of slime</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.00 ± 0.00 Ab</td>
<td>2.33 ± 0.47 Ab</td>
<td>4.00 ± 0.00 Ac</td>
<td>5.67 ± 1.05 Ad</td>
<td>6.67 ± 0.67 Ac</td>
<td>7.33 ± 0.82 Ac</td>
<td>7.33 ± 0.82 Ac</td>
</tr>
<tr>
<td>PAST 1</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.67 ± 0.47 Ab</td>
<td>2.00 ± 0.00 Ab</td>
<td>2.33 ± 0.47 Ab</td>
</tr>
<tr>
<td>PAST 2</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.00 ± 0.00 Ab</td>
<td>1.33 ± 0.47 Ab</td>
<td>1.67 ± 0.67 Ab</td>
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</tbody>
</table>

Sensory evaluation. Table 3 shows the results of a sensory analysis. At least one of the sensory parameters exceeded the threshold of 4 in the control samples on day 14. Significant signs of spoilage (P < 0.05) with particularly sour odor appeared in the control samples on day 14. The other signs of spoilage were appeared from day 28. From day 28, all the spoilage parameters, such as sour odor, the presence of slime and gas production have been considered unacceptable (P < 0.05) by the panelists. The yellow color was the last indication of spoilage which appeared from day 56. The results demonstrated that sour odor reached an unacceptable level of 9 at the end of the experiment. On the contrary, in both pasteurized groups, none of the sensorial parameters were rated higher than the consumer-rejection threshold of 4 throughout 84 days of the study. The result of the current study agrees with other investigators findings [14], as it is shown in Fig. 1 and Fig. 6, from day 14, that when the LAB count in the control samples reached 10^7 CFU/g, an immediate drop in pH was observed. The pH value in the control samples decrease from 6.7 on day 0 to 5.6 on day 14. The result from the sensorial analysis is well correlated with pH values. It was observed that on day 14, sour odor exceeded the threshold of 4 in the control samples. Different small letters indicate statistically significant differences in columns (p < 0.05). Different capital letters indicate statistically significant differences between days in each sample (p < 0.05). Throughout 84 days of experiment, in-package pasteurization at 60 and 68°C had no undesirable effects such as changing color, cooking loss and package damage in PAST 1 and PAST 2 samples and extend the shelf life of PAST 1 and PAST 2 samples to more than 3 months, while the control samples were considered as spoiled products 2–3 weeks after manufacturing, on the contrary, in-package pasteurization. The results of the current study suggest in-package pasteurization that can be used as a decontamination method for preventing spoilage microorganism growth and extending product shelf life.

In summary, we consider in-package pasteurization as an effective method for preventing spoilage caused by LAB growth and extending emulsion vacuum packaged sausages shelf life. Although no substantial differences in the bacterial population or organoleptic analysis were seen between PAST 1 and PAST 2, heating at 68°C may be more practical in the modern, high volume meat industry due taking less time achieving 4 log reductions in LAB counts. Future research of other food models with different temperature and a diverse analysis in bacterial metabolic activity will contribute to clarify in-package pasteurization method effectiveness.

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CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

AUTHOR’S CONTRIBUTION
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REFERENCES


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OAT PROTEIN CONCENTRATE PRODUCTION

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Abstract: Oats, both separately and as part of other dishes, refer to that healthy food that is regularly consumed by the broad masses of the population of various countries. Oats are a grain crop with a high level of nutrients, such as proteins, fats, minerals and vitamins. The oat grain from other cereals is distinguished by a high protein content and the unique composition of essential amino acids. The high content of β-glucan-soluble fiber allows us to consider oats as part of the complex therapy and prevention of cardiovascular diseases. There are more than 70 types of oats in the world, one of the most cultivated is *Avena sativa* L. The objects of the study were the samples of oat grains of the Drug [Friend], Adamo and Rysak [Trotter] varieties grown on the territory of the Russian Federation (the crop of 2017). The results of the study of the chemical composition and nutritional value of the samples of oat grains of these varieties have been provided. In the samples of the studied oat grain varieties, the content of proteins, carbohydrates and fats is not inferior to other varieties. The record content of protein, among the samples studied, is noted in the samples of oat grains of the Rysak variety, the minimum content – in the samples of oat grains of the Drug variety. The oat grains of the Adamo variety are characterized by a high content of carbohydrates, their mass fraction in this variety is 76.40%. The protein concentrate of oat grains of the Rysak variety was obtained using two methods: alkaline and acid extraction. The highest efficiency was observed in the alkaline extraction in the presence of an extracting agent – 1 M of the aqueous sodium hydroxide solution. The protein yield was 75.53% at a temperature of 40 ± 2°C, an irrigation module of 1 : 10, an active acidity of pH equal to 11.5 and a processing time of 120 ± 2 min.

Keywords: Oats, Nutritional value, Protein product, Alkaline extraction


INTRODUCTION

Oats (*Avena sativa* L.) is an important grain crop which is among the top ten cultivated cereals in the world with such crops as corn, rice, wheat, barley and sorghum [1–3]. Oat grains are characterized by a high protein content, a balanced amino acid composition, they also contain fiber, carbohydrates, unsaturated fatty acids, essential micro- and other nutrients that vary depending on a variety and growth conditions [4–6].

It has been established [7] that oat proteins contain the fractions that are soluble in alkali – 78%, alcohols – 11%, salts – 2.9% and others – 4%. The process of wet grinding of oat grains results in the production of a protein concentrate with a content of 59–75%. The combined use of acetylation and succinylation improves oat protein solubility and fat fixation, the emulsifying properties, impairs water retention and foam stability, the nutritional value has not changed, and the digestibility has improved [8–10].

The study was aimed at the possibility of obtaining protein concentrates from the samples of the oat grains cultivated on the territory of the Russian Federation using two methods – acid and alkaline extraction.

STUDY OBJECTS AND METHODS

The experimental studies were carried out at the Research Institute of Biotechnology of the Kemerovo State University.

The objects of the study were the samples of common oat grains of the Drug, Adamo and Rysak varieties (the crop of 2017). The grain samples were preliminary ground in a laboratory mill so that all the ground grains passed through a sieve with a hole diameter of 1 mm when sieved.

The mass fraction of protein in oat grains was determined using the spectrophotometric method with a biuret reagent forming the complexes of peptide bonds with copper ions (II) colored in violet in an alkaline medium according to a calibration schedule. Based on the data obtained, the mass concentration of protein in the studied solution was calculated taking into account the dilutions.

Plotting a calibration graph. A standard protein solution containing 10 mg protein in 1 ml was prepared from which a number of protein solutions of the known concentration were prepared. The studied protein solution was poured into three test tubes: 1.0 ml (Sample No. 1) in the first tube, 0.5 ml in the second (Sample No. 2) and 0.25 ml in the third (Sample No. 3). Then, 0.5 and 0.75 ml of water were added to the second and third tubes, respectively (the volume of the contents should be the same in each tube and amount to 1 ml at this stage). In the second and third tubes, the analyzed protein solution diluted 2 and 4 times, respectively, was prepared. These dilutions were taken into account in the calculation.
4 ml of biuret reagent (4 ml of reagent per 1–10 mg of protein) were poured into the control tube with the samples with the known protein concentration and with the samples of the test protein solution. The contents of each tube were mixed and left at room temperature for 30 minutes to develop color.

The optical density of the solutions (extinction) was measured using a UV 1800 spectrophotometer (Shimadzu, Japan) at 540–650 nm against the control (Sample No. 1). The results obtained for the protein solutions of the known concentration were shown graphically, plotting optical density along the ordinate axis and the protein mass that corresponds to this value – along the abscissa axis. The calibration curve was constructed using the average data of standard solution colorimetric extinction (triple replication).

To recalculate the results (mg/ml) as a percentage, it is necessary to know the mass of the raw material or the product and solvent taken for protein extraction. The protein content (%) was determined using the formula:

\[ C = \frac{9.2 \cdot V \cdot 100}{1000 - m}, \]

where \( V \) is the volume of the extract obtained; \( m \) is the weight of the sample; 1000 is a mg to g conversion factor; 100 is a 100 g conversion factor.

The extraction degree was calculated using the formula:

\[ X_{\text{protein}} = \frac{X_p + X_{pp}}{X_{\text{theor}}}, \]

where \( X_{\text{protein}} \) is an extraction degree; \( X_p \) is a high-molecular protein fraction in the solution, g/l; \( X_{pp} \) is a low-molecular protein fraction in the solution, g/l; \( X_{\text{theor}} \) is a 100% protein content in the solution, g/l.

The mass fraction of carbohydrates in oat grains was determined in accordance with GOST 31683-2012. The carbohydrates contained in the grains were dissolved in a hot dilute hydrochloric acid solution, the protein substances were precipitated and the optical density of the resulting solutions was measured using a UV 1800 spectrophotometer (Shimadzu, Japan) at 540–650 nm against the control.

The mass fraction of fiber and fat in oat grains was determined in accordance with GOST 32749-2014.

The method is based on recording the reflection spectra of the analyzed samples in the near infrared region and determining the mass fractions of fat and fiber therein.

The mass fraction of the measured indicator (excluding moisture), in terms of absolutely dry matter \( X_i \), %, was calculated using the formula:

\[ X_1 = \frac{X \cdot 100}{100 - W}, \]

where \( X \) is the mass fraction of the measured indicator that corresponds to the indication of the device, %; \( W \) is the mass fraction of moisture and volatile substances, %.

The mass fraction of ash was determined in accordance with GOST 10847-74. The essence of the method consists in burning the sample of the ground grain followed by the quantitative determination of a noncombustible residue.

The ash content (X) in percents of grain sample, in terms of dry matter, was calculated using the formula:

\[ X = \frac{m_3 \cdot 100 \cdot 100}{m_n \cdot (100 - W)}, \]

where \( m_3 \) is the weight of ash, g; \( m_n \) is the weight of the sample of the ground grains, g; \( W \) is the moisture content of the ground grains, %.

The mass fraction of water was determined in accordance with GOST 13586.5-93. The method consists in dehydrating the sample of the ground grains in an air-heating cabinet with the fixed parameters: temperature, drying time and determining the loss of its mass.

The moisture content of the grains (%) was calculated using the formula:

\[ X_1 = 100 - m_1 \cdot m_2, \]

where \( m_1 \) is the weight of the sample of whole grains after pre-drying, g; \( m_2 \) is the weight of the sample of the ground grains after drying, g.

The oat grain protein complex was studied by means of electrophoresis in polyacrylamide gel according to Laemmli. Necessary reagents for electrophoresis: THAM (tris(hydroxymethyl)aminomethane), acrylamide, bis acrylamide, SDS, glycine, hydrochloric acid, ammonium persulfate, tetramethylethylenediamine, glycerol, 2-mercaptoethanol and bromophenol blue. A sample buffer and a gel solution are prepared from the resulting solutions.

When preparing separating gel, all the solutions for preparation must be of room temperature before mixing. The gel solution was mixed to prevent the formation of bubbles and 100 μl of 10% SDS was added. A block was collected for gel pouring. First, 100 μl of 10% PSA was added to the gel solution, and then 10 μl of TMEDDA was stirred without bubbles and poured into a gel block. Then we waited until the gel polymerized. A concentrated gel was prepared from the previously prepared reagents.
The excess water was removed from the block with the polymerized separating gel by flowing onto filter paper. A comb and block were prepared to pour the stacking gel. 50 μl of 10% PSA and 5 μl of TMEDA were added to the concentrated gel solution, mixed and poured into the block, and a comb was put in to form wells. Then we waited for the stacking gel to polymerize.

To conduct electrophoresis, the filled block was placed in a chamber and the required volume of an electrode buffer was prepared. To this end, a 10x electrode buffer was diluted in a ratio of 1 : 9. An electrode buffer was poured into the chamber. 10% SDS was added to the upper chamber in a volume of 0.01 of the volume of the buffer in the upper chamber.

The samples were placed in the well with the gel using a micro syringe and the camera was connected to the power unit.

The completion of electrophoresis was controlled by the approach of the front of the bromphenol blue dye to the lower mark of the gel.

The gel electropherograms were developed using bright blue Coomassie. A boiling water bath installed in a fume hood was prepared. A dye solution was prepared. The gel was placed in a beaker and filled with a five-fold volume of the dye solution. The beaker with the gel was placed on the water bath in the fume hood and boiled for 10 minutes. The dye was poured out from the beaker, the gel was washed with distilled water and filled with a 7% acetic acid. The acetic acid solution was boiled in the water bath for 5 minutes, then changed to a fresh one twice and boiled again. Adding small pieces of the filter paper that absorbs the dye to the acetic acid solution makes washing faster.

The active acidity was determined and the activity of hydrogen ions was measured using a potentiometric analyzer. To this end, the test sample was added to the beaker, an electrode was placed in a way to avoid the contact of the electrode with the walls of the beaker.

The amino acid composition was determined using an Aracus PMA GmbH automatic amino acid analyzer. The principle of the method consists in the cation-exchange separation of amino acids with a pH step gradient and post-column derivatization with ninhydrin.

The elemental composition was determined by means of capillary electrophoresis using a Kapel-105/105M capillary electrophoresis system.

The mass fraction of phospholipids was determined by HPLC with UV detection using an LC-10 liquid chromatograph (Shimadzu, Japan).

The fatty acid composition was determined in accordance with GOST 30418-96 "Vegetable oils. Method for determination of fatty acid content".

RESULTS AND DISCUSSION

Oat grains are widely used both in cereals, in beverages and in bakery products. Oat protein is considered more nutritious than most of the crops consumed today [11, 12]. The chemical composition and nutritional value of the samples of different types of cereals vary greatly depending on growth, climatic and geographical conditions, and well as the variety of a cereal crop. Fig. 1 presents the results of determining the chemical composition of oat grains.

The highest amount of protein, carbohydrates and fat is contained in the samples of oat grains of the Rysak, Adamo and Drug variety, respectively. The lowest – in the samples of grain oats of the Drug, Adamo and Rysak variety, respectively.

The elemental composition of common oat grains is presented in Fig. 2. The main ash elements of the samples of common oat grains are potassium and phosphorus. There is also silicon, magnesium, manganese, calcium, sulfur, iron, copper and zinc in oat grains. Phosphorus is found in oat grains in an organic and mineral state. The total phosphorus content varies from 409 to 472 mg/100 g, herewith, the content of organic phosphorus varies from 297 to 329 mg per 100 g, and the content of phosphorus in the mineral form varies from 31 to 39 g per 100 g of seeds. Organic phosphorus is found in phytin, lipid and nucleic acid complexes. The percentage of organic phosphorus in phytin reaches 62%, in the lipid complexes – up to 1%, and in the nucleic acids – from 9.5 to 10.5% of total of phosphorus compounds.

Fig. 1. Chemical composition of oat grains: (1) the mass fraction of protein, %; (2) the mass fraction of fat, %; (3) the mass fraction of carbohydrates, %; (4) the mass fraction of ash, %.

Fig. 2. Analysis of the mineral composition of common oat grains: (1) potassium, (2) calcium, (3) magnesium, (4) sulfur, (5) phosphorus, (6) chlorine, (7) iron, (8) manganese, (9) copper, (10) zinc, (11) silicon.
The carbohydrate composition of the samples of common oat grains was determined using the chromatography method. The results of the study are presented in Fig. 3.

Carbohydrates (Fig. 1) account for the largest part among the other substances in the samples. The carbohydrate complex of oats grains consists of starch, hemicellulose, cellulose, lignin and simple saccharides. A starch fraction predominates in common oat grains, the mass fraction of starch in the common oat grains of the Drug variety is 36.37%, and the content of starch in the oat grains of the Adamo variety is 12% higher than in the common oat grains of the Rysak variety. The mass fraction of hemicellulose in common oat grains varies from 12.6 to 14.51%, moreover, the content of this carbohydrate in the oat grains of the Drug variety is 14.18%, which is 2.3% lower than the content of hemicellulose in the oat grains of the Adamo variety. Hemicellulose is represented in oat kernels mainly by non-starchy water-soluble beta-glucan – a polysaccharide that is able to reduce a high blood cholesterol level. The presence of beta-glucan causes the viscosity of aqueous solutions and the jelly-like properties of oat grain derivative products. The chromatographic analysis showed that hemicellulose is 20–80% of beta-glucan, its amount depends on the variety, location and growth conditions of oats. The content of beta-glucan in the oat grains of the Drug variety is 17.8% lower than in the oat grains of the Adamo variety and 2% higher than in the oat grains of the Rysak variety. Hemicellulose and fiber activate the motility of the gastrointestinal tract and promote the secretion of digestive juices in a living body. The oat grains of the Adamo variety dominates in a fiber content, the mass fraction of fiber in the common oat grains of the Adamo variety is 10.92%, and the mass fraction of fiber in the oat grains of the Drug variety is 10.19%, which is 1.2% lower than the fiber content in the oat grains of the Rysak variety.

The carbohydrate composition of the samples of common oat grains of the Drug variety is 20–80% of beta-glucan, its amount depends on the variety, location and growth conditions of oats. The presence of beta-glucan causes the cholesterol level. The presence of beta-glucan causes the secretion of digestive juices in a living body. The oat grains of the Drug variety have the lowest value among the studied varieties of common oat grains was determined using the chromatography method. The results of the study are presented in Fig. 3.

The analysis of the fractional composition of proteins of the samples of common oat grains (Fig. 5) shows that the protein complex of oat grains, regardless of a variety, consists mainly of albumins, globulins, glutelins and prolamins, moreover, the storage proteins (globulins and glutelins) make the largest part in oat grains. Thus, the endosperm accounts for up to 51%, the aleurone layer – 56% and the embryonic axis – only 2–4% of nitrogenous substances.
(the mass fraction was 33.76%) and globulins (the mass fraction of globulins was 28.19%), which is 7% more than the content of globulins in the oat grains of the Drug variety and 13.5% less than in the oat grains of the Rysak variety. The record amount of albumins is contained in the oat grains of the Drug variety (the mass fraction of albumins was 23.37%), which is 2.1 times more than that in the protein part of the oat grains of the Rysak variety and 2.3 times more than in the oat grains of the Adamo variety.

Fig. 4. Chromatogram of the fatty acid composition of lipids of oat grain samples: (a) the Drug variety; (b) the Adamo variety; (c) the Rysak variety.
The qualitative amino acid composition of grains does not depend on the type and variety of oats. Oat varieties differ in the quantitative content of amino acids. The analysis of the results shows that the composition of oat grains includes all the eight essential amino acids and they can be referred to complete proteins. The most complete protein is a Rysak variety, the biological value of this variety is 66 in relation to the protein standard – a chicken egg. The quantitative content of essential amino acids in the oat grains of the Rysak variety is 29.4% in relation to the total amino acid content. The oat grains of the Drug variety contain 27.6% of essential amino acids in relation to the total content, and the oat grains of the Adamo variety are characterized by a low content of essential amino acids.

Oats are considered as a source of high quality proteins. The protein content varies within the range of 12–15%. There are mutagenized varieties with a high protein content of up to 24%. They have a balanced composition of amino acids and a high fat content in comparison with other cereals. Our results are consistent with the reports of other authors [13–14] and the studied samples of grains of the oats cultivated on the territory of the Russian Federation are not inferior to other varieties and growth territories in the nutritional value. The studies on the chemical composition of oat grains of the Rysak variety, oats grains of the Drug variety and oat grains of the Adamo variety showed that oat grains contain a large amount of protein, carbohydrates and lipids. The record content of protein among the studied objects has been found in the oat grains of the Rysak variety, the minimum mass fraction of protein has been noted for the oat grains of the Drug variety. The oat grains of the Adamo variety are characterized by a high content of carbohydrates, their mass fraction in this variety is 76.40%. According to the content of essential amino acids, the grains of the Rysak variety are also a leader. Therefore, the oat grains of the Rysak variety have been chosen for further studies.

Alkaline and acid extraction was used to isolate proteins from the samples of oat grains of the Rysak variety. The main parameters of an alkaline extraction process are active acidity (pH is 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5 and 13.0), temperature (35, 40, 45 and 50°C), process duration (30, 60, 90 and 120 min) and an extractable substance and extractant-hydromodule ratio (1 : 05, 1 : 10, 1 : 15 and 1 : 20). The main parameters of an acid extraction process are active acidity (pH is 1.0, 2.0, 3.0 and 4.0), temperature (30, 35, 40, 45 and 50°C), process duration (30, 60, 90, 120 and 150 min) and an extractable substance and extractant-hydromodule ratio (1 : 5, 1 : 10, 1 : 15 and 1 : 20). The most common and economically sound extractants were used as alkaline solutions: a 1M potassium hydroxide solution and a 1M sodium hydroxide solution. An aqueous hydrochloric acid solution and an aqueous sulfuric acid solution – as aqueous inorganic acid solutions. The results of the studies are presented in Fig. 7–10.
Fig. 7. Effect of the amount of hydromodule in the process of alkaline (a) and acid (b) extraction on the yield of protein substances from the oat grains of the Rysak variety in the presence of: (1) 1 M of a potassium hydroxide solution; (2) 1 M of a sodium hydroxide solution; (3) of an aqueous hydrochloric acid solution; (4) of an aqueous sulfuric acid solution.

Fig. 8. Effect of pH of a medium in the process of alkaline (a) and acid (b) extraction on the yield of protein substances from the oat grains of the Rysak variety in the presence of: (1) 1 M of a potassium hydroxide solution; (2) 1 M of a sodium hydroxide solution; (3) of an aqueous hydrochloric acid solution; (4) of an aqueous sulfuric acid solution.

Fig. 9. Temperature effect of the process of alkaline (a) and acid (b) extraction on the yield of protein substances from the oat grains of the Rysak variety in the presence of: (1) 1 M of a potassium hydroxide solution; (2) 1 M of a sodium hydroxide solution; (3) of an aqueous hydrochloric acid solution; (4) of an aqueous sulfuric acid solution.
Thus, in the course of the studies of the technological approach of isolating protein from the oat grains of the Rysak variety using the alkaline and acid extraction method, an extraction agent has been selected – a 1 M aqueous sodium hydroxide solution and an aqueous hydrochloric acid solution, respectively. The rational values of the technological parameters of the extraction process have determined a temperature of 40 ± 2°C and 35 ± 2°C, a hydromodule of 1 : 10 and 1 : 10, an active acidity of 11.5 and 3.0 and a duration of 120 ± 2 and 90 ± 2 min, respectively, for alkaline and acid extraction. The use of these technological regimes allows us to achieve the maximum yield of protein substances from oat grains to 75.53% and 54.60% for the studied processes.

The obtained results are in agreement with the results of the authors of [3] who reported that up to 78% of oat proteins are soluble in alkalis and the alkaline extraction with the use of a sodium hydroxide solution was more effective for the sample of oat grains of the Rysak variety.

CONCLUSIONS

The carried out studies have confirmed that the oat varieties cultivated on the territory of the Russian Federation are not inferior to other oat varieties grown on other territories in a nutritional value. The study of the chemical composition of the samples of oat grains of the Drug, Adamo and Rysak varieties has distinguished the Rysak variety in terms of the content of proteins and essential amino acids.

The parameters of alkaline and acid extraction of protein from the oats grains of the Rysak variety have been optimized and an extraction agent and optimal technological regimes have been selected. It has been shown that the use of a 1 M aqueous sodium hydroxide solution and the selected technological regimes (the temperature is 40 ± 2°C, the hydromodule is 1 : 10, the active acidity is 11.5 and the duration is 120 ± 2 min) allows us to achieve the maximum yield of protein substances from oat grains (the yield of protein is 75.53% ). It has been established that the use of an aqueous hydrochloric acid solution and the selected technological regimes (the temperature is 35 ± 2°C, the hydromodule is 1 : 10, the active acidity is 3.0 and the duration is 90 ± 2 min) allows us to achieve the maximum yield of protein substances from oat grains (the yield of protein is 54.60%). The most effective method for obtaining a protein product from the samples of oat grains of the Rysak variety is the alkaline extraction method.

ACKNOWLEDGMENTS

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DEVELOPMENT AND STUDY OF FOOD PRODUCT WITH ANTI-ANEMIC AGENT BASED ON FARM ANIMAL BLOOD

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Abstract: Due to natural resources scarcity, farm animal blood processing to make functional food products is current task. The most promising direction is the isolation of iron-containing components from blood to obtain food products for the prevention of iron deficiency. Having analyzed disadvantages of existing techniques, we decided to use easily digestible heme iron and amino-peptide complexes obtained after the hydrolysis of red blood cells as an anti-anemic base. The scientific novelty of the research work consists in using the dried hydrolysate of packed red blood cells in developing a confection intended for the prevention of iron deficiency. Common methods were used in the investigation. Analysis of physicochemical, microbiological, and toxicological properties of the dried hydrolysate of red blood cells has been performed. Also, work on formulations of a confection, namely, sherbet-type bar containing the dried hydrolysate of red blood cells as an anti-anemic component has been carried out. In order to understand an effect of ingredients and develop the optimal processing technique, rheological properties of final bar have been studied. According to the results of the research, the dried hydrolysate of red blood cells conforms to safety requirements of SanPiN 2.3.2.1078-01. Two optimal formulations of the sherbet-type bar with anti-anemic agent based on farm animal blood have been chosen. It has been proved that such toxic elements as lead, cadmium, zinc, copper, arsenic, and mercuric, as well as pathogenic organisms are absent or within normal range.

Keywords: Confection, anti-anemic properties, farm animal blood, formulation, processing technique


INTRODUCTION

In the light of natural resources scarcity, the use of secondary raw materials in manufacturing functional food products is of relevance [1]. It is a matter of importance to give functional properties to these products to increase their marketability and profitability in the future.

Slaughter animal blood processing allows various products to be obtained [2]. One of common used ones is a product for the prevention of iron deficiency in humans. This direction has not yet been studied sufficiently hence, searching for new technical solutions is actual task [3].

The main drawback of existing products from slaughter animal blood consists mainly in using black food albumin, which is stabilized and dried whole blood or packed red blood cells of slaughter animals (packed RBCs), as an iron-containing component.

The low efficiency of food albumin is due to fairly complicated digestion for proteolytic enzymes because of the membrane stability of dried aggregated red blood cells. It is known that the absorption of iron takes place in a duodenum only, where black food albumin is mainly in an undigested form, which leads to pathogenic microorganism growth [4].

Thus, the use of easily digestible iron form and amino-peptide complexes obtained by the hydrolysis of red blood cells is a decision, which can exclude the disadvantages mentioned above.

It should be taken into consideration that protein hydrolysate is a good source of amino acids, which are being in a free state [5, 6]. Under conditions of human anemic states, amino acids would have a positive effect on both iron absorption and general state of man [7].

In previous works on obtaining an easily digestible form of iron from slaughter animal blood, the method of blood stabilization followed by fractionation for maximum yield of red blood cells from total blood bulk has been presented. Also, hydrolysis parameters for maximum proteolysis of protein components of packed RBCs have been described [8, 9].

The research is aimed at making the confection, namely, a sherbet-type bar containing the dried hydrolysate of packed RBCs as a component for the prevention of iron deficiency. Also, the aim of the work is to study physicochemical, microbiological, toxicological, and rheological properties of final product.

STUDY OBJECTS AND METHODS

Theoretical and experimental investigations are carried out at the Research Institute of Biotechnology and the Department “Bionanotechnology” (Kemerovo Institute of Food Science and Technology (University), Kemerovo, Russia).
The subjects of the study were dried hydrolysate of packed red cells from cattle blood and a confection made of such ingredients as full-cream evaporated milk, sugar, dried hydrolysate of packed red cells from cattle blood, vanillin, and ascorbic and citric acid.

The following equipment was used in conducting the investigation: analytical balance AND HR-202i (A&D, Japan), double distillation apparatus TKH25-11.15.92-81 BC.W39 (Russia), drying cabinet SH5vL-80 (Russia), Tarr-Baker gelometer (Russia), electrical oven SNOL («Umega», Lithuania), pH-meter SeweCompact (MetterToledo, the U.S.A), and spectrophotometer UV 1800 (Shimadxy, Japan).

Solids content in dried hydrolysate of packed RBCs was determined according to GOST 30648.3-99.

Moisture and solids content in the bar were determined in accordance with GOST 5900-73.

Water activity was determined indirectly by means of the unit, the schematic diagram of which is showed in Fig. 1. The indirect method is based on tentative determination of equilibrium relative humidity in the working space of the unit.

A silicone hose (3) joins all the elements of the unit, forming a closed circuit where a ventilator (4) forces air to move. Sensitive measuring elements IT5 – TR-2 “Termit” (2), being at the inlet and outlet of the working chamber (3), display measured values of temperature and relative air humidity.

Determination of water activity in foods includes two stages – preparatory and principal. The former involves draining the working circuit, while the latter determines the equilibrium relative air humidity in the working circuit. The water activity value $a_w$ was calculated as follows:

$$a_w = \frac{ERH}{100},$$

where $ERH$ is the equilibrium relative air humidity in the working circuit, %.

![Fig. 1. Schematic diagram of the unit for determination of water activity index in foods: (1) indicators of temperature and relative air humidity IT5 – TR-2 “Termit”; (2) sensitive measuring elements of indicators; (3) working chamber; (4) ventilator; (5) silicone hose.](image)

RESULTS AND DISCUSSION

Before including the dried hydrolysate of packed RBCs in the bar formulation, physicochemical, microbiological, and toxicological parameters of the hydrolysate were studied.

Results of physicochemical parameters are showed in Table 1.

**Table 1. Physicochemical parameters of dried hydrolysate**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids content, %</td>
<td>98.0 ± 0.9</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>Water activity, units</td>
<td>0.15 ± 0.9</td>
</tr>
<tr>
<td>Average molecular weight of peptide complexes, kDa</td>
<td>21.6</td>
</tr>
<tr>
<td>Mass fraction of iron, %</td>
<td>5.5 ± 0.9</td>
</tr>
<tr>
<td>Impurities visible to the unaided eye</td>
<td>not detected</td>
</tr>
</tbody>
</table>
It is seen from the data that the dried hydrolysate of packed RBCs is a dehydrated mixture of amino-peptide complexes heme iron. From this, the hydrolysate is relatively resistant to spoilage due to low water activity and moisture content.

Further, amino-acid analysis of the dried hydrolysate of packed RBCs was studied (Table 2), which indicated that all the existing amino acids can be conditionally divided into three groups: high mass fraction amino acids, small mass fraction amino acids, and amino acids which are not present in the hydrilysate.

The first group includes such amino acids (in g/100 g of protein) as valine (10.7), leucine (12.5), lysine (11.3), phenylalanine + tyrosine (10.9), glutamine acid (9.0), and asparagines acid (9.8).

The second group consists of the following amino acids (in g/100 g of protein): methionine + cystine (2.0), threonine (5.7), tryptophane (1.9), arginine (4.5), histidine (7.6), and isoleucine (0.3).

Glycine, oxyproline, proline, serine, and alanine are in the third group.

Microbiological properties and toxic substances content were studied as the quality parameters of dried hydrolysate of packed RBCs. The results are in Tables 3 and 4.

From the results it can be concluded that the dried hydrolysate conforms to safety requirements of SanPiN 2.3.2.1078-01 in microbiological parameters.

The next stage was the development of formulations of sherbet-type bars containing the dried hydrolysate of packed RBCs as an anti-anemic agent.

Vanillin, ascorbic and citric acids amount 5 g. Consequently, the amount of full-cream evaporated milk, sugar, and dried hydrolysate is 145 g. Varying their proportions, the change of such rheological properties as the viscosity of bar mixture, relative deformation, characteristic time of cutting, hardness as well as density, solids content, and mass fraction of iron were estimated. The viscosity of the mixture was determined at different temperatures (20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C). The data are presented in Fig. 2–9.

### Table 2. Amino-acid composition of dried hydrolysate of packed RBCs

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Content in the hydrolysate, g/100 g of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>10.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>12.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>11.3</td>
</tr>
<tr>
<td>Methionine + cystine</td>
<td>2.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.7</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>1.9</td>
</tr>
<tr>
<td>Phenylalanine + tyrosine</td>
<td>10.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>0</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.5</td>
</tr>
<tr>
<td>Asparagines acid</td>
<td>9.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>7.6</td>
</tr>
<tr>
<td>Glutamine acid</td>
<td>9.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>0</td>
</tr>
<tr>
<td>Oxyproline</td>
<td>0</td>
</tr>
<tr>
<td>Proline</td>
<td>0</td>
</tr>
<tr>
<td>Serine</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3. Microbiological safety indicators of dried hydrolysate of packed RBCs

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Standard value</th>
<th>Actual value</th>
</tr>
</thead>
<tbody>
<tr>
<td>QMAFAnM, CFU/g, no more than</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.5 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coliform bacteria in 0.1 g</td>
<td>not allowed in 0.1 g of product</td>
<td>not detected</td>
</tr>
<tr>
<td>Pathogenic (incl. salmonella) in 25.0 g</td>
<td>not allowed in 25.0 g of product</td>
<td>not detected</td>
</tr>
<tr>
<td>Sulfite-reducing clostridia in 1.0 g</td>
<td>not allowed in 1.0 g of product</td>
<td>not detected</td>
</tr>
<tr>
<td>Mold fungi, CFU/g, no more than</td>
<td>not allowed in 0.1 g of product</td>
<td>not detected</td>
</tr>
</tbody>
</table>

### Table 4. Toxicological safety indicators of dried hydrolysate of packed RBCs

<table>
<thead>
<tr>
<th>Indicator, mg/kg</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>0.05 ± 0.006</td>
</tr>
<tr>
<td>Lead</td>
<td>0.01 ± 0.003</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.10 ± 0.005</td>
</tr>
<tr>
<td>Mercuric</td>
<td>0.01 ± 0.0005</td>
</tr>
</tbody>
</table>

Full-cream evaporated milk and sugar have the largest effect on texture. The weight of one bar is planned to be 150 g. Vanillin, ascorbic and citric acids amount 5 g. Consequently, the amount of full-cream evaporated milk, sugar, and dried hydrolysate is 145 g. Varying their proportions, the change of such rheological properties as the viscosity of bar mixture, relative deformation, characteristic time of cutting, hardness as well as density, solids content, and mass fraction of iron were estimated. The viscosity of the mixture was determined at different temperatures (20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C). The data are presented in Fig. 2–9.
Fig. 3. Dependence of bar viscosity on ingredient proportion at 30°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).

Fig. 4. Dependence of bar viscosity on ingredient proportion at 40°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).

Fig. 5. Dependence of bar viscosity on ingredient proportion at 50°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).

Fig. 6. Dependence of bar viscosity on ingredient proportion at 60°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).

Fig. 7. Dependence of bar viscosity on ingredient proportion at 70°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).

Fig. 8. Dependence of bar viscosity on ingredient proportion at 80°C (1 – 100 : 43 : 2; 2 – 105 : 38 : 2; 3 – 110 : 33 : 2; 4 – 110 : 32 : 3; 5 – 105 : 37 : 3; 6 – 100 : 42 : 3).
According to the data in Figures 2–9, the bar viscosity depends on temperature; the higher temperature the lower the viscosity value, which corresponds to general theory of viscosity. Also, viscosity characteristics of the bar increase with the increase of full-cream evaporated milk content and the decrease of sugar amount. It should be noted that in samples with increased amount of the dried hydrolysate of packed RBCs viscosity value has increased; in other words, increased quantity of iron in the bar causes increased internal friction of bar layers.

In order to determine strength of the bar mixture, elastic limit for different proportions of full-cream evaporated milk, sugar, and dried hydrolysate was analyzed. The diagram of force distribution over time is shown in Fig. 10.

The results demonstrate the curve of the dependence of characteristic time of cutting the bar on ingredient proportions to have the minimum value when proportions are 110 : 32 : 3. Also, the time of cutting has minimum value for samples with ingredient proportions 110 : 33 : 2 and 105 : 37 : 3. Consequently, bars with such proportions of components have maximum elastic properties.

Fig. 11 demonstrates changes of elastic-plastic properties of the mixture when changing ingredient proportions.

On the basis of the results in Fig. 11, it can be stated that optimum proportions are 110 : 33 : 2 and 110 : 32 : 3.

Also, dependence of hardness of the mixture on ingredient proportions was studied (Fig. 12).

The mixture with higher sugar content has the greatest hardness. It should be noted that increased quantity of dried hydrolysate reduces the bar hardness.

Changes in density, solids content, and mass fraction of iron in the bar mixture at varying ingredient proportions were also studied. The results are represented in Table 5.

The data in Table 5 show density and solids content to be increased with higher sugar content and lower amount of full-cream evaporated milk. Optimum values of density (1265 ± 30 kg/m³, 1285 ± 30 kg/m³) and solids content (63.0 ± 0.1%,
64.0 ± 0.1%) take place when ingredient proportions are 110 : 33 : 2 and 110 : 32 : 3 where mass fraction of iron in the first and the second samples is 12 ± 0.5 and 17 ± 0.5 respectively.

Thus, from the results of the study of such properties of bar mixture as viscosity, characteristic time of cutting, relative deformation, hardness, density, solids content, and mass fraction of iron, proportions 110 : 33 : 2 and 110 : 32 : 3 (full-cream evaporated milk : sugar : dried hydrolysate) were chosen as optimal.

Formulations of bars are represented in Table 6.

The technological process of the bar consists of several interrelated stages.

(1) Preparation of ingredients. All the ingredients are checked for compliance with the certificate of quality in relation to the presence of extraneous impurities. In case of non-compliance, that ingredient is rejecting.

(2) Syrup making: Sugar and water are heated, full-cream evaporated milk is added and syrup obtained is boiled out until solids content is 85%.

(3) The making of bar mixture: Vanillin and ascorbic and citric acids are added into the syrup cooled to 45–65°C then all is thoroughly whipped. The fondant obtained is heated up to 70–75°C with constant stirring, mixed with dried hydrolysate of packed RBCs, and stirred at constant temperature until candy mass is obtained. The candy layer is formed, cooled in a refrigerator to 35–40°C, and then cut to pieces which are subjected to cooling to 20–25°C for 30 minutes.

The next stage included the study of organoleptic and physicochemical, microbiological and toxicological parameters in final bars. Organoleptic and physicochemical properties of the bar are presented in Tables 6 and 7.

Analysis of the results indicate the bar with anti-anemic agent from farm animal blood, produced by using developed formulations, to conform to requirements of GOST 30058-95 in organoleptic (surface, texture, color, consistency, taste, and flavor) and physicochemical (moisture content and mass fraction of ash) characteristics.

Toxic elements content in the bar with anti-anemic properties is shown in Table 8.

According to the data in Table 8, the bar made by using developed formulation complies with requirements of SanPiN 2.3.2.1078-01.

Microbiological parameters of the bar are reflected in Table 9.

The data show that the bar made by using developed formulation conforms to requirements of GOST 30058-95 in microbiological parameters.

### Table 5. Changes in density, solids content, and mass fraction of iron in the bar mixture at varying ingredient proportions

<table>
<thead>
<tr>
<th>Proportion</th>
<th>Density at 25°C, kg/m³</th>
<th>Solids content, %</th>
<th>Mass fraction of iron, mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 : 43 : 2</td>
<td>1325 ± 30</td>
<td>70.0 ± 0.1</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>105 : 38 : 2</td>
<td>1295 ± 30</td>
<td>67.0 ± 0.1</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>110 : 33 : 2</td>
<td>1265 ± 30</td>
<td>63.0 ± 0.1</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>110 : 32 : 3</td>
<td>1285 ± 30</td>
<td>64.0 ± 0.1</td>
<td>17 ± 0.5</td>
</tr>
<tr>
<td>105 : 37 : 3</td>
<td>1305 ± 30</td>
<td>68.0 ± 0.1</td>
<td>17 ± 0.5</td>
</tr>
<tr>
<td>100 : 42 : 3</td>
<td>1345 ± 30</td>
<td>71.0 ± 0.1</td>
<td>17 ± 0.5</td>
</tr>
</tbody>
</table>

### Table 6. Formulations of bars containing dried hydrolysate of packed RBCs as an anti-anemic agent (in 100 g of final product)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Weight, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-cream evaporated milk</td>
<td>73.4</td>
</tr>
<tr>
<td>Sugar</td>
<td>22.0</td>
</tr>
<tr>
<td>Dried hydrolysate packed RBCs</td>
<td>1.3</td>
</tr>
<tr>
<td>Vanillin</td>
<td>1.3</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>1.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1.0</td>
</tr>
</tbody>
</table>

### Table 7. Organoleptic properties of bar with anti-anemic agent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>Typical of this type of product, without hard setting of sides and syrup exudation</td>
</tr>
<tr>
<td>Texture</td>
<td>Typical of this type of product, uniform</td>
</tr>
<tr>
<td>Shape</td>
<td>Typical of this type of product</td>
</tr>
<tr>
<td>Color</td>
<td>Light brown, uniform</td>
</tr>
<tr>
<td>Consistency</td>
<td>Elastic, slightly viscous</td>
</tr>
<tr>
<td>Taste and flavor</td>
<td>Typical of this type of product, sweet, with light vanilla aftertaste</td>
</tr>
</tbody>
</table>

### Table 8. Physicochemical parameters of bar with anti-anemic properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample No. 1</th>
<th>Sample No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content, %</td>
<td>37.5 ± 0.6</td>
<td>36.6 ± 0.6</td>
</tr>
<tr>
<td>Density, kg/m³</td>
<td>1265 ± 30</td>
<td>1285 ± 30</td>
</tr>
<tr>
<td>Water activity, units</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Mass fraction of iron, %</td>
<td>0.012</td>
<td>0.017</td>
</tr>
<tr>
<td>Mass fraction of ash, insoluble in solution of hydrochloric acid with concentration of 10%, %</td>
<td>not detected</td>
<td>not detected</td>
</tr>
</tbody>
</table>

### Table 9. Content of toxic substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>lead, mg/kg</td>
<td>0.060 ± 0.003</td>
</tr>
<tr>
<td>Cadmium, mg/kg</td>
<td>0.025 ± 0.001</td>
</tr>
<tr>
<td>Mercuric, mg/kg</td>
<td>0.0010 ± 0.0001</td>
</tr>
<tr>
<td>Arsenic, mg/kg</td>
<td>0.090 ± 0.004</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>2.00 ± 0.23</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>7.50 ± 0.38</td>
</tr>
</tbody>
</table>
Table 10. Microbiological characteristics of bar with anti-anemic agent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Standard value (according to GOST 30058-95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QMAFAnM, CFU/g</td>
<td>$2.5 \times 10^1$</td>
<td>no more than $5.0 \times 10^3$</td>
</tr>
<tr>
<td>Coliform bacteria in 0.1 g</td>
<td>not detected</td>
<td>not allowed</td>
</tr>
<tr>
<td>Pathogenic (incl. salmonella) in 25.0 g of product</td>
<td>not detected</td>
<td>not allowed</td>
</tr>
<tr>
<td>Yeasts, CFU/g in 1 g of product</td>
<td>$5.0 \times 10^1$</td>
<td>no more than $5.0 \times 10^2$</td>
</tr>
<tr>
<td>Mold fungi, CFU/g</td>
<td>$1.0 \times 10^2$</td>
<td>no more than $1.0 \times 10^2$</td>
</tr>
</tbody>
</table>

Thus, the following conclusions can be made:

1. The confection, namely, a sherbet-like bar containing the dried hydrolysate of packed RBCs as an anti-anemic agent, was developed. The following ingredients were used: full-cream evaporated milk, sugar, vanillin, dried hydrolysate of packed RBCs, and citric and ascorbic acids. Full-cream evaporated milk, sugar, and vanillin give the product taste characteristics, while the acids were used to enhance taste and stimulate iron assimilation by a human body.

2. Quality characteristics of the dried hydrolysate of packed RBCs as an anti-anemic agent were studied. The results obtained show the hydrolysate to be a dehydrate mixture of amino-peptide complexes with heme iron.

3. Rheological properties of the sherbet-like bar, such as viscosity, elastic properties, hardness, and characteristic time of cutting, were studied. On the basis of the results obtained, two optimum formulations of the sherbet-like bar with anti-anemic agent on farm animal blood were chosen (Table 6).

4. Organoleptic, physicochemical, microbiological, and toxicological properties of the sherbet-like bar with anti-anemic agent were investigated. It is proved that the bar produced by using the developed formations conforms to requirements of GOST 30058-95 in organoleptic, physicochemical, and microbiological characteristics and SanPiN 2.3.2.1078-01 in toxicological parameters.

REFERENCES


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STUDY OF THE EFFECT OF SEX AND TYPE OF MUSCLES ON THE DEVELOPMENT OF QUALITY DEFECTS IN TURKEY MEAT AFTER THE SLAUGHTER

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Abstract: The defects in the quality of the processed meat lead to economic losses of enterprises. With an increase in the share of meat of the turkey sent to the production of meat products, the study of its technological properties becomes an urgent problem. Until now, data on the amount of domestic turkey meat with quality defects have not been collected. In this regard, the pH and microstructure of turkey meat were studied during maturation, which showed that the raw materials that go through processing have had significant quality deviations, which were based on some intravital factors. Thus, the carcasses of turkey cocks with pH20 below 5.6; from 5.6 to 5.9; above 5.9 were, respectively, 26%, 47% and 26% of their total. The carcasses of turkey hens were only divided into two groups: the carcasses with pH20 below 5.6–80%; from 5.6 to 5.9 – only 20%. The histological studies made it possible to describe in detail the nature of autolytic changes in the microstructure of the muscular tissue of pectoral and femoral muscles and confirmed the effect of poultry stress on the formation of technological properties of meat during maturation. The meat of turkey hens, especially pectoral muscles, was most susceptible to the development of quality defects. The presence of overcontraction knots and bands testified to the severity. The study showed that the problem of defects in the quality of turkey meat requires an increase in the responsible attitude towards the compliance with the technologies of poultry pre-slaughtering.

Keywords: Turkey meat, recyclability, muscle fibers, destructive changes

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The carcasses of turkey cocks had the values of pH20 ranging from 5.21 to 6.19, turkey hens – from 5.08 to 5.84. At the same time, there was no correlation between the values of pH20, body weight and body temperature (the correlation test showed that the absolute value of the correlation coefficients was not more than 0.2220). According to the results of pH20 measurement, the selected carcasses were conditionally divided into 3 quality groups (Fig. 1).

The analysis of the obtained carcass division depending on pH20 showed that, among the carcasses of turkey cocks, the proportion of meat presumably with PSE signs (pH from 5.6 to 5.9) was 47%. The rest of the carcasses was equally presented by two quality groups: 26% had pH values below 5.6 and, accordingly, were turkey meat with PSE properties; and 26%, on the contrary, had pH values above 5.9. The latter group of carcasses could be both meat with the signs of DFD, and meat with normal autolysis.

Among the carcasses of turkey hens, there was a different pattern of distribution among the quality groups: the carcasses with the value of pH from 5.6 to 5.9 were only 20%; the carcasses with pH20 below 5.6–80%. The carcasses with pH30 above 5.9 were not detected during the study. This confirmed the fact that turkey hens are more susceptible to stress than turkey cocks [2], and therefore the quality stability and producibility of the meat obtained from turkey hens is lower than the turkey meat obtained from turkey cocks.

Thus, even by measuring pH20 it was found that a significant part of carcasses of turkey cocks and turkey hens had some deviations in the development of autolytic processes, and, consequently, in quality. Moreover, the influence of sex on the autolytic changes in meat was significant: the number of carcasses from turkey cocks with a non-critically low level of pH20 (from 5.6 inclusive and higher) was 3.7 times higher than that from turkey hens (respectively, 47%, + 26% and 20%). In general, of the total number of the studied carcasses, only 13%, according to pH20 measurement, were close to the normal course of autolysis, 33% did not have strongly pronounced deviations from the norm and 54% were meat raw materials with the pronounced properties of PSE, which is hardly suitable for use in meat processing due to low functional and technological properties.

It should be noted that according to foreign literature data [3, 4], in order to achieve the optimal technological characteristics of turkey meat, it is necessary to ensure that the bulk of carcasses (over 85%) should have a pH value not lower than 6.2 and not higher than 6.7 20 minutes after the slaughter. With such pH values, it can be expected 20 minutes after the slaughter that the matured meat will have a pH value from 5.7 to 5.9 inclusive and the best organoleptic and technological characteristics.
Table 1. Results of pH-metry of the carcasses of turkey cocks and turkey hens selected for histological studies

| Identification number of a carcass in the experiment | Measurement results |  
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH₂₀ | pH₂₄ |  
| | Pectoral muscles | Femoral muscles | Pectoral muscles | Femoral muscles | Pectoral muscles | Femoral muscles |  
| The carcasses of turkey cocks with pH₂₀ above 5.9 (1m group) |  
| 5898 | 6.16 | 5.21 | 5.30 | 5.23 | 5.57 |  
| 5892 | 6.09 | 5.30 | 5.57 | 5.55 | 5.65 |  
| The carcasses of turkey cocks with pH₂₀ from 5.6 to 5.9 inclusive (2m group) |  
| 5894 | 5.81 | 4.95 | 4.90 | 5.43 | 5.70 |  
| 5902 | 5.87 | 5.00 | 5.00 | 5.10 | 5.80 |  
| The carcasses of turkey cocks with pH₂₀ below 5.6 (3m group) |  
| 5891 | 5.29 | 5.04 | 5.27 | 5.07 | 5.61 |  
| 8910 | 5.21 | 4.90 | 5.00 | 4.97 | 5.51 |  
| The carcasses of turkey hens with pH₂₀ from 5.6 to 5.9 inclusive (2f group) |  
| 7031 | 5.84 | 4.75 | 4.90 | 4.89 | 5.25 |  
| 7039 | 5.83 | 4.70 | 5.10 | 5.00 | 5.46 |  
| The carcasses of turkey hens with pH₂₀ below 5.6 (3f group) |  
| 7043 | 5.10 | 4.80 | 4.90 | 4.99 | 5.30 |  
| 7046 | 5.08 | 4.75 | 4.84 | 4.75 | 4.99 |  

For a histological study, two carcasses from each quality group (10 carcasses in total) were selected. Table 1 shows the data on the determination of pH₂₀, pH₂₄ and pH₄ for the selected samples.

The results of 24-hour pH measurement in the carcasses of turkey cocks showed that the lowest decrease in pH₂₀ in 1m group was recorded 5 hours after slaughter and was 5.2–5.3 for pectoral muscles and 5.3–5.6 for femoral muscles. After 24 hours, there were no significant changes in pH in the pectoral muscles, or there was an insignificant increase in pH (0.13 on average). In the femoral muscles, there was an increase in pH₂₄ in comparison with pH₂₀ on average by 0.25. 2m group was characterized by a decrease in the value of pH₂₀ to 4.9–5.0 both in the pectoral and femoral muscles. Then the pH₂₄ measurement showed significant differences during the autolysis between the pectoral (5.1–5.4) and femoral (5.7–5.8) muscles. In 3m group, pH₂₀ and pH₂₄ remained in the same interval, the upper value of which did not exceed 5.3. Moreover, in the pectoral muscles, the low values of pH remained unchanged after 24 hours, and in the femoral muscles pH₂₄ increased to 5.50–5.65.

The following changes in pH were characteristic of the carcasses of turkey hens of 2f group: during the first 2 hours after the slaughter there was already a sharp decrease and pH₂₀ was 4.7–4.8 for pectoral muscles and 4.9–5.1 for femoral muscles; after 24 hours, pH did not increase in pectoral muscles, but it increased to 5.2–5.6 in femoral muscles. 3f group showed the extremely low values of pH for 24 hours, while pH₂₄ was not higher than 5.3 even for femoral muscles.

It should be noted that the pattern of a change in pH that was noted during the study was not typical for turkey meat, and indicated the pronounced effect of intravital stress factors on the course of autolysis [2, 5, 6, 7, 8, 9].

The histological studies of the muscle tissue samples taken in 2, 5, 8 and 24 hours aimed at studying the nature of development of autolytic processes depending on the sex and type of poultry muscles. In this study, two types of muscles were selected: pectoral and femoral muscles as those that contain, respectively, the largest and smallest number of glycolytic "white" muscle fibers. In the samples studied, they were on average 74.6 ± 3.5% and 64.0 ± 2.5%, respectively, in the pectoral and femoral muscles. Such a number was small and was explained by the genetics of fowl (according to the literature data [7], their number is up to 99.8% and 87.5% in pectoral muscles).

1m group. During the microstructural study of the muscle tissue of pectoral muscles, muscle fibers of a wavy or convoluted shape were noted 2 hours after the slaughter. The boundaries between the fibers were clearly distinguishable. The cores of the fibers had a round or oval shape. The muscle tissue was characterized by fine cross striation. The length of sarcomeres was 1.8–2.0 μm. Separate fibers were characterized by increased longitudinal striation. The cross section of the muscle fibers had a polygonal shape. The connective-tissue layers were wavy. The change in the shape of muscle fibers, the shortening of the length of sarcomeres, the increase in longitudinal striation and the decrease in cross striation of fibers indicated the beginning of post mortem rigor (rigor).

Five hours after the slaughter, the changes characteristic for rigor were detected in the bulk of the fibers. The longitudinal striation increased, the length of sarcomeres decreased to 1.5–1.7 microns. The muscle fibers took on a straight shape due to the uniform contraction of myofibrils throughout the sample. 8 hours after the slaughter, there was some swelling of muscle fibers and an increase in the diameter of a fiber and the length of sarcomeres (1.9–2.2 μm) in muscle tissue. There was a restoration of cross striation and a decrease in longitudinal striation. The noted microstructural changes were characteristic for the post-slaughter resolution stage (postrigor).
After 24 hours of autolysis, the muscle tissue of pectoral muscles was characterized by straight or slightly wavy muscle fibers with distinct cross striation, the length of sarcomeres was 2.5–2.7 μm. The boundaries between the fibers were clearly distinguishable, the cores of the fibers took on a rod or oval shape. The destructive changes were detected in the form of microcracks or cross cracks with an integral sarcolemma. The destructive changes in the structure of muscle fibers was characteristic for this stage of maturation of muscle tissue.

The samples of muscle tissue of femoral muscles were characterized by less pronounced dynamics of microstructural changes for the same time after the slaughter. Thus, the first signs of post-mortem contraction of muscle fibers were only noted 5 hours after the slaughter of fowl. The muscle fibers took on a wavy or folded shape. As a result of the unstable process of contraction of myofibrils, the longitudinal striation of fibers intensified. The length of sarcomeres was 1.9–2.0 μm. The muscle fibers lay loosely in relation to each other. The muscle tissue was at the initial stage of rigor. 8 hours after the slaughter, the muscle fibers took on a slightly wavy or straight shape. The longitudinal striation was expressed in the bulk of the fibers. The length of sarcomeres decreased to 1.7–1.9 μm. According to the microstructural parameters, muscle tissue was in a state of rigor. After 24 hours of autolysis, the muscle tissue was characterized by straight muscle fibers, the cross striation was distinct, the length of a sarcomere was 2.7–2.9 μm. Separate muscle fibers showed destructive changes in the form of microcracks that indicated post rigor and the beginning of the maturation stage.

The nature of microstructural changes in the carcasses of 1m group was the closest to the changes that take place during the normal course of autolysis. Table 2 presents the photos that show these changes. However, there were still some separate segments of myofibril overcontraction in the microstructure of muscle tissue samples (mainly in the pectoral muscle of carcass No. 5889), usually found in PSE meat, but their pronouncement was insignificant in comparison with the samples of other groups.

**Table 2.** Microstructure of the muscular tissue of turkey meat at different stages of autolysis with its history close to normal

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Microstructure of muscle tissue</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Longitudinal section</td>
<td>Cross section</td>
<td></td>
</tr>
<tr>
<td>Pectoral muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h after the slaughter</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>5 h after the slaughter</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>8 h after the slaughter</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td>24 h after the slaughter</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Microstructure of the muscular tissue of turkey meat at different stages of autolysis with its history characteristic of meat with the pronounced symptoms of PSE

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Pectoral muscles</th>
<th>Femoral muscles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Longitudinal section</td>
<td>Cross section</td>
</tr>
<tr>
<td>2 h after the slaughter</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>5 h after the slaughter</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>8 h after the slaughter</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>24 h after the slaughter</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

2m group. Two hours after the slaughter, the muscle fibers of pectoral muscles were characterized by a folded or straight shape. The longitudinal striation was distinct, the cross striation was reduced and was not uniform. The length of sarcomeres was 1.6–2.0 μm. The muscle fibers lay loosely in relation to each other. 5 hours after the slaughter, the fibers took on a straight shape, the longitudinal striation was sharply strengthened in the bulk of the fibers, the cross striation was connivent, the length of a sarcomere was 1.3–1.5 μm. In the structure of muscle tissue, separate overcontraction knots of a rectangular shape and cross bands were revealed in varying degrees of pronouncement. The muscle tissue was at the stage of post mortem rigor. 8 hours after the slaughter, the muscle fibers became straight or slightly wavy, the cross striation was restored, the length of sarcomeres was 1.9–2.0 μm, which is typical for the post rigor stage.

Twenty-four hours after the slaughter, the muscle fibers had a straight shape, the cross striation was clearly pronounced in the bulk of the fibers, the length of sarcomeres was 2.0–2.5 μm. In the structure of muscle fibers, separate cross cracks were detected, peculiar to the beginning of the maturation stage.

The microstructure of muscle tissue of the femoral muscles 2 hours after the slaughter was characterized by straight or wavy muscle fibers. The cross striation was reduced and was not uniform, the length of sarcomeres was 1.9–2.5 μm. The longitudinal striation was strengthened in the straight-lying fibers. The muscle tissue was at the initial stage of postmortem contraction. The longitudinal striation increased in 5 hours after the slaughter. The fibers became straight. The cross striation was non-uniform and fine. The muscle fibers lay loosely in relation to each other. The length of sarcomeres was 1.9–2.3 μm. The muscle tissue was at the stage of rigor development. 8 hours after the slaughter, the muscle fibers had a straight shape, the longitudinal striation was pronounced in the bulk of the fibers, the length of sarcomeres decreased to 1.7–2.0. According to the microstructural parameters, muscle tissue was in a state of rigor. 24 hours after the slaughter, the muscle fibers lay in a
straight line, the boundaries between them were well pronounced. The cross striation was clearly revealed, the length of sarcomeres was 2.2–2.7 μm. The destructive changes were detected in the form of microcracks and separate cross cracks without the violation of integrity of the sarcolemma.

The microstructure of muscle tissue samples from the carcasses of 2m group was characterized by a more pronounced formation of multiple overcontraction knots both in the pectoral and femoral muscles. According to the results of histological studies, the carcasses of this group were classified as turkey meat with moderately prominent signs of PSE.

The carcasses of 3m group. Two hours after the slaughter, the muscle fibers of the pectoral muscles were straight and lay loosely in relation to each other. The nonuniform cross striation was clearly pronounced in the bulk of the fibers, the length of sarcomeres was 1.5 to 2.6 μm. In the structure of muscle tissue, there were multiple homogeneous overcontraction bands or knots characterized by ruptures of myofibrils and sarcolemma in the adjacent segments of the fibers and the destruction of the myofibrillar substance to a fine-grained protein mass. The nuclei in the fibers were well distinguishable.

In the subsequent terms of sampling after the slaughter, the muscle fibers did not change their configuration, the length of sarcomeres did not differ significantly either, which was a characteristic microstructural indicator of meat with a PSE defect. Twenty-four hours after the slaughter, multiple cross cracks were formed in the structure of the muscle fibers, which indicated the maturation of the meat.

In the femoral muscles, the microstructural signs of meat with a PSE defect were less distinct, but there were also multiple overcontraction cross sections with an integral sarcolemma. Two hours after the slaughter, the muscle fibers were characterized by a straight or slightly wavy shape, the cross striation was clearly pronounced, the length of sarcomeres was 2.3–2.7 μm. In the structure of the fibers, overcontraction bands were noted, which remained throughout the entire maturation period. After 5 and 8 hours of autolysis, there was an increase in longitudinal striation in separate muscle fibers, the cross striation was distinct, the length of sarcomeres varied from 2.0 to 2.5 microns. The post rigor stage was observed after 8 hours of autolysis. The signs of the maturation stage were noted 24 hours after the slaughter of the poultry in the form of multiple cross cracks.

According to the results of histological studies, the carcasses of 3m group were classified as turkey meat with the pronounced signs of PSE – the formation of multiple overcontraction bands and knots (in the pectoral and femoral muscles), sarcolemma ruptures in the knot formation segments, the destruction of myofibrils in the segments adjacent to the contraction knots and the dynamics of a change in the length of sarcomeres. Table 3 presents the photos that describe the microstructure of the samples of this group.

Groups 2f and 3f. The samples of muscle tissue from the carcasses of turkey hens had the same microstructural characteristics as the samples from the carcasses of turkey cocks of the groups 2m and 3m, respectively. However, in turkey hens, the signs of PSE meat and muscle fiber overcontraction were much more pronounced than in turkey cocks (Fig. 2). This fact was consistent with the foreign studies on the effect of turkey sex on the development of quality defects in meat. It is believed that turkey cocks are less susceptible to stress factors than turkey hens.

The microstructural study of the samples from the pectoral muscles of the carcasses of 2f group showed that 5 hours after the slaughter, the muscle fibers were predominantly of a straight shape. The non-uniform longitudinal striation was only in part of the fibers, the cross striation was reduced. The length of sarcomeres was 1.9–2.0 μm. The muscle fibers lay loosely in relation to each other. In some segments, some overcontraction bands or knots of the myofibrillar substance 0.6–0.8 mm in length were revealed without the violation of the integrity of sarcolemma. Muscle fibers of various (round or polygonal) shapes, as well as wavy connective-tissue layers were noted in cross sections. After 24 hours of storage, the muscle fibers took on a straight shape, the cross striation was clearly pronounced in the bulk of the fibers and the length of a sarcomere was 2.0–2.2 μm. In the structure of muscle fibers separate cross cracks were detected, characteristic of the beginning of the maturation stage.

Muscle fibers of a straight or slightly wavy shape were noted in the tissue of the femoral muscles 5 hours after the slaughter. The cross striation was distinct. The longitudinal striation was strengthened in separate fibers. The overcontraction segments were mainly found in the form of narrow cross bands or separate rectangular knots without the violation of the integrity of sarcolemma. In 24 hours of autolysis, the muscle fibers took on a straight shape, the cross striation was restored, the longitudinal striation was not pronounced, the length of a sarcomere was 2.0–2.3 μm. The destructive changes that characterized the stage of maturation of muscle tissue were detected in the form of microcracks or cross cracks.
The microstructure of the samples of muscle tissue of the pectoral and femoral muscles of the carcasses of 3f group 5 hours after the slaughter was characterized by straight muscle fibers lying loosely relative to each other, the cross striation was non-uniform and distinct in the bulk of the fibers, the length of sarcomeres varied within a considerable range – from 1.3 to 2.9 μm. In the structure of the muscle tissue, multiple overcontraction knots with the violation of the integrity of sarcolemma, the ruptures of myofibrils in the adjacent segments of the fibers and the destruction of the myofibrillar substance to a fine-grained protein mass were noted. In cross sections, the muscle fibers had in some places a round shape, which was due to the formation of multiple overcontraction knots and bands. The connective-tissue layers were folded or wavy in shape. After 24 h of autoysis, microcracks or cross cracks characteristic of the maturation stage of muscle tissue were detected in separate muscle fibers.

Thus, the turkey meat from 2f and 3f groups was assigned to the meat with pronounced PSE properties, moreover, the presence of strongly pronounced destructive changes in the form of overcontraction knots and bands was especially characteristic for these groups.

According to the literature data [10], such segments are formed as a result of conformational changes in actin myofilaments in the area of A-disks (zigzag folding). In this case, myosinous myofilaments diverge and become deformed. In the overcontraction segments, muscle fiber proteins such as myosin, actin, C-protein, tropomyosin, troponin, a-actinin, desmin and other proteins aggregate, forming a densified protein complex. The linear dimensions of sarcomeres decrease by 2–3 times, their diameter increases by nearly the same factor.

Overcontraction bands and knots are a negative factor that has a negative effect on the functional and technological properties of meat raw materials [11]. The presence of multiple overcontraction knots in muscle tissue leads to the rupture of sarcolemma, the denatural changes in proteins and a decrease in their functional properties, in particular, the ability to retain water. The formation of contraction knots and bands in muscle tissue was an additional factor in reducing the hydrophilicity of proteins, since some of the free bonds are used to form a densified protein complex with the aggregation of its structures. At the same time, the moisture that is not bound in the overcontraction segments leaves the muscle fibers through the damaged sarcolemma, and the structure of the densified protein complex is not subjected to any significant changes even in the process of prolonged autoysis.

Since such overcontraction knots and bands are not relaxed in the process of autoysis and also under any technological effects (salting, heat treatment, etc.), their presence adversely affects the formation of the technological properties of meat raw materials during maturation, and then the organoleptic characteristics of the finished product [11].

It should be noted that there were microcolonies of predominantly coccal microorganisms between the fibers, in the segments of sarcolemma rupture and in the connective-tissue layers in all the samples of muscle tissue 5–8 hours after the slaughter. The penetration and spread of microorganisms in the muscle tissue was a consequence of a decrease in the permeability of the intestinal walls as a result of the stress of fowl, as well as destructive changes in the muscle tissue as a result of deviation from the normal development of autoysis.

In recent years, similar defects in the microstructure of muscle tissue in farm animals and poultry (from a partially visually imperceptible change in the structural elements of a muscle fiber in a separate muscle to the deep lysis of muscle cells in an anatomically integral muscle group, manifested dependently or independently of the rate of autoysis) have been associated with the intensification of reproduction, rearing, fattening of animals, as well as other intravital and post-mortem factors, including those with the progressive diseases of muscles in mammals, birds and human [12–16].

On the one hand, the overcontraction of muscle fibers is characteristic of PSE meat and is explained by a distinct reaction to the formation of lactic acid in the process of rapid glycolysis. Overcontraction knots are the histological evidence of the increased stress sensitivity of turkey, especially of female turkey. Overcontraction can be caused by intravital factors – the violation of keeping and fattening technology (myopathy, the violation of calcium metabolism), a breed or stunning conditions [17, 18, 19]. In the presented study such destructive changes were less characteristic for the femoral muscles than for the pectoral muscles. This means that, first of all, it is necessary for the personnel to pay attention to correct fowl pre-slaughtering and the observation of the conditions of its transportation. The observed deviations in the quality of turkey meat are not the norm for this type of raw materials, but are directly related to non-compliance with requirements for fowl pre-slaughter preparation and transportation.

CONCLUSION

The problem of the quality of meat products is complex, as its quality is formed in a living organism that grows and develops according to the biological programs embedded therein that are self-correcting depending on internal and external factors. The deep destructive changes in the structure of muscle tissue are the result of the effect of a whole complex of consequences that are as a result of the intensifications and technological solutions used in specific production conditions on the living organism. Their presence today results in the loss of the quality of meat and meat products, the loss of its functional, technological and consumer properties, and, ultimately, in a decrease in the economic efficiency of livestock product processing.

As shown by the studies carried out, special attention should be paid to the study of the conditions for the formation of quality of the turkey meat obtained as a result of the slaughter of turkey hens, in particular, when the delivery of the thoracic part of the carcass to further processing is of primary interest.

The characteristics of the structure of muscle tissue are of primary importance for the quality and safety of meat and meat products. In the technological practice of output from the raw materials of unstable quality, all the undesirable changes in the structure of muscle tissue are
compensated by the use of food supplements and ingredients. Reasonable limitations in the use of food supplements in the production of both functional and traditional meat products should be based on the further study and use of mechanisms of formation, including intravital formation, and the stabilization of meat quality.

REFERENCES


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OPTIMIZATION OF PRESCRIPTION COMPOSITION OF JELLY MASSES USING THE SCHEFFE'S SYMPLEX PLAN

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Abstract: Manufacturers are faced with the task of creating new types of functional marmalades enriched with useful ingredients. The studies were carried out at the department of technology of bakery, confectionary, macaroni and grain processing industries of Voronezh State University of Engineering Technology. The formulation of "Jelly-fruit" marmalade was taken as control. A part of apple puree (the substance content is 15%) was replaced with juice from seabuckthorn berries (the substance content is 12%) and concentrated apple juice (the substance content is 80%) in terms of solids. The effect of various ratios of apple puree, concentrated apple juice and juice from sea-buckthorn berries on the process of gelation of jelly masses based on pectin and agar using the experimental-statistical approach has been studied. The values of plastic strength were chosen as the output parameters that characterize the properties of jelly mass. The experiments were carried out using the Sheffe's simplex-centroid plan. The use of the simplex optimization method allowed to determine the optimum values of the mass fraction of the introduced formulation components that provide the preparation of jelly masses with the maximum plastic strength of 31 kPa if based on agar, and 33.23 kPa if based on pectin. The organoleptic and physicochemical indicators of product quality have been determined. The content of antioxidant activity in marmalade has been determined experimentally. The nutritional and energy value of new products and the degree of meeting daily needs have been calculated. The products can be recommended for dietary and diabetic nutrition, as they do not contain sugar and molasses, as well as for all who look after their health.

Keywords: Optimization, the Scheffe's simplex plan, Rosenbaum concentration triangles, jelly masses, agar, pectin, stevioside

INTRODUCTION

The Russian Federation pays much attention to the development of a healthy lifestyle for the population. Malnutrition is one of the main factors worsening the health of the nation. Adult people consume large amounts of food high in vegetable fat and simple carbohydrates, whereas there are not enough vegetables and fruits in the diet, which leads to an increase in overweight, obesity, increasing the risk of developing diabetes and cardiovascular diseases.


Marmalade confectionery is loved by most consumers. Therefore, manufacturers are faced with the task of creating new types of this category of products enriched with useful ingredients.

Sea buckthorn juice containing carbohydrates, fats, proteins, fiber, organic acids, flavonoids, catechins, sterols, coumarins, vitamins A, C, H, PP, β-carotene, the vitamin B complex, minerals – potassium, calcium, phosphorus, silicon, iron, titanium, zinc, etc. [1]; concentrated apple juice and puree were chosen as a fruit filler. Puree contains a lot of mono- and disaccharides, vitamins C, E, PP, B1, B2 and minerals. Concentrated juice is rich in amino acids and monosaccharides, which decompose under the influence of a high temperature and low pH into components with the formation of 5-hydroxymethylfurfural.

The processing of confectionery masses is followed by the complex chemical, physico-chemical, thermophysical and mechanical processes, the study of which allows for effective and objective rheological control and the control of technological production cycles [2].

Thanks to the works of the Russian scientists P.A. Rebinder, M.P. Volarovich, K.P. Guskov,
of the sample for the analysis is 20 g. A filtrate was prepared for the analysis and five consecutive measurements of the signals of standard quercetin solutions were made. The arithmetic mean was taken as the result. A calibration graph was plotted in the coordinates: X is the signal of quercetin; Y is the concentration of quercetin, mg/dm$^3$. The antioxidant activity (mg/dm$^3$) of the study object was calculated according to the calibration curve of quercetin and calculated using a formula.

The optimal ratio of fruit fillers was chosen according to the Scheffe's simplex-centroid plan.

## RESULTS AND DISCUSSION

The samples of jelly-fruit marmalade were prepared in laboratory conditions. "Jelly-fruit" marmalade produced according to GOST 6442-2014 was chosen as the basis. When preparing marmalade based on pectin, a part of apple puree was replaced with juice from sea-buckthorn berries and concentrated apple juice, and apple puree was completely replaced with juice from sea-buckthorn berries in marmalade based on agar. In addition, sugar and molasses were excluded from the formulation and replaced with stevioside [4].

Stevioside, which is derived from stevia honey grass, is low in calories and does not increase the blood glucose level. The low doses evoke a sense of a sweet taste, the high doses have an unpleasant bitter aftertaste. Stevia contains iron, potassium, phosphorus, calcium, zinc, magnesium, selenium, chromium, cobalt, vitamins E, P, C, D, the vitamin B complex, essential oils and amino acids. The use of stevia helps to reduce blood sugar, strengthen immunity and reduce cholesterol in the body.

Sea buckthorn berries are valuable and healthy berries with a pleasant sweet and sour taste and a unique aroma [1]. These berries improve metabolism, strengthen the protective properties of the body and help to optimize the work of the gastrointestinal tract. Flavonoids that are found in all parts of the plant are anti-cancer antioxidants and can be used for the prevention of cancer.

Apple juice is a means of preventing viral diseases; its use helps in recovery after fatigue, physical exertion, improves the cardiovascular system and normalizes all kinds of metabolic processes in the body. Table 1 shows the chemical composition of fruit and berry supplements.

To develop a new kind of marmalade, apple puree with a mass fraction of solids of 15%, juice from sea-buckthorn berries with a mass fraction of solids of 12%, concentrated apple juice with a mass fraction of solids of 80% were chosen.

One of the basic physicochemical processes in the production of marmalade products is a gelation process.

When stevioside is applied instead of sugar, there is a slight decrease in plastic strength of 4 kPa (Fig. 1 and 2).
Table 1. Chemical composition and energy value of enrichers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Apple puree</th>
<th>Juice from seabuckthorn berries</th>
<th>Concentrated apple juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>0.6</td>
<td>1.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Fat, g</td>
<td>0.2</td>
<td>5.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>19.0</td>
<td>5.2</td>
<td>11.54</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>1.1</td>
<td>1.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Organic acids, g</td>
<td>–</td>
<td>1.8</td>
<td>–</td>
</tr>
<tr>
<td>Minerals, mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>12.0</td>
<td>20.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Potassium</td>
<td>124.0</td>
<td>180.0</td>
<td>126.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.0</td>
<td>3.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7.0</td>
<td>28.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>17.0</td>
<td>8.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Iron</td>
<td>1.3</td>
<td>1.2</td>
<td>0.26</td>
</tr>
<tr>
<td>Manganese</td>
<td>–</td>
<td>–</td>
<td>0.063</td>
</tr>
<tr>
<td>Selenium</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
</tr>
<tr>
<td>Zinc</td>
<td>–</td>
<td>–</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamins, mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, μg</td>
<td>–</td>
<td>240</td>
<td>–</td>
</tr>
<tr>
<td>B1</td>
<td>0.01</td>
<td>0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>B2</td>
<td>0.02</td>
<td>0.04</td>
<td>0.015</td>
</tr>
<tr>
<td>B3</td>
<td>–</td>
<td>0.10</td>
<td>0.063</td>
</tr>
<tr>
<td>B4</td>
<td>–</td>
<td>0.9</td>
<td>0.033</td>
</tr>
<tr>
<td>B5, μg</td>
<td>–</td>
<td>9.0</td>
<td>–</td>
</tr>
<tr>
<td>C</td>
<td>1.6</td>
<td>190.0</td>
<td>25.0</td>
</tr>
<tr>
<td>PP</td>
<td>0.5</td>
<td>0.4</td>
<td>0.038</td>
</tr>
<tr>
<td>F</td>
<td>0.2</td>
<td>4.0</td>
<td>–</td>
</tr>
<tr>
<td>H, μg</td>
<td>–</td>
<td>3.0</td>
<td>–</td>
</tr>
<tr>
<td>Energy value, kcal (kJ)</td>
<td>82 (342.76)</td>
<td>52 (217.36)</td>
<td>47 (196.46)</td>
</tr>
</tbody>
</table>

Fig. 1. Change in the proving of the plastic strength of jelly mass of the composition agar and: (1) sugar + molasses + apple puree; (2) stevioside + apple puree.

Fig. 2. Change in the proving of the plastic strength of jelly mass of the composition of pectin and: (1) sugar + molasses + apple puree; (2) stevioside + apple puree.

Fig. 3. Change in the proving of the plastic strength of jelly mass of the composition agar and: (1) molasses + sugar + apple puree (control); (2) stevioside + apple puree; (3) stevioside + juice from sea buckthorn berries.

Fig. 4. Change in the proving of the plastic strength of jelly mass of the composition pectin and: (1) molasses + sugar + apple puree (control); (2) stevioside + apple puree; (3) stevioside + apple puree + juice from sea buckthorn berries + concentrated apple juice.
When sea buckthorn or concentrated apple juice is added to jelly mass, there is a decrease in plastic strength of 8.5 kPa. But this does not affect the form-retaining ability (Fig. 3 and 4).

The effective viscosity of jelly mass based on pectin and agar decreases with an increase in the shear rate by 20% and 30%, respectively. The samples have a lower effective viscosity compared with the control (Fig. 5 and 6).

The organoleptic and physicochemical indicators of product quality have been determined: the samples of marmalade have a pleasant taste and smell, the original color, a gelatinous consistency, the content of solids is: if based on agar – 78%, if based on pectin – 80%.

The effect of various ratios of apple puree, concentrated apple juice and juice from sea buckthorn berries on the process of gelation of jelly masses based on pectin and agar was studied using the experimental statistical approach [5].

Since in this case the term of independence of factors – the dosages of prescription components – is violated (that is, the content of each component depends on the sum of the rest), the traditional methods of planning (the full factorial experiment, its fractional replicas, orthogonal and rotatable planning) are unsuitable. Therefore, the decision has been made to use simplex-based planning, which allows us to take the following condition into account in the mathematical model

\[ z_1 + z_2 + z_3 = 1, \]  

where \( z_1, z_2 \) and \( z_3 \) are, respectively, the mass fraction of apple puree, juice from sea buckthorn berries and concentrated apple juice, %.

If the number of factors \( q = 3 \), then the planning is carried out using the right simplex – an equilateral triangle. Each point of the triangle corresponds to a certain formulation of the triple system, and, conversely, each formulation is represented by a certain point.

The values of plastic strength (kPa) of the masses based on pectin \( y_1 \) and agar \( y_2 \) were taken as the output parameters that characterize the properties of jelly mass.

The experiments were carried out according to the Sheffe's simplex-centroid plan (Table 2). The order of the experiments is randomized through the table of random numbers. The Sheffe's plan provides for experiments both with pure compounding components and experiments at the center of the entire factor simplex and at the centers of all other lower simplexes that form the general simplex [6].
Table 2. Simplex-centroid planning conditions

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Mass fraction, %</th>
<th>Plastic strength of jelly mass, kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>of apple puree $z_1$</td>
<td>concentrate apple juice $z_3$</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>0.333</td>
<td>0.333</td>
</tr>
</tbody>
</table>

Having processed the experimental data, we obtained mathematical dependences of the plastic strength of jelly mass on the mass fraction of the studied compounding components in the form of incomplete third-order polynomials:

$$y_1 = 28.6z_1 + 33.07z_2 + 29.34z_3 - 1.06z_1z_2 - 3.52z_1z_3 - 3.54z_2z_3 + 95.49z_1z_2z_3,$$

$$y_2 = 27.78z_1 + 31.02z_2 + 28.22z_3 + 0.76z_1z_2 - 2.44z_1z_3 - 2.02z_2z_3 + 39.06z_1z_2z_3,$$

Since the Sheffe's simplex centroid plan is full (the number of experiments equals the number of the regression coefficients to be determined), then there is no required number of degrees of freedom to verify the adequacy of the obtained polynomial. In this regard, to check the adequacy, the additional experiments were carried out in 6 control points, the coordinates of which correspond to the combinations of values of the mass fraction of compounding components 0; 0.333 and 0.666. The choice of these conditions for checking the adequacy is explained (if necessary) by the possibility of transition to higher-order planning and the construction of complete polynomials of the third degree [7].

The results of the control experiments confirmed (according to Student's criterion) the adequacy of the equations (2) and (3) to the experimental data.

The graphic interpretation of incomplete third-order polynomials (2) (3) is shown in Fig. 7 and 8 in the form of Rosenbaum concentration triangles.

For a 3-component mixture, each diagram is a factor space in the form of an equilateral triangle. The content of each separate component $z_i$ at the corresponding vertex of the triangle is 1 (or 100%) [8].

Along the side of the triangle $z_1$, $z_2$, from the vertex $z_2$ (in percent of the length of the triangle side taken as 100%), the relative content of the component $z_i$ in the 2-component mixture $z_1$, $z_2$ is plotted. The points inside the triangle correspond to the 3-component mixture $z_1$, $z_2$, $z_3$. The percentage of the component $z_i$ that corresponds to an internal point $A$ (Fig. 7) is determined by the length of the side of an equilateral triangle, similar to the original triangle $z_1$, $z_2$, $z_3$. It is constructed in such a way that the point $A$ is one of the vertices, and one of the sides is the segment of the side $z_1$, $z_2$. The values of the plastic strength (kPa) of the jelly mass prepared based on pectin $y_1$ and $y_2$ agar are shown by contour isolines in each of the diagrams.
The required diagrams allow, on the one hand, to predict the values of the plastic strength of jelly masses for the given values of the mass fraction of apple puree \( z_1 \), juice from sea buckthorn berries \( z_2 \) and concentrated apple juice \( z_3 \). On the other hand, it is possible to select the values of the mass fraction of the applied compounding components that make it possible to prepare jelly masses based on pectin or agar with the given plastic strength.

Using the simplex method of optimization made it possible to determine the optimum values of the mass fraction of the applied compounding components that provide the preparation of jelly masses with the maximum plastic strength (Table 3) [2, 3, 9].

With the found optimal ratios of apple puree, juice from sea buckthorn berries and concentrated apple juice, jelly masses have the best jelly-forming ability, do not lose their functional properties and contain the sufficient number of useful substances (fiber, macro- and microelements and vitamin B and C complexes) [10].

A formulation (Table 4) and a method for producing the fruit jelly marmalade "Solnechnoe utro" and "Uslada" have been developed.

Table 3. Optimization results

<table>
<thead>
<tr>
<th>Type of jelly mass based on</th>
<th>Plastic strength, kPa</th>
<th>Mass fraction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>apple puree ( z_1 )</td>
<td>juice from seabuckthorn berries ( z_2 )</td>
</tr>
<tr>
<td>pectin</td>
<td>33.23</td>
<td>0.28</td>
</tr>
<tr>
<td>agar</td>
<td>31.0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4. Formulation of marmalade

<table>
<thead>
<tr>
<th>Name of raw materials</th>
<th>Marmelade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;Uslada&quot;</td>
</tr>
<tr>
<td></td>
<td>&quot;Solnechnoe utro&quot;</td>
</tr>
<tr>
<td>Agar</td>
<td>–</td>
</tr>
<tr>
<td>Pectin</td>
<td>+</td>
</tr>
<tr>
<td>Stevioside</td>
<td>+</td>
</tr>
<tr>
<td>Apple puree</td>
<td>+</td>
</tr>
<tr>
<td>Juice from seabuckthorn berries</td>
<td>+</td>
</tr>
<tr>
<td>Concentrated apple juice</td>
<td>–</td>
</tr>
</tbody>
</table>

Figure 9 shows that the highest value of antioxidant activity, compared to the control, in "Uslada" marmalade is 0.35 g quercetin/100 g of product, which is due to a high content of vitamins in the source raw materials.

The content of antioxidant activity was determined experimentally in the marmalade "Uslada" based on pectin and "Solnechnoe utro" based on agar (Fig. 9).

The nutritional and energy value of new products has been calculated (Tables 4 and 5), the diagram of their comparative estimation has been presented (Fig. 10).

The energy value of "Solnechnoe utro" marmalade is lower than that in the control by 211 kcal (881.98 kJ), and "Uslada" – by 226 kcal (944.68 kJ).

The degree of meeting the daily needs when consuming 100 g of the marmalade "Solnechnoe utro" and "Uslada" increases in comparison with the content control: protein – by 23.64 and 6.5 times; fiber – by 1.34 and 1.26 times; sodium – by 4.03 and 6.72 times; potassium – by 16.06 and 3.41 times; magnesium – by 11.27 and 16.64 times; phosphorus – by 9.74 and 8.70 times; iron – by 4.23 and 4.17 times; A vitamins – by 54.92 and 42.34 times; C – by 17.1 and 25.9 times; E – by 1.23 and 6.13 times; \( B_1 \) – by 31 and 29 times; \( B_2 \) – by 32 and 14.5 times; \( B_9 \) – by 1.35 and 1.56 times, respectively.

The obtained products have been tasted (Table 6). Table 6 shows that the best example is "Uslada" marmalade. It has the highest total score – 8.5, which indicates the highest organoleptic properties.

Fig. 9. Change in antioxidant activity in the marmalade samples: (1) agar-based marmalade (control); (2) agar-based marmalade "Solnechnoe utro"; (3) pectin-based marmalade (control); (4) pectin-based marmalade "Uslada".

Fig. 10. Diagram of comparison of the energy value of marmalade: (1) "Jelly-fruit" based on agar (control); (2) "Solnechnoe utro" based on agar; (3) "Jelly-fruit" based on pectin (control); (4) "Uslada" based on pectin.
Table 4. Nutritional and energy value of marmalade

<table>
<thead>
<tr>
<th>Name of nutrients</th>
<th>Content of nutrients in marmalade, g (mg) / 100 g</th>
<th>Jelly-fruit agar-based (control)</th>
<th>Jelly-fruit agar-based &quot;Solnechnoe utro&quot;</th>
<th>Jelly-fruit pectin-based (control)</th>
<th>Jelly-fruit pectin-based &quot;Uslada&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>0.17</td>
<td>4.02</td>
<td>0.63</td>
<td>4.07</td>
<td></td>
</tr>
<tr>
<td>Fat, g</td>
<td>0.06</td>
<td>0.74</td>
<td>–</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>82.4</td>
<td>71.12</td>
<td>72.75</td>
<td>69.93</td>
<td></td>
</tr>
<tr>
<td>Organic acids, g</td>
<td>1.17</td>
<td>0.21</td>
<td>0.79</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>Fiber, g</td>
<td>7.36</td>
<td>9.65</td>
<td>13.25</td>
<td>16.70</td>
<td></td>
</tr>
</tbody>
</table>

Minerals, mg

| Sodium            | 20.1                                          | 80.99                         | 12.51                                    | 84.06                             |
| Calcium           | 30.88                                         | 27.23                         | 16.0                                     | 26.99                             |
| Potassium         | 23.5                                          | 377.46                        | 116.14                                   | 396.66                            |
| Magnesium         | 4.6                                           | 51.88                         | 3.45                                     | 57.42                             |
| Phosphorus        | 10.80                                         | 105.17                        | 11.38                                    | 99.05                             |
| Iron              | 0.82                                          | 3.47                          | 1.01                                     | 4.22                              |

Vitamins, mg

| PP                | 0.013                                         | 0.13                          | 0.44                                     | 0.06                              |
| A, μg             | –                                             | 54.92                         | –                                        | 42.34                             |
| B₁               | 0.0011                                         | 0.31                          | 0.01                                     | 0.29                              |
| B₂               | 0.0011                                         | 0.32                          | 0.02                                     | 0.29                              |
| B₃               | –                                             | 0.59                          | –                                        | 0.66                              |
| B₄               | –                                             | 0.62                          | –                                        | 0.66                              |
| B₅               | 1.35                                          | –                             | –                                        | 1.56                              |
| F                | –                                             | 1.23                          | 0.22                                     | 1.35                              |
| C                | 0.23                                          | 39.50                         | 1.72                                     | 44.55                             |

Energy value, kcal (kJ)

<table>
<thead>
<tr>
<th>Jelly-fruit agar-based (control)</th>
<th>Jelly-fruit agar-based &quot;Solnechnoe utro&quot;</th>
<th>Jelly-fruit pectin-based (control)</th>
<th>Jelly-fruit pectin-based &quot;Uslada&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>313 (1308)</td>
<td>102 (426.36)</td>
<td>355 (1484)</td>
<td>129 (539.22)</td>
</tr>
</tbody>
</table>

Table 5. Degree of meeting daily needs

<table>
<thead>
<tr>
<th>Name of nutrients</th>
<th>Degree of meeting daily needs, %</th>
<th>Jelly-fruit agar-based (control)</th>
<th>Jelly-fruit agar-based &quot;Solnechnoe utro&quot;</th>
<th>Jelly-fruit pectin-based (control)</th>
<th>Jelly-fruit pectin-based &quot;Uslada&quot;</th>
<th>Norm, g (for minerals and vitamins, mg) in accordance with TR TS 022/2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g</td>
<td>0.02</td>
<td>0.7</td>
<td>4.47</td>
<td>4.52</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>Fat, g</td>
<td>0.08</td>
<td>–</td>
<td>0.80</td>
<td>0.50</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>18.31</td>
<td>16.7</td>
<td>16.54</td>
<td>15.54</td>
<td>530</td>
<td></td>
</tr>
<tr>
<td>Organic acids, g</td>
<td>59.0</td>
<td>39.5</td>
<td>11.00</td>
<td>14.00</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fiber, g</td>
<td>36.8</td>
<td>48.25</td>
<td>66.25</td>
<td>83.5</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

Minerals, mg

| Sodium            | 1.55                             | 0.96                          | 6.23                                     | 6.47                              | 1300                              |                                                            |
| Calcium           | 3.1                              | 1.6                           | 2.7                                      | 2.7                               | 1000                              |                                                            |
| Potassium         | 0.94                             | 4.67                          | 15.10                                    | 15.87                             | 3500                              |                                                            |
| Magnesium         | 1.16                             | 0.86                          | 14.36                                    | 14.36                             | 400                               |                                                            |
| Phosphorus        | 1.35                             | 10.1                          | 26.29                                    | 24.77                             | 800                               |                                                            |
| Iron              | 8.30                             | 1.42                          | 34.70                                    | 42.2                              | 14                                |                                                            |

Vitamins, mg

| A, R.E., μg       | –                                | –                             | 16.14                                    | 13.45                             | 800                               |                                                            |
| C                 | 0.27                             | 2.22                          | 43.88                                    | 49.44                             | 60                                |                                                            |
| B₁               | 0.08                             | 0.69                          | 20.66                                    | 13.33                             | 1.4                               |                                                            |
| B₂               | 0.07                             | 0.68                          | 17.78                                    | 16.11                             | 1.6                               |                                                            |
| B₃               | –                                | –                             | 7.38                                     | 8.25                              | 6                                 |                                                            |
| B₄               | –                                | –                             | 32.63                                    | 34.74                             | 2                                 |                                                            |
| B₅               | –                                | –                             | 67.5                                     | 78.00                             | 200                               |                                                            |
| F                | –                                | 1.38                          | 72.35                                    | 79.41                             | 10                                |                                                            |
| PP (niacin)      | 0.06                             | 2.44                          | 0.65                                     | 0.30                              | 18                                |                                                            |
Table 6. Results of a scoring of the organoleptic indicators of jelly-fruit marmalade

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Significance factor</th>
<th>Number of degrees of quality</th>
<th>Number of tasting participants</th>
<th>Estimate, score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste, flavor</td>
<td>4</td>
<td>3</td>
<td>20</td>
<td>68</td>
</tr>
<tr>
<td>Structure and consistency</td>
<td>3</td>
<td>3</td>
<td>20</td>
<td>53</td>
</tr>
<tr>
<td>Color</td>
<td>2</td>
<td>3</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Form</td>
<td>1</td>
<td>3</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Overall estimate</td>
<td></td>
<td></td>
<td></td>
<td>172</td>
</tr>
<tr>
<td>Total score</td>
<td></td>
<td></td>
<td></td>
<td>8.5</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND RECOMMENDATIONS

Thus, based on the studies carried out, the technology of marmalade has been developed with new types of vegetable raw materials and the optimal ratio of fruit and berry fillers has been chosen: apple puree, concentrated apple juice and juice from sea buckthorn berries.

The experimental and calculated data showed that marmalade has an increased nutritional value. Its use will enrich the diet with natural biologically active ingredients. The developed products can be recommended for dietary and diabetic nutrition, since they contain no sugar and molasses, as well as for all who look after their health.

REFERENCES


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DEVELOPMENT OF A TECHNICAL AND TECHNOLOGICAL SOLUTION FOR THE PRODUCTION OF CARROT NECTAR

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Abstract: The modern food market forms a need for two trends for the development, production and sale of products: taking into account the demand of consumers and taking into account lack of irreplaceable (functional) micronutrients in the diet of different population groups. It is advisable to develop technical and technological solutions for innovative ideas at a higher institution, which are tested in business incubators (small innovative enterprises) with the interaction of scientists and production workers. The study objects included: the carrots of different varieties of the crop of 2015–2017 grown in the climatic conditions of the Kemerovo region; the syrup based on Lesovichok plant raw materials; pectolytic, amylolytic and cytolytic enzymatic preparations. Modern research methods have been used in the study. The antioxidant activity was determined using the coulometric method; the content of dry soluble substances – using the refractometric method; the size of carrot particles in the semi-finished products and the finished product – by means of microscopy using a CT-2200 electron microscope; the strength characteristics of raw materials – using Structurometer St-1. As a result of the studies, a product with a high antioxidant activity – carrot nectar developed using hybrid technologies – has been obtained. On the basis of the results of the study of the structural and mechanical properties of carrots, two-stage grinding has been proposed with the subsequent biotechnological processing of raw materials (semi-finished products), which provides specified consumer properties. The high antioxidant activity of the developed nectar has been experimentally confirmed.

Keywords: Carrot nectar, nutritional value, antioxidant activity, hybrid technologies, innovative project

INTRODUCTION

One of the factors contributing to the development of innovative food technologies is the theory of specialized and functional nutrition. This direction is developed by trial and error, but the level of innovative technologies achieved in this area allows us to conclude that it is unconditionally promising. This trend tends to be broadly differentiated; from the idea of balanced mass nutrition to nutrigenomics [1]. The personal approach to nutrition is also conditioned by the physiological and psychological features of food perception and different sensory sensitivity to the same food products.

Summarizing all the trends for the development of specialized and functional food products, two main groups can be distinguished:

– the technology of mechanistic formation of a set of macro- and micronutrients necessary for human life and its addition to a food product [2, 3]. A classic example of this trend is programs for fortifying food with deficient macro- and micronutrients, while food fortification is determined by Codex Alimentarius [4] as “... adding one or more necessary nutrients to the foods that contain or do not contain these elements in their natural state, aiming to prevent or correct the demonstrated deficiency of one or more necessary nutrients in the population as a whole or in certain population groups...”;

– the technologies based on studying and taking into account the preferences of consumers in the development of food products. These technologies are developed in close cooperation with sociologists, psychologists, marketers and other specialists who actively study such an area as consumption psychology in general and nutrition psychology, in particular [5, 6, 7].

At present, the first trend has a sufficiently developed theoretical, normative-methodological and socio-psychological basis. The disadvantage of this trend is the mainstreaming of general ideas of nutritional science, without taking into account the interests and preferences of the population.

The second trend is only formed as the basic ideas and trends of its development. Its main task is to develop a technology for designing a food product based on the...
individual or group preferences of consumers. Herewith, in order to develop a competitive product, special attention should be paid to the so-called "trends" – the most popular, fashionable stereotypes of eating behavior. One of the most popular world trends in nutrition was a "healthy diet", the principles of which include the emphasis on increasing the consumption of vegetables rich in fiber, focusing on local raw materials, reducing the proportion of simple carbohydrates in the diet, etc. Considerable attention is paid to the development of foods with high antioxidant activity [8, 9]. In this trend, the task of forming water-disperse stable systems is urgent, in which all the substances necessary for easy uptake contained in native structures have already been revealed. To this end, the development of modern science-intensive technologies for food production is necessary.

Considering the prospects for the production of functional food products, it is advisable to carry out studies in the conditions of business incubators and small innovative enterprises created on the basis of higher institutions. Business incubators are one of the organizational forms for the development of an innovative activity based on the formation of favorable conditions for the development, testing and implementation of innovative ideas. The development of higher institution business incubators, on the one hand, makes it possible to effectively use the intellectual capital and privileges for scientific institutions, on the other hand, it allows to attract young scientists, students and graduate students to innovative activities. The main objective of a business incubator is to help innovators in the most risky period of the formation and development of business; this means when an innovation came out of a scientific laboratory, but an economic study has not yet been obtained for its commercialization.

Open and closed business incubators are designed to create effective small innovative enterprises that develop and test innovative projects taking into account the resources of local industry, intellectual property, etc.

In the regional context, the projects for processing local raw materials that create food products are urgent for the food industry, taking into account the stereotypes of eating behavior based on the production and processing of agricultural raw materials to meet the demand of different groups of the region's population, create new jobs, etc.

The study aims at developing a technical and technological solution for the production of nectar with a high nutritional value from carrots based on the formation of its conceptual image. The study topic corresponds to the priority areas of science, technics and technology and the critical technology of the Russian Federation "Technologies for reducing losses from socially significant diseases".

Carrot nectar with a high nutritional value was developed within the framework of the START grant (H.5 area) of the Fund for the Promotion of the Development of Small Forms of Enterprises in the Scientific and Technical Sphere in the conditions of the BATAT small innovative enterprise (Kemerovo).

The expedient of such a scheme for developing and introducing new food products to the market corresponds to the "science – production – market" industry / region generally accepted in the conditions of innovative development. Abroad, an analogous model – Triple Helix (the triple helix model) – "state – science – business" is effective [10, 11, 12].

**STUDY MATERIALS AND METHODS**

The materials of the study at different stages were:
- garden carrot (lat. *Daucus carota subsp. Sativus*) of the varieties Nantes, Toucheon and Forto collected within the period of 2015–2017 on the fields of the Kemerovo Research Institute of Agriculture (*Novostroyka settlement, Kemerovo district*) according to GOST 32284-2013;
- syrup based on medicinal and vegetable raw materials Lesovichok (based on a mixture of extracts from the herbs of yellow melilot (lat. *Melilotus officinalis*), peppermint (lat. *Mentha piperita*), meadowsweet (lat. *Filipendula ulmaria*), wormwood tarragon (lat. *Artemisia dracunculus*) and Siberian larch needles (lat. *Lärix sibirica*) according to STO 10912245-2-2009;
- enzyme preparations – alpha amylase ES 3.2.1.1, endo - 1.3 (4) – beta-glucanase ES 3.2.1.6, fructozym (in the modifications of Master, Color and P6L) – produced by Erbslohe Geissenheim AG, Germany.

**Study methods.** The antioxidant activity was determined using the coulometric method of titration according to Fisher with an Expert-006 – antioxidants precision coulometer developed and serially produced by NPK OOO Ekoniks-Ekspert, Moscow, No. 23192-02 in the State Register of Measuring Instruments of the Russian Federation. The studies were carried out according to the methods for determining antioxidant activity (MVI 01-44538054-07). The methods are used to quantify the total antioxidant activity in terms of a standard sample of food products. The instrument is calibrated with a standard rutin sample.

The content of dry soluble substances in the raw materials was determined using the standard refractometric method.

The size of carrot particles in the semi-finished products and the finished product was determined by microscopy using an ST-2200 electron microscope.

The strength characteristics of raw materials were determined using Structometer St-1. For the purpose of finding and analyzing the strength characteristics of carrots, the loading diagrams were recalculated by means of compression and cutting in the coordinates $y$ – $\varepsilon$ and $P_{spec}$ – $d$. The strain was calculated using the formula:

$$y = F/S,$$

where $F$ is force, N; $S$ is the surface area of the sample, m$^2$.

The relative movement $\varepsilon$ was calculated using the formula:

$$\varepsilon = (l_2 - l_1)/l_1,$$
where \( l_1 \) is the original length of the element; \( l_2 \) is the length of the element after deformation.

The specific force \( P_{\text{spec}} \) was calculated using the formula:

\[
P_{\text{spec}} = \frac{P}{l},
\]

where \( P \) is force, N; \( l \) is the length of the sample surface under the blade.

The relative movement \( \vartheta \) was calculated using the formula:

\[
\vartheta = \frac{l_2 - l_1}{l_1}.
\]

To estimate the organoleptic properties of the finished nectar samples, a 20-point scale has been developed to provide the estimation of such parameters as color, appearance and consistency, taste and aroma.

The experiments were carried out in 5–10 fold replicates, the results were processed with the help of the Statistica software package.

**RESULTS AND DISCUSSION**

It is known that vegetable juices and nectars are characterized by a high content of a whole complex of essential nutrients including ballast and mineral substances and vitamins. In the conditions of the growing trend of healthy nutrition, consumers' awareness of a need to preserve health, this product is of special importance due to its dietary and prophylactic properties.

Among the biologically active substances contained in vegetable juices, vitamins C, E and provitamin A, which are natural protectors that protect the human body from oxidative damage, are of particular importance. Due to this, on the one hand, the methods for processing raw materials must be effective for the maximal extraction and transfer of biological active substances into the finished product, on the other hand, the production technology should maximize their preservation.

Carrots are a traditional vegetable raw material throughout Russia and are widely used in public catering as an ingredient of dishes and culinary products, however, the assortment of its derivative products (including juices and nectars) in the market is limited. This is due to a number of reasons:

– the features of the anatomical and morphological structure of carrots. The absence of high-tech methods for providing storage at the enterprises results in the fact that the losses during the storage of fresh carrots reach 40%. A way out of this situation can be the processing of carrots in the shortest possible time after harvest, while the content of vitamins and other biologically active substances is maximum;

– the full production cycle of juice from fresh carrots requires a large number of technological operations, which, in comparison with the production of juice products from concentrates, is more costly both in terms of material and time resources, and therefore less attractive to producers;

– juices and nectars from carrots have specific flavor characteristics, which limits their spread in the mass market. This feature can be eliminated by blending carrot juice with the products the taste characteristics of which are more palatable to the consumer, but this can lead to the additional financial and production costs.

The reasons listed above have led to the fact that in the Russian market, the imported carrot juice for baby food is widely offered, as well as the carrot juice of domestic producers in limited quantities, while the sales volumes and assortment cannot meet the growing demand.

While forming the "conceptual image" of the product being developed, the following was taken into account at the design stage:

– the characteristics and features of the choice of raw materials and ingredients with the specified properties that determine the antioxidant properties of the finished products and their storage stability;

– the specificity of the technological regimes and parameters of processing carrots for the purpose of obtaining the sizes of pulp that promote the stabilization of carrot juice consistency during storage;

– the biotechnological ways of processing carrots (using macerating enzyme preparations);

– imparting competitive advantages by improving the consumer properties of the finished product: functional, social, safety, organoleptic, etc.;

– the prospects for including a new product in the preventive programs at a regional and other levels;

– the presence of the novelty of a technical solution for securing intellectual property as a component of an innovative project.

**Study of the structural and mechanical properties of carrots.** The characteristics of carrots that determine the consumer properties of its derivative products are the higher, in comparison with other vegetables, content of solids and a coarse fibrous structure. In this regard, when developing carrot nectar with pulp, special attention was paid to the formation of the optimal consistency of the finished product.

At the first stage, based on the analysis of literature data, the carrots varieties most suitable for nectar production were selected. The comparative characteristics of the composition, weight and size of root crops of eighteen varieties of carrots and their specific features (a keeping quality, juicy pulp, the high content of carotenoids, vitamin C, etc.) made it possible to choose the most appropriate ones: Nantes, Forto and Toucheon. The structural and mechanical properties of the source raw materials were preliminary determined.

When processing rough vegetable raw materials, one of the main tasks is its disintegration. To this end, it is necessary to destroy the native structure to a state when the insoluble dietary fibers are dispersed to sizes from \( 10^{-3} \) to \( 10^{-5} \) m and simultaneously bind solidly with the dispersion medium.

The cubes of freshly harvested carrots with a face size of 10 mm and the carrots after 3 months of storage (the storage conditions are 3 ± 1°C and the relative air humidity of 92 ± 1%) – of the varieties Nantes, Forto, Toucheon – were tested. As a result of experimental studies, the loading curves of carrot samples for compression and cutting have been obtained (Figures 1–4).
The nature of graphical dependencies shows that carrots exhibit the nonlinear strength properties described by the Kelvin law regardless of a variety:

$$\tau_0 = \eta \frac{d\gamma}{dt} - G\tau,$$

where $\tau = \tau_0 = \text{const}$ is the initial stress applied to the body; $\eta$ is the dynamic viscosity coefficient; $\frac{d\gamma}{dt}$ is the angular deformation velocity; $G$ is the elasticity modulus of the second kind; $\gamma$ is the deformation angle.

It has also been revealed that carrots exhibit elastic properties with some nonlinearity in individual areas and correspond to a creep curve according to the Kelvin-Voigt model. Herewith, the deviations from the nonlinearity are insignificant and to simplify the calculations, the experimental data were approximated by some linear dependences of the form $y = ax$.

Table 1 presents the results of the experiments.

The analysis of the experimental dependences of the strength characteristics of carrots presented in the table allows us to state that, with an increase in deformation velocity, the ultimate failure strain values for carrots increase, reaching their maximum at the average grinding rates, and then decrease, which is explained by the adhesive nature of strength, the presence of natural stress concentrators in the surface interaction zones of material structures both at the micro and macro levels. Thus, the results of carrots study using quasistatic loading allow us to justify the choice of equipment, to reveal the reserves of reducing its material consumption, thereby ensuring significant resources saving, which is of no low importance in the organization of production in the conditions of a small enterprise.

**Determining the carrots grinding parameters.**

One of the mandatory technological stages of production of nектars with pulp is the availability of such a technological operation as grinding. The formation of consumer properties of nектars with pulp, especially organoleptic ones, directly depends on a consistency that has a colloidal structure. Such a consistency can be obtained if carrot pulp is ground to a size of 1–10 microns. The available literature data and our own experience show that such a particle size is difficult to obtain using standard grinding methods. The possibility of solving this problem was based on the use of hybrid technologies for processing root crops – combining mechanical grinding and the enzymatic hydrolysis of pulp with the use of pectolytic enzymes.
Fig. 5. Nantes, Forto and Toucheon carrots (a, b, c, respectively) after grinding using a centrifugal juicer at a screen rotation rate of 1800 rpm, a 200-fold increase.
Table 1. Strength characteristics of carrots

<table>
<thead>
<tr>
<th>Variety of carrots</th>
<th>Type of loading</th>
<th>Approximation dependence, ( y = ax )</th>
<th>RMS deviation, ( R^2 )</th>
<th>Limiting strain, KPa</th>
<th>Limiting force, N*m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes Pressure</td>
<td>( y = 955.86x )</td>
<td>0.9128</td>
<td>484.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forto Pressure</td>
<td>( y = 1034.2x )</td>
<td>0.9516</td>
<td>628.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toucheon Pressure</td>
<td>( y = 1505.6x )</td>
<td>0.9757</td>
<td>817.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes Cutting</td>
<td>( y = 0.0199x )</td>
<td>0.8281</td>
<td></td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td>Forto Cutting</td>
<td>( y = 0.0206x )</td>
<td>0.9181</td>
<td></td>
<td></td>
<td>0.027</td>
</tr>
<tr>
<td>After 3 months of storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes Cutting</td>
<td>( y = 0.0244x )</td>
<td>0.8542</td>
<td></td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>Forto Cutting</td>
<td>( y = 0.0355x )</td>
<td>0.8143</td>
<td></td>
<td></td>
<td>0.187</td>
</tr>
<tr>
<td>Toucheon Cutting</td>
<td>( y = 0.0151x )</td>
<td>0.6846</td>
<td></td>
<td></td>
<td>0.068</td>
</tr>
<tr>
<td>Nantes Pressure</td>
<td>( y = 648.9x )</td>
<td>0.9053</td>
<td>425.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toucheon Pressure</td>
<td>( y = 784.2x )</td>
<td>0.8779</td>
<td>481.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Structure of carrot pulp of different varieties after grinding using a centrifugal juicer at a screen rotation rate of 1800 rpm

<table>
<thead>
<tr>
<th>Variety of carrots</th>
<th>Juice yield, %</th>
<th>% of solids in juice</th>
<th>Average particle length, μm</th>
<th>Average particle area, μm^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nantes</td>
<td>55.0</td>
<td>9</td>
<td>586.3 ± 20.6</td>
<td>227397.7 ± 99.3</td>
</tr>
<tr>
<td>Forto</td>
<td>54.5</td>
<td>10</td>
<td>657.6 ± 39.2</td>
<td>163310.5 ± 123.5</td>
</tr>
<tr>
<td>Toucheon</td>
<td>48.0</td>
<td>10</td>
<td>735.0 ± 37.5</td>
<td>203465.0 ± 146.6</td>
</tr>
</tbody>
</table>

The algorithm of the carrot processing technology included the initial stage of grinding root crops using a centrifugal juicer at a screen rotation rate of 1800 rpm followed by the separation of pulp and cell sap. Grinding carrots at the indicated parameters promoted the yield of juice from 48 to 55%, depending on a variety. After grinding, the carrot pulp was microscopized at a two-hundred-fold increase, the results are shown in Fig. 5 and in Table 2.

The data presented in the table show that Nantes carrots yield the highest amount of juice – 55%, and Toucheon carrots – the lowest (48%). At the same time, the content of dry substances in carrot juice of different varieties is practically the same and amounts to 9–10%.

The microscopy of carrot pulp after grinding using a centrifugal juicer showed that it was flakes of various shapes. It has been found that the particles most uniform in size and shape are in Nantes carrots, the least uniform – in Toucheon carrots. Thus, various structural and mechanical properties of the carrots of the studied varieties affect juice yield. Since, in the conditions of production, it is practically impossible to process the carrots of only one variety (mixed varieties are used), it is necessary to choose the parameters of a raw material processing technology that level these differences. A hypothesis has been put forward that complex two-step mechanical grinding with the subsequent biochemical effect allows us to obtain nectar with the required organoleptic characteristics.

A rotary disperser (the rotor speed is 1200 rpm) operating in closed mode was used for the fine grinding of carrot pulp. After dispersion, the carrot pulp was microscopized again. When analyzing the results, a reduction in the size of pulp particles to 3–400 microns was established (Fig. 6).

The analysis of distribution of particle sizes of carrot pulp after dispersion showed that 50% of particles have a size of up to 10 μm, the proportion of particles larger than 150 μm is only 5%. Thus, more than 85% of the particles have a size of less than 100 μm. However, in order to form the colloidal structure of pulp to ensure the stability of nectar consistency during storage, it is necessary to increase the number of particles up to 10 μm in size.

Determination of parameters for carrot pulp maceration. The efficiency of pulp processing using enzyme preparations in order to increase the yield of juice providing the correct ratio of raw materials: enzyme preparation is confirmed in a number of studies [13, 14]. To achieve the specified level of the particle size of carrot pulp that causes the colloidal stability of the finished product and an increase in the yield of juice from the pulp, it was macerated. It is known that when affected by pectolytic enzymes, the parenchyma tissue cells are partially hydrolized, which contributes to a reduction in the particle size.

Fig. 6. Distribution of particle sizes of carrot pulp after dispersion.
The use of enzyme preparations is a promising research trend in the development of technologies of juice products. The choice of an enzyme preparation in each specific case is determined by the chemical composition of raw materials, their structural and mechanical properties, as well as the desired properties of the finished product. Processing is carried out using both mono preparations and a complex of enzymes that encompass the action of amylolytic, proteolytic, hemicellulolytic, and other enzymes. In this case, insoluble high-molecular carbohydrate and protein substances, as well as the substances related to the structural elements of cells, become soluble. The paper studied the effect of five names of enzyme preparations on the structural elements of cells, becoming soluble. The paper presents the results of experimental studies. The visual estimation of the mass of the fermented pulp allowed us to give a positive estimate, as the appearance became a more homogeneous pureed mass. Based on the complex of organoleptic and instrumental methods for estimation (the yield of solids), the rational concentration of the enzymatic preparation was determined during maceration: 0.005–0.01% to the mass of pulp. The processing was carried out without impairing the organoleptic characteristics of the semi-finished product, a resultant was an increase in the yield of juice. It has been shown that the concentrations of the enzymatic preparation of more than 0.01% make it possible to obtain a pureed pulp consistency, but this is followed by a change in the color of pulp from bright orange to dark orange, the appearance of a bitter aftertaste and unpleasant odor that impair the organoleptic characteristics. This is probably due to the deep hydrolysis of sugars and the formation of oxymethylfurfural.

The established concentration of the enzyme preparation was used during the maceration of samples of the semi-finished carrots of different varieties for 3 hours at a temperature of 55°C. The content of solids in juice was determined every 30 minutes. Fig. 7 presents the results of experimental studies.

Table 3. Juice yield from the pulp of Nantes carrots after processing with enzymatic preparations

<table>
<thead>
<tr>
<th>Name of the enzyme preparation</th>
<th>Juice yield, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54 ± 1.2</td>
</tr>
<tr>
<td>Alpha amylase</td>
<td>57 ± 2.0</td>
</tr>
<tr>
<td>Endo – 1.3 (4) – beta gluconase</td>
<td>58 ± 1.4</td>
</tr>
<tr>
<td>Fructozym Master</td>
<td>60 ± 2.1</td>
</tr>
<tr>
<td>Fructozym Color</td>
<td>66 ± 2.1</td>
</tr>
<tr>
<td>Fructozym R6L</td>
<td>61 ± 1.5</td>
</tr>
</tbody>
</table>

Note: * – the detailed composition of the preparations is a commercial secret of the manufacturer.
Fig. 7. Dynamics of juice yield and solids content during the maceration of pulp of different varieties of carrots using a Fructozym Color enzymatic preparation in an amount of 0.01% of the mass, T = 55°C: (a) Nantes variety carrots, (b) Forto variety carrots, and (c) Toucheon variety carrots.

As the provided data show, the increase in maceration duration for more than 120 minutes does not lead to an increase in the yield of juice and an increase in the content of solids. The maximum juice yield is when Forto carrot pulp is processed using an enzymatic product – 66%, processing the pulp of Nantes and Toucheon carrots contributes to the yield of juice of 60% and 63%, respectively. At the same time, the maximum increase in dry substances was in Nantes carrot juice. On average, the use of a Fructozym Color enzyme preparation increases the yield of juice by 6.6 ± 2%, depending on a carrot variety, the growth of dry substances is 1.5–2.0%.

The use of enzyme preparations in juice production implies their elimination from the finished product by inactivation. To this end, the carrot pulp was heated at a temperature of 90–100°C for 1.5–2.0 minutes and then microscopized using an electron microscope with a two-hundred-fold increase. Fig. 8 and Table 6 present the microscopy results.

The results show that, as a result of enzymatic processing, the particle sizes of carrot pulp decreased on average by 10–15%. This indicates that in the process of fermentation, there is the surface hydrolysis of parenchyma tissue, which does not significantly affect the size of pulp particles. However, the set parameters are: the increase in juice yield and the increase in solids indicate that hydrolysis is successful. In general, the number of pulp particles with the corresponding specified sizes (less than 10 microns) increased to 75–80%, which indicates the stability of pulp in the finished product.

**Table 6. Characteristics of the structure of carrot pulp after maceration**

<table>
<thead>
<tr>
<th>Variety of carrots</th>
<th>Average particle length, μm</th>
<th>Average particle area, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nantes</td>
<td>515.9 ± 18.1</td>
<td>200109.9 ± 87.4</td>
</tr>
<tr>
<td>Forto</td>
<td>578.6 ± 34.4</td>
<td>143713.2 ± 134.5</td>
</tr>
<tr>
<td>Toucheon</td>
<td>646.8 ± 38.2</td>
<td>179049.2 ± 122.9</td>
</tr>
</tbody>
</table>

Determination of antioxidant activity of the finished product. Due to the fact that the functional properties of carrots are largely due to the content of carotenoids and vitamin C (soluble biologically active substances excluding cellulose), the high antioxidant activity of carrot nectar due to the complex and synergistic effect of these biologically active substances was initially predicted.

To determine the retention of biologically active substances and confirm the functional orientation of the nectar being developed, the antioxidant activity of carrots was determined before and after maceration, the results of the studies are presented in Table 7.

**Table 7. Antioxidant activity of carrots before and after maceration**

<table>
<thead>
<tr>
<th>Variety of carrots</th>
<th>Total antioxidant activity, mg of rutin</th>
<th>Total of carotenoids, mg per 100 g</th>
<th>Content of citric acid, mg per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>before maceration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes</td>
<td>161.0 ± 4.4</td>
<td>19.7 ± 2.3</td>
<td>22.35 ± 0.1</td>
</tr>
<tr>
<td>Forto</td>
<td>129.0 ± 5.4</td>
<td>18.3 ± 1.95</td>
<td>21.57 ± 0.1</td>
</tr>
<tr>
<td>Toucheon</td>
<td>126.7 ± 7.8</td>
<td>18.5 ± 4.6</td>
<td>27.43 ± 0.1</td>
</tr>
<tr>
<td>after maceration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nantes</td>
<td>172.6 ± 8.3</td>
<td>19.8 ± 2.4</td>
<td>26.1 ± 0.5</td>
</tr>
<tr>
<td>Forto</td>
<td>144.4 ± 6.85</td>
<td>17.9 ± 1.65</td>
<td>26.1 ± 0.35</td>
</tr>
<tr>
<td>Toucheon</td>
<td>131.7 ± 7.3</td>
<td>18.4 ± 0.41</td>
<td>28.1 ± 0.4</td>
</tr>
</tbody>
</table>
Fig. 8. Fermented carrot pulp, a 200-fold increase: (a) Nantes variety carrots, (b) Forto variety carrots, (c) Toucheon variety carrots.
Based on the data obtained, it has been established that in the process of maceration, there is a tendency for an increase in the antioxidant activity of the semi-finished product. The basis is the process of hydrolysis when dehydroascorbic acid and other biologically active substances are released from tissue cells. The higher antioxidant activity of Nantes carrots has been noted.

According to the Technical Regulations of the Customs Union TR TS 023/2011 "Technical Regulations for Juice Products from Fruits and Vegetables" the composition of nectar should include at least 25% of puree, and the puree may consist of different proportions of NFC juice and pulp.

The model samples of nectars were prepared from Nantes carrots by mixing the prepared puree with 10% invert sugar syrup in a ratio of 1 : 1, while the puree consisted of different proportions of NFC juice and the prepared pulp, but it was 25% in total. The carrot pulp was added in an amount of 5 to 25% of the volume of nectar, at a pitch of 5%.

The results of the estimation of the expert commission (tasters) made it possible to establish that when pulp is added in an amount of 5–10%, the good organoleptic properties of the finished nectar are formed, the increase in the pulp content from 15 to 25% significantly affects the organoleptic characteristics for the worse. The addition of pulp of 15% and above worsens the consistency of nectar and results in the worse. The addition of pulp of 15% and above significantly affects the organoleptic characteristics for the worse. The increase in the pulp content from 15 to 25% results in the decrease of organoleptic properties of the finished nectar.

The demand for juice products from carrots is affected by the specificity of its consumer properties (smell, taste), which necessitates carrot nectar blending. The analysis of the market of juice products in Kemerovo showed that it is mainly carrot juices and nectars that producers blend with apple juice.

In order to provide high consumer properties, it was suggested to use Lesovichok vegetable syrup manufactured at a small enterprise in Novokuznetsk, the Kemerovo region, as an ingredient. The syrup is a mixture of extracts from plant raw materials (yellow melilot herbs, peppermint, Siberian larch, meadowsweet and wormwood tarragon) and sugar syrup with a solids content of 50.5%. The tests carried out earlier in the accredited laboratory of the Rospotrebnadzor territorial directorate in the Kemerovo region confirmed a high content of vitamins and micronutrients, in particular, selenium in the syrup. It was also assumed that the addition of syrup would reduce the sugar laying rate and would have a positive effect on the stability of consistency of the finished nectar.

The choice of the rational amount of the syrup added was carried out on the basis of studying its effect on the organoleptic indicators of the finished nectar. To this end, the syrup was introduced in amounts of 1 to 20% by volume at a pitch of 2.5%, a 20-point scale was used to estimate the consumer properties of samples. The results showed that the syrup content of 5–15% gives the nectar a more pronounced harmonious and mild, nevertheless, recognizable and corresponding to the product name "Carrot nectar" taste. The increase in the proportion of the added syrup to 25% changes the organoleptic properties of nectar towards the predominance of taste qualities of the syrup, there is a change in color along with a change in taste. Given that Lesovichok syrup has a high content of extractives and a specific taste, a syrup sample with a concentration of Lesovichok syrup 15% by volume gained the maximum score – 17.9.

The antioxidant activity (AOA) of the developed product, confirming the functional purpose of nectar before and after heat treatment, was determined. The results are presented in Table 8.

On the one hand, the negative effect of nectar sterilization on antioxidant activity has been established, on the other hand, the sterilization of the product allows to provide microbiological purity and an increase in its shelf life (6 months).

The comparative analysis of the antioxidant activity of the analogues products of different manufacturers sold in the market: Tedi nectar, Gerber apple-carrot juice and the developed carrot nectar showed the advantage of the latter.

Thus, the use of hybrid technologies (the mechanical effect and biotechnological methods for processing raw materials) allowed us to effectively use carrots – vegetable raw materials with complex structural and mechanical properties – to obtain juice products with high consumer properties and functional orientation. The order of methods for affecting carrot pulp using the example of Nantes variety and the size limits of pulp particles ensuring the stability of nectar consistency have been established: the primary mechanical processing of pulp – coarse grinding and dispersion; biotechnological processing – maceration. The novelty of the technical solution is confirmed by Patent No. RU 2493747 "Carrot nectar production method".

Table 8. Antioxidant activity of ready-made carrot nectar

<table>
<thead>
<tr>
<th>Name of the product</th>
<th>Total antioxidant activity, mg of rutin</th>
<th>Total of carotenoids, mg per 100 g</th>
<th>Content of an antioxidant complex, mg per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before sterilization</td>
<td>after sterilization</td>
<td></td>
</tr>
<tr>
<td>Carrot nectar with Lesovichok syrup</td>
<td>124.2 ± 6.8</td>
<td>15.4 ± 0.56</td>
<td>24.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrot nectar with Lesovichok syrup</td>
<td>110.1 ± 4.5</td>
<td>12.6 ± 2.1</td>
<td>20.2 ± 0.6</td>
</tr>
<tr>
<td>ready-made nectars sold in the market</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tedi carrot nectar, OOO SMP MARK-IV, Russia</td>
<td>79.8 ± 10.8</td>
<td>11.9 ± 2.8</td>
<td>18.3 ± 0.67</td>
</tr>
<tr>
<td>Gerber apple-carrot juice, Nestle</td>
<td>81.6 ± 7.3</td>
<td>6.3 ± 2.7</td>
<td>17.5 ± 1.3</td>
</tr>
</tbody>
</table>

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It should be borne in mind that the use of multi-level technological methods and methods for processing carrots can lead to the higher costs of the finished products. This makes it advisable to use the search design methods, namely, the functional and physical as well as functional and cost analysis in the development of a technical and technological solution for carrot nectar.

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STUDY OF THE STABILITY OF FOAM AND VISCOELASTIC PROPERTIES OF MARSHMALLOW WITHOUT GELATIN

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Abstract: Marshmallow is a special kind of chewing souffle, prepared on the basis of sugar and gelatin, belongs to popular confectionery products. The study aims at developing a marshmallow technology with the replacement of gelatin (taking into account religious and ethnic restrictions in human nutrition) with various non-starch polysaccharides. Taking into account the data on the synergism of polysaccharides, some pairs of non-starch polysaccharides (xanthan gum, guar gum and locust bean gum) with the total concentration of 1–2%, based on which eight marshmallow samples had been produced, were experimentally selected. The organoleptic quality of these samples was estimated using the subjective estimation of shape retention, elasticity, and an increase in volume. The marshmallow texture indicators were analyzed using a tool-software complex "TA.XT plus Texture Analyzer". The shelf life was estimated by measuring the moisture content and water activity using a water activity analyzer "HygroPalmAw" (Rotronic, Switzerland), which is equipped with a dielectric moisture sensor. The study of the moisture content and water activity of eight selected samples with different concentrations of xanthan gum and vegetable gums allows to refer the corresponding samples to a class of products with a mid moisture content. Based on the study, the organoleptically acceptable and economically viable marshmallow samples were selected, each of which successfully reproduces the main attributes of the well-proven and widely consumed traditional marshmallow.

Keywords: Food hydrocolloids, synergism, organoleptic estimation, moisture, water activity, textural characteristics, surface tension

STUDY OBJECTS AND METHODS

Technology. The study was carried out on the basis of the chair "Food Technology" of Saratov State Agrarian University named after N.I. Vavilov (Saratov, Russia) and the Food industry laboratory of the Research Institute of Food Science and Technology (Mashhad, Iran).

The basis for sample preparation is a typical marshmallow formulation that includes the stages of the swelling and dissolution of gelatin and egg albumin and dissolution followed by boiling sugar, glucose syrup and water, adding inverted sugar to the boiled syrup and shaking the mixture [5]. The product obtained is characterized by the following ratio of its components: gelatin – 2.08%, water – 25.11%, albumin – 0.69%, sugar – 38.91%, the composition of syrups – 33.21%. These values were accepted as basic in the development of alternative marshmallow formulations without gelatin.

The gelling agents were selected taking into account both their individual effect and the synergistic effect exhibited when interacting with another polysaccharide [6]. The concentrations of the studied polysaccharides were taken within the range from 0.2% – the minimum value required for the exhibition of a gelling effect, up to 1% – the value above which the use of a polysaccharide is generally unprofitable.

Objects. The commercial samples of the following materials were used in the studies:

2. Agar, Fujian Quanzhou Quangang Chemical Plant, China;
3. Iota Carrageenan, Sarda Starch Pvd. Ltd. India;
4. Kappa Carrageenan, Sarda Starch Pvd. Ltd. India;
5. Guar Gum, Sarda Bio Polymers Pvt. Ltd., India;
6. Locust bean gum from Ceratonia siliqua seeds, Sigma-Aldrich Co. LLC, USA;
7. Sodium Alginate, DuPont Nutrition & Health, France;
8. HMP, Genu Pectin Factory, Copenhagen, Denmark;
9. LMP (apple and citrus origin), ZPOW Pektowin, Poland;
10. Xanthan Gum, Danisko, Spain;
11. Pea Protein Isolate, Shandong Jindu Talin Foods Co., Ltd., China;
12. Dry Albumen «Albumixo», Eurosab LLC, Russia;
13. Organic Brown Rice Protein, Jiangxi Golden Agriculture Biotech Co., Ltd., China;
14. Whey Protein Concentrate, Belorussia;
15. High Fructose Syrup F55, Shandong Scents Jinyuan Bio-Tech Co., Ltd., China;

Quality evaluation methods. For the preliminary characterization of structural and mechanical properties of the marshmallow samples, the point system of organoleptic estimation was developed reflecting the elasticity of the material, its ability to retain its shape and increase in volume. The following estimates are used in the system: "+" – indicates that the studied quality is exhibited in the best way in the sample and remains unchanged over time; "++" – the sample characterizes itself satisfactorily; "+/-" – the initially satisfactory quality of the sample gets lost with time; "-" – the material shows itself unsatisfactorily.

For the deeper study of the samples obtained by means of technical means, the following types of analysis were carried out: a water activity and moisture test, a texture analysis, the measurement of surface tension and the microphotography of sections.

Water activity is one of the most important physical characteristics that determines the texture properties of the product, as well as the rate of chemical and biological reactions therein [7]. Water activity is usually understood as the ratio of the pressure of water vapor above the studied material to that above pure water at the same temperature. To find this parameter, a "HygroPalmAw" (Rotronic, Switzerland) instrument equipped with a dielectric moisture sensor was used in our studies [8]. The working principle of the sensor is based on a change in the conductivity of the hygroscopic polymer depending on the relative humidity of the chamber [9]. The measurements were carried out in accordance with the generally accepted procedure [10]. Subsequently, for the reliable interpretation of the obtained data, the moisture content of the samples was additionally established using a method for drying to a constant mass in a drying cabinet [11].

Later on, to study the structural properties of control and experimental marshmallow samples, a textural analysis method was used, implemented in the hardware and software complex "TA.XT plus Texture Analyzer" manufactured by "Stable Micro Systems". The method aims at carrying out the overall estimation of the samples from the point of view of their perception by man [12].

During the study, the samples were subjected to the controlled compression forces using a specially selected probe. The resistance of materials was controlled using a calibrated load cell. The loads determined as a result of the experiment are the functions of the properties and modes of testing the samples [13].

The surface tension value in the liquid medium that forms this foam has a higher value for the stability of the foam that is the basis of aerated confectionery products [14]. Thus, this parameter had been determined for the systems before they were shaken. The measurement was carried out using the high-precision surface tension meter K100 manufactured by "Kruss". This device implements a direct measurement method, continuously recording the resistance force tested by a specially selected probe when immersed in the studied medium [15].

RESULTS AND DISCUSSION

Table 1 presents the results of organoleptic estimation of the obtained marshmallow samples according to the described point system. The samples with the best indicators are highlighted in color.
Table 1. Results of the organoleptic estimation of the obtained marshmallow samples with the addition of egg albumin

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Hydrocolloid</th>
<th>Concentration, %</th>
<th>Parameters</th>
<th>Shape safety</th>
<th>Increase in volume</th>
<th>Elasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gelatin (water swelling)</td>
<td>2.0</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>i-carrageenan (water swelling)</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Agar (water swelling)</td>
<td>1.5</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>HMP (water swelling) with citric acid (40%)</td>
<td>1.0 / 1.2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>HMP (dry mixing with sugar) with citric acid (40%)</td>
<td>2.5 / 1.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>LMP (dry mixing with sugar)</td>
<td>1.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sodium Alginate (water swelling)</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Xanthan Gum &amp; Guar Gum (separate water swelling)</td>
<td>1.0 : 1.0</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Xanthan Gum &amp; Guar Gum (combined water swelling)</td>
<td>0.5 : 0.5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.5 : 1.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>1.0 : 0.5</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.8 : 0.7</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.6 : 0.9</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>14</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.4 : 1.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.2 : 1.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.7 : 0.8</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>0.9 : 0.6</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>1.1 : 0.4</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>1.3 : 0.2</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>1.0 : 1.0</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Xanthan Gum &amp; i-carrageenan (water swelling)</td>
<td>1.0 : 0.5</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Xanthan Gum &amp; Carob (water swelling)</td>
<td>0.5 : 0.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Xanthan Gum &amp; Carob (water swelling)</td>
<td>1.0 : 1.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Sodium Alginate &amp; HMP (dry mixing with sugar)</td>
<td>1.0 : 1.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Sodium Alginate &amp; Guar Gum (dry mixing with sugar)</td>
<td>1.5 : 1.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>26</td>
<td>Sodium Alginate &amp; Guar Gum (dry mixing with sugar)</td>
<td>1.0 : 1.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Composition of the studied marshmallow formulations, %

<table>
<thead>
<tr>
<th>Formulation number</th>
<th>Guar gum</th>
<th>Carob bean gum</th>
<th>Xanthan</th>
<th>Water</th>
<th>Whey protein</th>
<th>Egg albumin</th>
<th>Sugar</th>
<th>Invert syrup</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.80</td>
<td>–</td>
<td>0.70</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>2</td>
<td>0.80</td>
<td>–</td>
<td>0.70</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>3</td>
<td>0.70</td>
<td>–</td>
<td>0.80</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>–</td>
<td>0.80</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>5</td>
<td>0.90</td>
<td>–</td>
<td>0.60</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>6</td>
<td>0.90</td>
<td>–</td>
<td>0.60</td>
<td>35.16</td>
<td>0.64</td>
<td>–</td>
<td>35.77</td>
<td>30.53</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>1.00</td>
<td>1.00</td>
<td>35.47</td>
<td>0.60</td>
<td>–</td>
<td>33.41</td>
<td>28.52</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>1.00</td>
<td>1.00</td>
<td>35.47</td>
<td>0.60</td>
<td>–</td>
<td>33.41</td>
<td>28.52</td>
</tr>
</tbody>
</table>

As can be seen from Table 1, those samples have the best texture indicators which are prepared on the basis of agar and low-etherized pectin, the combinations of xanthan gum with guar gum and locust bean gum, and also the combination of sodium alginate and guar gum. The results obtained are in good agreement with the known data on the degree of synergism between hydrocolloids [16, 23]. For further studies, 8 formulations based on xanthan, guar and locust bean gum were selected as the cheapest options, Table 2.

Table 3 presents the data on the water and moisture activity of the samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>a_w</th>
<th>W, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6741 ± 0.0040</td>
<td>20.42 ± 0.0018</td>
</tr>
<tr>
<td>2</td>
<td>0.6813 ± 0.0080</td>
<td>21.14 ± 0.0040</td>
</tr>
<tr>
<td>3</td>
<td>0.6985 ± 0.0102</td>
<td>22.85 ± 0.0087</td>
</tr>
<tr>
<td>4</td>
<td>0.6893 ± 0.0022</td>
<td>21.91 ± 0.0110</td>
</tr>
<tr>
<td>5</td>
<td>0.7218 ± 0.0017</td>
<td>25.16 ± 0.0133</td>
</tr>
<tr>
<td>6</td>
<td>0.7221 ± 0.0067</td>
<td>25.20 ± 0.0026</td>
</tr>
<tr>
<td>7</td>
<td>0.7725 ± 0.0050</td>
<td>29.27 ± 0.0081</td>
</tr>
<tr>
<td>8</td>
<td>0.7773 ± 0.0017</td>
<td>29.73 ± 0.0183</td>
</tr>
</tbody>
</table>
As can be seen from Table 3, all the samples can be referred to a group of food products with intermediate moisture, since the water activity therein is higher than 0.6 and less than 0.9 [17]. At the same time, close correlation between the activity of water and the mass fraction of moisture can be seen from the data (Fig. 1).

The dependence of water activity on the mass fraction of moisture is approximated by a linear equation. It is known that in the aqueous solutions of monosaccharides and disaccharides the highest decrease in water activity is provided by fructose. Glucose and lactulose have a less pronounced effect, and sucrose rounds out the given sequence [18]. It should be noted that to some extent the mass ratio of sucrose and invert sugar also affects the nature of the dependence of water activity on moisture. This is consistent with the known data on the highest efficiency of invert sugar compared with sucrose as a component that reduces water activity [19]. In this case, the combination of invert sugar and sucrose has a synergistic effect and provides a decrease in water activity by about 85%. The reverse ratio of the above components at the same concentration makes it possible to reduce the water activity by about 35%. The application of various volumes of polysaccharides does not practically affect a change in the water activity in the system [20].

The data obtained in the course of the experiment, as well as the rheological curves, Fig. 2a, based on them, describe one compression cycle and, in accordance with Fig. 2b, allow to determine the following characteristics of the product:

1. the work spent on deformation and relaxation, as well as the overall work;
2. sample hardness;
3. the initial and final relaxation rates;
4. Young's modulus;
5. elasticity [21].

The force needed to chew the product is characterized by its rigidity and is equal to the peak value of the curve. The cohesiveness of the product determines the energy of molecular bonds therein, can be estimated from the energy necessary for product deformation and can geometrically be defined as the sum of the areas formed by the curve and the abscissa before and after reaching the rigidity peak [22]. In this case, the former area characterizes the adhesive force relative to the degree of density of the material being studied, and the inverse relation of the areas is elasticity, i.e., the ability of the product to undergo significant elastic deformations without destruction. More precise information about the elasticity of the product is provided using Young's modulus calculated as the ratio of the pressure on the sample to a change in its linear size during the test. Having constructed tangents to the initial and final points of the section that describes the relaxation time, it is possible to determine the angle of their slope and calculate the corresponding rates at which the sample is reduced. Table 4 presents the study results.

It follows from the obtained data that the properties of the samples vary significantly depending on the composition. Thus, Samples 3 and 8 significantly exceed the control in all the parameters, while samples 2 and 4 only slightly exceed the corresponding characteristics. In terms of total of its properties, sample 1 is the closest to the control and is only inferior to it in the final reduction rate. The behavior pattern of Sample 7 differs from that of the above being inferior to the control in a number of properties (work, hardness, the initial relaxation rate, Young's modulus) and in a number of properties – superior to it. Samples 5 and 6 surpass the control in terms of performance and rigidity, are approximately equal to it in the initial reduction and elasticity rate, but significantly surpass the control in terms of the final reduction rate and Young's modulus.
Table 4. Textural characteristics of the studied product

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hardness work done, J</td>
<td>0.11</td>
</tr>
<tr>
<td>Relaxation work done, J</td>
<td>1.95</td>
</tr>
<tr>
<td>Total work done, J</td>
<td>2.06</td>
</tr>
<tr>
<td>Hardness, g</td>
<td>214.68</td>
</tr>
<tr>
<td>Initial resilience rate, g/s</td>
<td>24.47</td>
</tr>
<tr>
<td>Final resilience rate, g/s</td>
<td>3.65</td>
</tr>
<tr>
<td>Young's modulus, Pa</td>
<td>2406.05</td>
</tr>
<tr>
<td>Resilience</td>
<td>18.04</td>
</tr>
</tbody>
</table>

Fig. 3. Surface tension forces in the samples before shaking.

Since it is known that gums used in the present paper refer to neutrally charged polysaccharides, the strong ionic and electrostatic types of interaction between their molecules are excluded [23]. This assumption is confirmed by the low energy of intermolecular bonds, which in turn clearly demonstrates the amount of energy consumed for the compression and relaxation of the sample. It follows therefrom that molecules interact mainly at the level of non-covalent associations with the formation of weak hydrogen bonds. At the same time, the rigidity of the obtained polymer systems reflects the quality of this interaction, when various amounts of these bonds cause changes in the rigidity of separate samples.

The reduction rate, different at the initial and final stages of relaxation, reflects the pattern of the highly elastic deformation of the sample as a result of a change in the shape of flexible polymer molecules that are curved or rolled up [24]. Accordingly, the material with a high relaxation rate has a more ordered structure. The latter indicates the degree of synergy between the components of the polymer system. Indeed, there is good correlation in the experimental results between the parameters of the relaxation rate and rigidity, which, as noted above, indicates the amount of stable bonds between the molecules of xanthan and locust bean gum.

As can be seen from Table 4, another consequence of a more dense and homogeneous texture is the high value of Young's modulus: the polymer networks with a higher concentration of knots are characterized by a higher resistance to compression under elastic deformation. At the same time, the elasticity of the material established as a result of the experiment gives a notion of the distance between the knots of the polymer network, which is approximately the same in all the analyzed samples.

Thus, in terms of total of properties, the samples the rheological curves of which are at the same level or higher than the curve for the control gelatin-based sample should be considered the most advanced from a technological point of view.

Figure 3 shows the curves obtained using a Krüss K100 apparatus for the surface tension forces acting on the probe, when immersed in the experimental system.

It can be seen from Fig. 3 that the surface tension forces decrease rather significantly over time, moreover, curve 1 decreases below such a low value as 10 mN/m within 35 s, and curve 7 becomes so close to that it brings these systems closer to classical gelatin-based ones. The time dependence is usually explained by the adsorption of one or more surface active components on the surface of the platinum plate hampered by the high viscosity of the system. However, the minimum value of \( \sigma \) is unusual.

To interpret these curves, let us consider the components of the mixture. After water, invert syrup based on sucrose (35.77%), glucose and fructose (10.38%) takes the first place. It is known that the 70% syrups of the mentioned sugars have a value of surface tension of 46, 55 and 43 mN/cm, respectively [25]. According to other data [26, 27], the addition of sucrose only increases the surface tension of solutions (the article [26] provides a linear nomogram, and the article [27] shows the effect of inorganic substances). This cannot explain the noted decrease in surface tension, but once again indicates the significant effect of the method for its measuring on the result.
The surface tension of egg protein is estimated as 46.1–50.3 mN/m [28], moreover, the equilibrium value was established after 600 s (in our case, $\sigma$ stops to change after 40 s). It should be noted that compositions 1, 7 and 3, the kinetic curves $\sigma$ of which are below the others, contain whey protein, but not egg protein. Even the small amounts of whey protein sharply reduce $\sigma$ of the aqueous solution [29]. Thus, 1% yields ~46 mN/m. The minimum value of $\sigma$ is ~43 mN/m at 5%, then it begins to grow. The measurement procedure is based on using a tensiometer with a platinum ring manufactured by the same company (Krüss). However, composition 5 is another one that includes whey protein that differs little from composition 1 in the content of guar and xanthan gums and is identical to it in the rest.

The third group of ingredients is gums. The book [30] provides surface tension values for guar gum (55 mN/m) and locust bean gum (50 mN/m) for 0.7%. These values cannot explain a decrease in $\sigma$ to 10 mN/m, in addition, various gums are used in compositions 1 and 7.

In the paper [31], the value $\sigma$ of xanthanum was measured using Wilhelmi method with a platinum plate, depending on time. It decreased with time, which is explained by the diffusion and adsorption of polymer macromolecules on the platinum surface. The addition of low molecular salt (0.1 M NaCl) accelerated the equilibrium and lowered the equilibrium value of $\sigma$. However, this time continued to be measured in hours, and the minimum value of the surface tension did not decrease below 45 mN/m.

Everything taken together makes it possible to propose that the noted decrease in surface tension to 10 mN/m is due to the rather rapid diffusion of a rather low-molecular component to the platinum plate and adsorption thereon. It is presumably a component of whey protein.

Figure 4 shows the photos of the cuts of the samples under study, on the basis of which it is possible to judge the quality of their texture. The following can be distinguished among the significant parameters: the thickness of the sample, the cleanliness of the cut, the size and distribution of gas cavities. Thus, the thickness of the sample indicates the ability of plastic mass for efficient aeration, the presence of various sags, burrs and torn edges on the cut – the adhesive properties of the product, and the size and uniformity of distribution of air bubbles in its volume – the consistency of the texture properties of the product.

In accordance with the above, the samples with the numbers 3 and 4 are characterized by the highest thickness, relatively clean cuts and the uniform distribution of the air bubbles that are approximately equal in size, which indicates quality texture. The samples with the numbers 1 and 2 are also quite large, they are easy to cut and they are evenly saturated with gas. In contrast to the former two couples, samples 5 through 8 exhibit high adhesion properties and, as can be seen from the figure, are less amenable to aeration.

Figure 5 presents a marshmallow preparation flow sheet.

![Sections of experimental marshmallow samples.](image)
The RF patent No. 2626580 and the Iran patent No. 93555 "Protein and carbohydrate confectionery base and the method of its preparation" (developed by Klyukina O.N., Ptichkina N.M., Kodatsky Yu.A., Nepovinnykh N.V., S. Yeganehzad and R. Kadkhodaei) have been received for the developed marshmallow formulations and technology.

CONCLUSIONS

The organoleptic estimation, taking into account the elasticity of the system, its ability to retain its shape and the fact that it beats well, confirmed the presence of the developed synergy in hydrocolloid agar: low-etherified pectin and guar gum: xanthan gum / carob bean gum / sodium alginate couples. The studies of moisture and water activity in eight selected formulations with various ratios of xanthan and vegetable gums make it possible to classify the corresponding samples as a class of products with intermediate moisture. In addition, a close correlation was found between the parameters studied, and a synergistic effect was found between sucrose and invert sugar that leads to a sharp decrease in water activity. On the other hand, there is no noticeable effect of different concentrations of polysaccharides on water activity in the final product.

The obtained profile of the texture analysis gives an idea of the degree of effect of the product composition on its viscoelastic properties that vary widely. As follows from the obtained data, Sample 3 has the most quality and ordered structure, which makes it the most promising alternative to gelatin-based marshmallow.

It is obvious that the graphs for surface tension indicate the prolonged adsorption, the pattern of which is still unclear and needs a further study. At the same time, it was found that the pattern of the gelatin curve...
is completely opposite to the curves that correspond to all other polysaccharides and this is of interest, too. In all other respects, the experimental data indicate that the liquid preparations under study have a surface tension sufficient to provide stable foaming in the final product, which is not inferior to the classical marshmallow formulation from this point of view.

Finally, the photos of the cuts clearly demonstrate the quality of the texture of the produced samples. In fact, the samples with the numbers 3 and 4 are characterized by the best structure: they are large, the air bubbles are distributed evenly inside them. Together with the obtained graphs, these images indicate that the claimed samples are the best in this experiment and can be recommended for production.

ACKNOWLEDGEMENTS

The study was carried out within the framework of the joint research agreement on the topic "Study on foam stability and viscoelastic properties of aerated confectionary products influenced by protein-polsaccharide interaction: gelatin-free marshmallow" between the scientific groups of the Russian Federation and Iran.

The studies have been supported by the grant of the President of the Russian Federation for young Russian scientists MD-2464.2018.8 on the topic "Designing the composition and technologies of the balanced food products aimed at the primary and secondary prevention of cardiovascular diseases and their complications".

REFERENCES


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DEVELOPMENT OF INTEGRATED TECHNOLOGY AND ASSORTMENT OF LONG-LIFE RYE-WHEAT BAKERY PRODUCTS

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Abstract: Insufficiently studied and at the same time promising is the use of an acidophilic starter for making the bakery products designated as military personnel’s food. An acidophilic sourdough starter is a mixture of the species and strains of yeast and lactic acid bacteria grown on the basis of saccharified pregelatinized flour and specially selected in certain proportions. The study aims at obtaining a dehydrated biopreparation of fermented pregelatinized flour for bakery production using the strain \textit{Lactobacillus helveticus H10} (dry fermented pregelatinized flour) and its application in the technologies of bakery products made from rye and a mixture of rye and wheat flour. The composition of the nutrient mixture for dry fermented pregelatinized flour has been optimized in terms of rheological, biotechnological and microbiological indicators. It has been found that fermented pregelatinized flour with a 50\% content of a starter has the least viscous structure, which allows us to dry the sample faster and to preserve lactic acid bacteria. It has been found that the sample prepared using rye wholemeal flour was characterized by a higher content of amine nitrogen (by 15\%), volatile acids (by 29\%) and mass fraction of sugar (by 27\%) compared to the sample of fermented pregelatinized flour made using rye bread flour. It has been revealed that microorganisms are destroyed least during convective drying. The main technological parameters for making dry fermented pregelatinized flour that provide stable biotechnological and microbiological properties have been developed: a drying temperature of 40–45°C for 170–200 min with an air flow rate of 1.5–1.8 m/s above, 0.3–0.5 m/s below, 0.8 m/s on the right and 0.1–0.15 m/s on the left. A complex technology of long-life rye-wheat bakery products has been developed.

Keywords: Strains, starter, complex biotechnology, rheology, thermoradiation drying, convection drying, freeze drying, drying kinetics, bakery products


INTRODUCTION

In one form or another, bread is used in the diet of military personnel of any country in the world. In the Russian Federation, the norms of bread consumption for military personnel approved by the Decree of the Government of the Russian Federation No. 946 of 29.12.2007 are 150–300 g for bread made of a mixture of rye and wheat flour and 150–400 g for bread made of wheat flour. Making rye-wheat bakery products with a high nutritional value and long-life products that can be included in packed meal is up-to-date for the Russian army. At the moment, this kind of bakery products is only army loaves (sea biscuits). The task of the study is dictated by the need to enlarge the assortment of rye-wheat bakery products, to improve their quality and safety and to increase a potential shelf life and microbiological resistance during storage. The solution to this problem is possible due to the use of the biological methods that provide the application of biological systems (starters) for dough making.

Starters are the specially selected, identified, non-pathogenic and nontoxigenic strains of microorganisms and their symbiotic associations that have a set of properties necessary for making bakery products. Sourdough bread is characterized by the improved: structure of porosity and properties of crumb, taste and aroma and ability to preserve freshness for long. Starters form organic acids, antibiotics and other inhibitors of pathogens of microbiological bread spoilage during dough making. The use of biological systems will increase the shelf life of products and improve its quality and safety with a significant decrease in food supplements as preservatives.

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The long-term baking experience has already shown the efficiency of applying technologies for making fermented dough. Starters are obtained by fermenting a nutritious flour mixture (a water-flour mixture and saccharified pregelatinized flour). The composition of such starters includes lactic acid bacteria and their compositions with yeast. The biodiversity of lactic acid bacteria and yeast of sourdough bread microbiota is described in detail in the articles of the scientists from the Belgian University [1, 2], as well as in a number of works by Russian scientists [3–8]. The microflora of starters and dough is represented by the lactic acid bacteria Lactobacillus and the yeast Saccharomyces for semifinished products from wheat flour and Saccharomyces and Candida for semi-finished products from rye flour [9–11].


The authors of [15] showed that the yeast Saccharomyces cerevisiae produces the growth factors that stimulate milk bacteria. The scientists of Nigeria [16] studied the functional properties (acid formation, gas formation, antibiotic production) of the microflora of a starter for corn bread. Lactobacilli and yeast were isolated from the spontaneously fermented corn dough on the basis of dominance during fermentation and their presence at the end of fermentation. The microorganisms were identified as Lactobacillus plantarum, Lb. brevis, Lb. fermentum, Lb. acidophilus, Pediococcus acidilactici, Leuconostoc mesenteroides, Leuconostoc dextranicum and Saccharomyces cerevisiae.

In Italy [17], the possibility of industrial application of lactic acid bacteria (Weissella cibaria strains PON10030 and PON10032, as well as Leuconostoc citreum PON 10079 and PON10080), isolated from local semolina, has been studied for producing sourdough bread. The joint study of scientists from Finland and Italy [18] provides a characteristic of the lactic acid bacteria isolated from wheat sourdough bread. Such lactic acid bacteria as Leuconostoc mesenteroides, Leuconostoc citreum, Lactobacillus brevis, Lactobacillus curvatus, Lactobacillus sakei, Lactobacillus plantarum and Pediococcus pentosaceus were studied, their growth, acid formation, carbohydrate fermentation, anti-fungalic properties, the specificity of exopolysaccharide production, and safety have been described.

The scientists from South Korea [19] have studied the possibility of using Leuconostoc citreum HO12 and Weissella koreensis HO20 isolated from kimchee (fermented vegetables similar to Russian sour cabbage) as a starter for wholemeal wheat bread. The bread made on the basis of this starter demonstrated the ability to suppress the growth of Penicillium roqueforti and Aspergillus niger, as well as the growth of Bacillus subtilis. It has been established that both types of lactic acid bacteria are capable of increasing the storage stability of whole grain bread, have unique fermentation characteristics and allow for producing bread of satisfactory quality.

In Ireland, together with the scientists of Greece [20], the possibility has been studied of using Kluyveromyces marxianus (IFO 288), Lactobacillus delbrueckii ssp. bulgaricus (ATCC 11842) and Lactobacillus helveticus (ATCC 15009) as starters for bread. The highest acid formation (3.41 g of lactic acid per 1 kg of bread) was obtained using 50% of the starter that contained 1% K. marxianus and 4% L. delbrueckii ssp. Bulgaricus. In addition, the use of these crops improved bread flavor, which was shown by sensory evaluation and a GC-MS analysis. The possibility of reducing the microbial damage of gluten-free bread and prolonging its shelf life by using Lactobacillus amylolavorus strain DSM19280 as a starter has been studied [21]. The carried out studies have confirmed this possibility.

Data on the effect of various strains of lactic acid bacteria in the composition of a starter on the physico-chemical and organoleptic indicators of the quality of bread and the shelf life of products are known [22]. In particular, the authors used the strains of Lactobacillus acidophilus and Lactobacillus sakei separately and as part of microbial compositions for making sourdough bread. The results showed that bread made using a starter with the microbial composition of these strains remained well-preserved for more days (12 days) than all the other samples of the products made within the framework of this study because it contained the higher concentrations of lactic acid, and also had a stronger texture, better flavor, taste and total quality compared to the other samples studied.

The analysis of literature data showed that the issue of using an acidoophilic starter for making bakery products designated as military personnel's food is rather insufficiently studied and at the same time promising. The starters made on the basis of acidoophilous bacterium strains are widely used in various branches of the food industry, which is due to its probiotic properties. An acidoophilic sourdough starter is a mixture of the species and strains of yeast and lactic acid bacteria grown on the basis of saccharified pregelatinized flour and specially selected in certain proportions. An acidoophilic starter is resistant to high temperature, which contributes to the better preservation of lactic acid bacteria cells, for example, during drying. The microflora of an acidoophilus starter consists of a mixture of two cultures. These are the strains of the lactic acid bacteria L. acidophilus-146 and the yeast Saccharomyces cerevisiae FR-3 that are in a ratio (by weight) of 2 : 1 [23]. According to the scientific and technical literature, at present, an acidoophilic starter with the strain L. acidophilus-146 is only used in the production of wheat bread.

The existing technologies for making traditional starters are long and labor-intensive for the enterprises.
operating on a single- or double-shift basis and remote production including field works of military personnel. One of the ways to reduce the production cycle for rye-wheat bread is the use of dried (dehydrated) fermented pregelatinized flour for bakery production and low-moisture starters, which allows us to provide quality, microbiological purity and an increase in the nutritional, including biological, value of products.

Thus, it is urgent to develop a technology and a method for obtaining dry fermented pregelatinized flour for bakery production and to develop a technology for its application in making long-life rye-wheat bakery products.

**Aim of the study.** Development of the technology and assortment of long-life rye-wheat bakery products based on the use of a dehydrated biopreparation with fermented pregelatinized flour for bakery production using the strain *Lactobacillus helveticus* H10.

**STUDY OBJECTS AND METHODS**

**Laboratory facilities.** The laboratory studies were carried out on the basis of the Center of Technologies, Biochemical and Microbiological Research of the Federal State Autonomous Scientific Institution "Scientific Research Institute of the Baking Industry"; the study of the rheological properties and the drying of the samples was carried out in the laboratories of the Kemerovo State University.

**Raw materials used in the study.** When carrying out the studies to develop the technology of bakery products, the following raw materials were used:
- rye bread flour (GOST R 52809-2007);
- stone ground whole grain rye flour, 50–70 µm (STO 12396977-003-2014) the residue on sieve No. 067 is 1.6%, passing through polyamide sieve No. 41/43–38%;
- first grade bread wheat flour (GOST R 52189-2003);
- pressed bakery yeast (GOST R 54731-2011);
- extra fine food common salt (GOST R 51574-2000);
- enzymatic preparations Novamyl 3D and Pentopan 500 BG (Novozymes, Denmark);
- sorbic acid (GOST 32779-2014);
- potable water (SanPiN 2.1.4.1074-01).

The indicators of quality of the raw materials used in the studies met the requirements specified in the technical documentation.

**Methods for making dough and bread.** To carry out the studies, the samples were prepared according to the formula developed in the Federal State Autonomous Scientific Institution "Scientific Research Institute of the Bakery Industry" presented in Table 1.

The acidophilic starter was prepared using the strain *Lactobacillus helveticus* H10. To prepare an acidophilic starter the food is added in the previous starter that consists of a mixture of rye bread flour and water in a ratio of 1 : 2.5. The acidophilic starter was left for fermentation until the acidity reached 14–16 deg. The pregelatinized flour was made in a ratio of rye bread flour: water (t = 95–98°C) – 1 : 2.5 and left for a few hours for saccharification until saccharified pregelatinized flour was made. Further on, fermented pregelatinized flour was being made. To this end, an acidophilic starter was added to the saccharified pregelatinized flour and left for a few hours to ferment until the acidity reached 12–14 deg. Then the dough was kneaded of bakery rye bread flour, first-grade wheat flour, food common salt and the whole amount of fermented pregelatinized flour. The bunching time is 8–20 minutes depending on the brand of equipment. The initial temperature of dough is 28–30°C. The total time of dough fermentation is 60 min, while the acidity is 8–10 deg.

The dough pieces were placed in molds and placed in a fermentation cabinet at a temperature of 36–38°C and relative air humidity of 70–85%. The proofing time was 40–70 min.

The bread was baked in a humidified baking chamber in two thermal modes: first at 210–220°C for 10–15 min, then at 160–170°C for 30–40 min.

**Table 1.** Formulation and mode of making dough on the basis of the pregelatinized flour fermented using a liquid acidophilic starter

<table>
<thead>
<tr>
<th>Name of raw materials and process parameters</th>
<th>Raw material consumption and process parameters by stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acidophilus starter</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Acidophilus starter, kg</td>
<td>17.1</td>
</tr>
<tr>
<td>Amount of flour per starter, kg</td>
<td>–</td>
</tr>
<tr>
<td>Rye bread flour, kg</td>
<td>5.0</td>
</tr>
<tr>
<td>First grade bread wheat flour, kg</td>
<td>–</td>
</tr>
<tr>
<td>Saccharified pregelatinized flour, kg</td>
<td>–</td>
</tr>
<tr>
<td>Pressed bakery yeast, kg</td>
<td>–</td>
</tr>
<tr>
<td>Extra fine food common salt, kg</td>
<td>–</td>
</tr>
<tr>
<td>Fermented pregelatinized flour, kg</td>
<td>–</td>
</tr>
<tr>
<td>Potable water, kg</td>
<td>12.1</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>74.0</td>
</tr>
<tr>
<td>Initial temperature, °C</td>
<td>28–30</td>
</tr>
<tr>
<td>Acidity, deg.</td>
<td>14–16</td>
</tr>
<tr>
<td>Duration of, h</td>
<td>–</td>
</tr>
<tr>
<td>– saccharification</td>
<td>–</td>
</tr>
<tr>
<td>– fermentation</td>
<td>12–14</td>
</tr>
</tbody>
</table>
Research methods used in the study. When studying the properties of raw materials, semi-finished products and quality of bakery products, the common physicochemical and organoleptic methods were used, as well as the special methods set forth in the text of the paper.

Methods for studying the properties of raw materials. All the samples of rye bread flour were analyzed for the organoleptic and physicochemical properties: moisture, acidity, the falling number; the samples of first-grade wheat flour – for the organoleptic and physicochemical properties: moisture, acidity, the quantity and quality of raw gluten.

The moisture content of the flour was determined according to GOST 9404-88, the acidity of flour – according to GOST 27493-87, the falling number – according to GOST 27676-88, the content of raw gluten – using MOK-3M according to GOST 27839-2013, the properties of raw gluten – using IDK-3M according to GOST 27839-2013.

The pressed yeast was analyzed for the time of a dough rise in accordance with GOST R 54731-2011.

The common food salt and wholemeal stone ground rye products and drinking water were estimated organoleptically in accordance with the current documentation.

Bread quality estimation methods. The bread was analyzed after 14–16 hours after baking for the organoleptic and physicochemical properties: the moisture content of crumb, the acidity of crumb, the porosity of crumb, the volume yield, the specific volume and the total deformation of crumb thickening.

The volume yield of bread was determined according to GOST 27669-88, the specific volume of bread was determined according to GOST 27669-88 and expressed in terms of cm³/g.

The moisture of crumb was determined according to GOST 21094-75, the acidity of crumb – using the accelerated method according to GOST 5670-96, the porosity of crumb – according to GOST 5669-96.

The deformation of bread crumb thickening was determined using Structurometer ST-2 (Russia). Structurometer ST-2 is designed to determine the rheological characteristics of food media. The operation principle of the device is based on measuring the mechanical load on the nozzle-indenter when it is inserted at a given rate into the prepared sample of the product. The necessary indenter is attached to the strain-gage, which is moved in the vertical direction by means of a ball screw according to the given programme. Selecting, setting the mode and displaying information is carried out using a personal computer connected to the device's data acquisition board via the USB interface. When determining the rheological properties of the analyzed media, both the motion rate of the indenter and the loading rate of the product can be specified. Using Structurometer ST-2, the total ($H_{total}$), plastic ($H_{plastic}$), and elastic deformation ($H_{elastic}$) of bread crumb thickening was determined.

The organoleptic indicators of bread quality were estimated in accordance with GOST 5667-65.

Methods for studying the properties of semi-finished products. The mass fraction of sugar in fermented pregelatinized flour, saccharified pregelatinized flour and dry fermented pregelatinized flour was determined using the permanganate method according to the methods set forth in GOST 5672-68.

The mass fraction of moisture in dry fermented pregelatinized flour was determined using the methods set forth in GOST 9404-88.

The acidity of dry fermented pregelatinized flour was determined according to the methods set forth in GOST 5670-96.

The microbial titer of dry fermented pregelatinized flour was determined by counting the number of cells after inoculation on some nutrient media as described in [23].

The rheological characteristics of fermented pregelatinized flour were determined using the rotational viscometer RHEOTEST-2 (Germany) with an S/S1 measuring system in accordance with the following methods. The measuring (inner) cylinder 2 (Fig. 1) is mounted on the drive shaft 5. The predetermined volume of the studied product is poured into the measuring container (outer cylinder) and it is set in the operating position coaxially to the cylinder 2. In this case, the annular space is filled with the studied mass. With the help of the shift lever 9, the measuring cylinder 2 is rotated. Rotation at this speed is carried out until the indications of the secondary instrument that is used to measure the angle $\alpha$ of the relative rotation of the cylinders 2 and 3 are stabilized. The frequency of the output signal of the strain-gage sensor is recorded and the value of the shear stress $\tau$ is determined for the fixed shear rate $\gamma$ and temperature. Then, the shear rate $\gamma$ in the layer of the investigated product is increased by $\Delta\gamma$. The rheological characteristics of fermented pregelatinized flour are determined using the developed computer program "Flow curve virtual model" (Certificate of official registration of the computer program "Flow curve virtual model" No. 2008612695, registered on May 29, 2008).

Fig. 1. Functional diagram of the measuring unit: (1) a bed; (2) a measuring cylinder (inner); (3) a measuring tank (outer cylinder); (4) a measuring shaft; (5) a drive shaft; (6) a dynamometer (spiral spring); (7) a potentiometer; (8) a thermostatic tank; (9) a shift lever; (10) an indicating instrument.
SPECIAL STUDY METHODS

Methods for determining volatile acids in fermented pregelatinized flour. Volatile acids are the product of the life of lactic acid bacteria. The volatile acids of rye dough and bread consist mainly of acetic acid and a small amount of lactic acid. The content of volatile acids characterizes the partially flavoring acidity of bread.

The sample of the analyzed substance (20 g) is pre-ground in a mortar with distilled water to obtain a homogeneous mass and transferred quantitatively to a 100 cm³ volumetric flask. Distilled water is added to the mark and stirred for 3 minutes, whereafter the suspension is filtered. 10 cm³ is taken for distillation and 3–4 drops of orthophosphoric acid (H₃PO₄) and 1 drop of refined vegetable oil are added (to prevent foaming of the extract while passing steam). The obtained distillation is immediately heated to the boiling point and titrated with a 0.05 N sodium hydroxide solution for phenolphthalein to obtain a slightly pink color. The content of volatile acids is expressed in terms of degrees, i.e. in cm³ of 1 N sodium hydroxide solution per 100 g of the substance and determined using the following formula:

\[ X = \frac{v \cdot 100 \cdot K}{p \cdot 20} \]

where \( v \) is the amount of cm³ of a 0.05 N sodium hydroxide solution used for distillation titration; \( p \) is the amount of the substance per 10 cm³ of the extract (2 g); \( K \) is the correction factor for the alkali titre.

Methods for determining amine nitrogen in fermented pregelatinized flour. The method is based on the fact that the resulting soluble copper salt, when reacting with KI in the presence of CH₃COOH, reduces iodine. The released iodine is determined by titration with a Na₂S₂O₃ solution. When titrating, it is considered that 1 ml of a 0.01 N solution of Na₂S₂O₃ corresponds to 0.28 mg of amine nitrogen providing that the solution contains only amino acids. It is known that, in addition to amino acids, soluble salts can form various peptides, which introduces an error during determination.

From 1 to 5 ml of the extract or solution is poured in a 50 ml volumetric flask, 4 drops of the indicator is added and the extract is neutralized to obtain a slightly blue color using a 1N solution of NaOH. 30 ml of a copper phosphate suspension is added to the neutral solution, the content of the flask is brought to the mark with water and mixed well. The mixture is filtered using dense filter paper or centrifuged. In the course of the study, the resulting solution can be left until the next day. 10 ml of the solution are taken from the resulting centrifugate (filtrate), 0.5 ml of an 80% solution of CH₃COOH and 1 g of KI (powdered) are added. The isolated iodine is titrated with a 0.01 N solution of Na₂S₂O₃ with 5 drops of starch as an indicator. The presence of turbidity in the centrifuges or filtrates can introduce errors during determination, so it must be ensured that they are transparent. In addition, it must be taken into account that not all the amino acids form soluble copper salts, for example, cystine salt is hardly soluble. To make a correction for the purity of the reagents, a control experiment is made. The amount of nitrogen of amino acids \( X \), in mg, is calculated using the formula:

\[ x = \frac{(a - b) \cdot 0.28 \cdot 5 \cdot 100}{v} \]

where \( a \) is the amount of a 0.01 N solution of Na₂S₂O₃ consumed for the titration of the working solution, ml; \( b \) is the amount of a 0.01N solution of Na₂S₂O₃ consumed for the titration in the control experiment, ml; \( v \) is the volume of solution (or hydrolyzate) taken for determination, ml; 0.28 is a coefficient for recalculating the titration data in terms of the nitrogen of amino acids.

Methods for studying drying kinetics. When drying the starter using a “Всц-100” convective drier, a drying agent heated to temperatures of 55, 60, and 65°C was used.

When using freeze (lyophilic) drying by means of Sublimator 3–4–5, a 2-gram starter was packed in the sterile glass bottles covered in compliance of sterility and frozen at a temperature of 18°C. After complete freezing, the bottles were evacuated. In the course of vacuum drying, there was sublimation (lyophilization) when dehydrating the samples.

RESULTS AND DISCUSSION

The paper includes the studies of:
- optimizing the composition of a nutrient mixture for dry fermented pregelatinized flour in terms of rheological, biotechnological and microbiological properties;
- the development of the main technological parameters of making dry fermented pregelatinized flour providing stable biotechnological and microbiological properties;
- various methods for drying fermented pregelatinized flour for use in the technologies of bakery products made of rye and a mixture of rye and wheat flour;
- the development of a method for preserving the freshness and microbiological stability of bakery products from a mixture of rye and wheat flour;
- the development of integrated technology and assortment of long-life rye-wheat bakery products.

Below are the results of the studies and their analysis.

Optimization of a nutrient mixture composition for dry fermented pregelatinized flour in terms of rheological, biotechnological and microbiological properties. The use of pregelatinized flour in bread making makes it possible to obtain the following results: to increase the content of sugars in dough; to increase fermentation activity; to improve the baking
properties of flour; to increase bread yield; to improve bread quality; to slow down its staling. The use of pregelatinized flour has a beneficial effect on the fermentation microflora of dough.

According to the modern technologies of production of rye-wheat varieties of bread, pregelatinized flour is used as fermented one [12]. Such pregelatinized flour is called fermented pregelatinized flour. Below are the results of the studies on the development of a method for obtaining dry fermented pregelatinized flour from the saccharified pregelatinized flour fermented with an acidophilic starter for use in the technology of long-life rye-wheat varieties of bread.

Bakery pregelatinized flour is traditionally made of rye bread flour. To increase the nutritional value of a semi-finished product and, accordingly, the finished products, the possibility of replacing rye bread flour with rye wholemeal bread flour characterized by a higher content of vitamins and minerals was studied.

The indicators of the falling number and the content of reducing sugars in rye wholemeal flour have been determined in comparison with rye bread flour. It has been revealed that the falling number of rye bread flour was 48% higher than that of wholemeal bread flour, and the sugar content was 19% higher.

It has been established that making saccharified pregelatinized flour with the use of rye wholemeal bread flour improves the content of reducing sugars by 1.2 times in comparison with the saccharified pregelatinized flour made of rye bread flour. The high content of reducing sugars in saccharified pregelatinized flour will contribute to the more intensive growth of microorganisms, which will intensify the process of making semi-finished products.

The effect of wholemeal flour on the content of volatile acids, amine nitrogen and sugar in fermented pregelatinized flour has been studied. The fermented pregelatinized flour was made with the addition of an acidophilic starter in the amount of 50% of the weight of saccharified pregelatinized flour.

Based on the results of the analyses, it has been revealed (Table 2) that the sample prepared using rye wholemeal flour was characterized by a higher content of amine nitrogen, volatile acids and mass fraction of sugar in comparison with the sample of the fermented pregelatinized flour made of rye bread flour. Therefore, making saccharified pregelatinized flour of rye wholemeal flour has a beneficial effect on the life of lactic acid microorganisms.

**Development of the basic technological parameters for making dry fermented pregelatinized flour to provide stable biotechnological and microbiological properties.** To optimize the parameters of drying fermented pregelatinized flour, the effect of the amount of an acidophilic starter (from 0 to 50% of the weight of saccharified pregelatinized flour) on the rheological properties of fermented pregelatinized flour has been studied. The pregelatinized flour was made of rye wholemeal flour, saccharified for 4 hours and then an acidophilic starter was added. In the process of fermentation, an acidophilic starter was added to the samples of saccharified pregelatinized flour in the amount of: 1–0%, 2–10%, 3–15%, 4–20%, 5–25%, 6–30%, 7–35%, 8–40%, 9–45%, 10–50%.

For clarity, let us present some flow curves obtained as a result of the studies (Fig. 2)

Figure 2 shows the general trend for all the samples. In the initial period of time, the viscosity of the fermented pregelatinized flour decreases, then the structure is stabilized and, at the final stage, there is a slight decrease in the mobility of the medium. This is due to the biochemical reactions in the medium. The difference in the readings of the shear stress scale is directly related to the consistency of the samples. In the denser media of the samples with a mass fraction of a starter of 20%, 30% and 35% (samples No. 4, 6 and 7, respectively), there are higher shear stresses within the range from 500 to 3000 Pa. The samples numbered 2, 3, 8 and 10 had a medium consistency during mixing of the starter, and therefore the shear stress varies from 300 to 500 Pa. The samples with a starter content of 0%, 15% and 45% had the most liquid consistency as evidenced by the shear stress values within 150–300 Pa. In each individual case, there is a difference in the values of shear stress in a certain time interval which is explained by the chemical reactions at the molecular level. As a rule, this happens at the final stage, 8–10 hours after batching.

Analyzing the obtained curves, it can be concluded that viscosity is affected not only by the addition of a starter in percent, but also by such external factors as a starter technological process.

It has been established that the samples of fermented pregelatinized flour refer to the non-Newtonian or anomalous viscous liquids described by the Ostwald-de-Waele rheological relationship, which is written in general form as:

$$\tau = K \cdot \dot{\gamma}^n,$$

where $\tau$ is shear stress, Pa; $K$ is a consistency coefficient proportional to viscosity; $\dot{\gamma}$ is a shear rate, s$^{-1}$; $n$ is a process index.

The values of coefficients are presented in Table 3.

**Table 2. Effect of different types of flour on the content of volatile acids, amine nitrogen and sugar in fermented pregelatinized flour**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fermented pregelatinized flour made of rye bread flour</td>
</tr>
<tr>
<td>Volatile acid content, %</td>
<td>13.5</td>
</tr>
<tr>
<td>Amine nitrogen content, mg</td>
<td>210.0</td>
</tr>
<tr>
<td>Mass fraction of sugar, %</td>
<td>11–13</td>
</tr>
</tbody>
</table>
To analyze the optimal composition of pregelatinized flour, some flow curves have been plotted for the values of dependence of rate on shear stress at the end of fermentation of pregelatinized flour, as shown in Fig. 3.

Analyzing the data obtained, it can be concluded that the sample numbered 10 has the lowest shear stress, hence the fermented pregelatinized flour with a 50% content of a starter has the least viscous structure, which allows the sample to be dried more quickly using a moisture analyzer and to preserve lactic acid bacteria.

Study of various methods for drying fermented pregelatinized flour for use in the technologies of bakery products made of rye and a mixture of rye and wheat flour. When determining the conditions of drying fermented pregelatinized flour, a number of studies were carried out at the Kemerovo State
University. The pregelatinized flour was dried in three ways: a thermoradiation, convective and sublimation one. After each drying, the number of cells of beneficial microflora was determined in the samples. For this purpose, limiting dilution inoculation was carried out with plating and filling with a differential diagnostic medium. Since the main microflora of a starter consists of thermophilic lactobacilli, the agar MRS medium has been chosen for the analysis. The microflora of a starter is a group of conditionally anaerobic microorganisms, therefore, conditionally anaerobic conditions were created to determine the number of cells. The account was carried out by counting and microscopy. The analysis of the obtained data showed that in all the samples the number of cells did not exceed $1 \times 10^6$ CFU/g. For example, in the samples of pregelatinized flour subjected to thermoradiation drying, the number of cells was $(2-6) \times 10^4$ CFU/g, in the samples obtained by convection drying – $(5-9) \times 10^5$ CFU/g. Whereas, when drying using the sublimation method, the titer of beneficial microflora showed $1 \times 10^6$ CFU/g, which is ten times lower compared to the other two types of drying. Given that the temperature of the process is not destructive for sourdough microorganisms – it is most likely that the low survival rate of microflora is related specifically to the radiation activity of IR lamps.

Thus, it has been experimentally established that microorganisms are destroyed least during convective drying. The survival rate of microorganisms is maximum in the case of this method for concentrating.

As a result, the optimal method and modes for drying fermented pregelatinized flour have been determined: convective drying at a temperature of 40–45°C for 170–200 min and an air flow rate of 1.5–1.8 m/s above, 0.3–0.5 m/s below, 0.8 m/s on the right and 0.1–0.15 m/s on the left.

An organoleptic estimation has been carried out and the physicochemical and microbiological indicators of the obtained dry fermented pregelatinized flour have been determined.

In terms of organoleptic indicators, the obtained dry fermented pregelatinized flour was polydisperse powder non-uniform in a particle size, with no foreign impurities, of a cream color, with a taste and odor peculiar to fermented pregelatinized flour.

The microflora of the obtained dry fermented pregelatinized flour is mono-shaped and consists of the lactic acid bacteria *Lactobacillus helveticus* H10 adapted to high temperatures (40–45°C), the microbial titer is $1 \times 10^6$ CFU/g, the acidity is 58–60 deg., the moisture is 7.0–9.0% and the mass fraction of sugar is 34–36%.

**Development of aggregate technology and assortment of long-life rye-wheat bakery products.**

To develop an aggregate technology and assortment of long-life rye-wheat bakery products, the effect of the obtained dry fermented pregelatinized flour on the physicochemical, organoleptic and microbiological indicators of the quality of bakery products made of a mixture of rye and wheat flour, as well as the effect of the food ingredients that preserve the freshness of products increasing their storage stability and nutritional value have been studied.

Studies have been carried out to determine the optimal dosage of dry fermented pregelatinized flour. The dry fermented pregelatinized flour was added to the dough in the amount of 10, 20, 30 and 40% of the weight of flour (at the same time the amount of rye wholemeal flour in the dry fermented pregelatinized flour was subtracted from the total quantity of rye bread flour according to the recipe to maintain the ratio of rye bread flour: first grade wheat flour equal to 70 : 30). The fermentation time was 60 minutes. Table 4 gives the results of the physico-chemical analysis of the quality of rye-wheat bread.

As a result of the analysis of the data, it has been established that the indicators of the volume yield of bread, the specific volume and the porosity of crumb decrease in direct proportion with an increase in the percentage addition of dry fermented pregelatinized flour. The acidity of crumb of the bread sample with a 40% content of dry fermented pregelatinized flour (Sample No. 4) is more than twice as high as that of the bread sample with the addition of dry fermented pregelatinized flour in the amount of 10% (Sample No. 1). The samples with a mass fraction of dry fermented pregelatinized flour of 20% and 30% (the samples numbered 2 and 3, respectively) have optimal characteristics.

As a result of the analyses, the technological possibility of adding dry fermented pregelatinized flour in a dosage of 20–30% has been established. The samples were characterized by optimal acidity, a specific volume and porosity for long-life rye-wheat bakery products.

The next stage of the study was the choice of an optimal method for making dough. The dough was made using the batter method with a shortened fermentation time and using the quick dough method. As the control sample, a dough sample with no starters was used.

The formulations and modes for making dough are given in Table 5 and the bread quality indicators – in Table 6.

**Table 4. Effect of dry fermented pregelatinized flour on the physicochemical indicators of rye-wheat bread**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>The value of indicators for the bread samples with the addition of dry fermented pregelatinized flour in the amount of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>Volume yield, cm$^3$</td>
<td>300</td>
</tr>
<tr>
<td>Specific volume of bread, cm$^3$/g</td>
<td>1.4</td>
</tr>
<tr>
<td>Porosity of the crumb, %</td>
<td>54</td>
</tr>
<tr>
<td>Acidity of the crumb, deg.</td>
<td>4.0</td>
</tr>
</tbody>
</table>
Table 5. Formulation and mode for making dough with dry fermented pregelatinized flour using various methods for making dough

<table>
<thead>
<tr>
<th>Name of raw materials, semi-finished products and process parameters</th>
<th>Consumption of raw materials and process parameters by dough making stage using the batter method</th>
<th>using the quick dough method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1) predough</td>
<td>(2) dough</td>
</tr>
<tr>
<td>Rye bread flour, kg</td>
<td>–</td>
<td>52</td>
</tr>
<tr>
<td>First grade bread wheat flour, kg</td>
<td>30</td>
<td>–</td>
</tr>
<tr>
<td>Dry fermented pregelatinized flour, kg</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>Rye bread flour in a starter, kg</td>
<td>–</td>
<td>18</td>
</tr>
<tr>
<td>Pressed bakery yeast, kg</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Food common salt, kg</td>
<td>–</td>
<td>1.5</td>
</tr>
<tr>
<td>Water, kg</td>
<td>30 as per calculation</td>
<td>as per calculation</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>45.0</td>
<td>$W_{bread} + (0.5–1.0)$</td>
</tr>
<tr>
<td>Final acidity, deg.</td>
<td>12.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Fermentation time, min</td>
<td>120</td>
<td>–</td>
</tr>
</tbody>
</table>

It has been found that various methods for making dough did not have a significant effect on the physicochemical indicators of the quality of bakery products. In terms of organoleptic properties, all the test samples were characterized by a correct form without cracks, the brown color of crusts, the developed porosity, elastic baked crumb and a characteristic taste and odor. The obtained data allow us to recommend the quick dough method to make bakery products of a mixture of rye and wheat flour, since the samples were characterized by a higher acidity. The increase in the acidity index will prevent their microbiological spoilage during storage.

Development of a method for preserving the freshness and microbiological resistance of the bakery products made of a mixture of rye and wheat flour. To increase the shelf life of more than five days, a number of studies have been conducted on the effect of food ingredients (enzyme preparations (EP) and preservatives) on the quality of bakery products and the preservation of freshness and microbiological safety.

The products were prepared according to the formula with the addition of sorbic acid in the amount of 0.2% of the weight of flour, Novamyl 3D – 0.01% of the weight of flour and Pentopan 500 BG – 0.01% of the weight of flour according to the chosen technology. As the control sample, a flour sample with no conditioners was used. The effect of the addition of conditioners on the preservation of freshness and microbiological safety of bakery products was judged by the moisture, the total, elastic and plastic deformation of bread crumb, determined using Structurometer ST-2 and the surface indications of mold formation on the 1st, 7th and 14th days of storage. The bakery products were packed in plastic bags (PA/HV/PE) with a thickness of 0.08 mm. Table 9 presents the moisture content and deformation of bread crumb.

According to the data presented in Table 7, it can be seen that the indicators of the total, elastic and plastic deformation of crumb decreased to a lesser extent in the products made using Novamyl 3D and sorbic acid as a preservative.

Table 6. Quality indicators of bread with dry fermented pregelatinized flour

<table>
<thead>
<tr>
<th>Name of indicators</th>
<th>Using the batter method</th>
<th>Using the quick dough method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Moisture of the crumb, %</td>
<td>46.0</td>
<td>45.5</td>
</tr>
<tr>
<td>Specific volume of bread, cm$^3$/g</td>
<td>1.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Porosity of the crumb, %</td>
<td>55</td>
<td>57</td>
</tr>
<tr>
<td>Acidity of the crumb, deg.</td>
<td>5.0</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Table 7. Moisture and deformation indicators of bread crumb during storage

<table>
<thead>
<tr>
<th>Name of indicators</th>
<th>Value of indicators during storage using control (without conditioners)</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
<td>7th day</td>
<td>14th day</td>
<td>1st day</td>
</tr>
<tr>
<td>H$_{total}$, mm</td>
<td>1.117</td>
<td>1.039</td>
<td>–</td>
<td>1.164</td>
<td>1.117</td>
</tr>
<tr>
<td>H$_{elastic}$, mm</td>
<td>0.436</td>
<td>0.282</td>
<td>–</td>
<td>0.448</td>
<td>0.421</td>
</tr>
<tr>
<td>H$_{elastic}$, mm</td>
<td>0.757</td>
<td>0.671</td>
<td>–</td>
<td>0.726</td>
<td>0.696</td>
</tr>
<tr>
<td>Moisture of the crumb, %</td>
<td>46.5</td>
<td>46.0</td>
<td>–</td>
<td>45.0</td>
<td>44.5</td>
</tr>
</tbody>
</table>
The first signs of microbiological damage were observed on the 7th day of storage in Samples 1, 2 and 3. In the case of the use of sorbic acid and Novamyl 3D in the formulation of bakery products, Sample 4 did not show any molding signs after 14 days.

Thus, the joint use of Novamyl 3D and sorbic acid helped to suppress microbiological spoilage and preserve the freshness of bakery products for 14 days.

CONCLUSION

Based on the results of the studies:
(1) The composition of the nutrient mixture for dry fermented pregelatinized flour has been optimized in terms of rheological, biotechnological and microbiological indicators. It has been found that fermented pregelatinized flour with a 50% content of a starter has the least viscous structure, which allows to dry the sample faster using a moisture content analyzer and to achieve the preservation of lactic acid bacteria.
(2) It has been found that the sample prepared using rye wholemeal flour was characterized by a higher content of amine nitrogen (by 15%), volatile acids (by 29%) and a mass fraction of sugar (by 27%) compared to the sample of the fermented pregelatinized flour made of rye bread flour. Therefore, making saccharified pregelatinized flour of rye wholemeal flour has a beneficial effect on the life of lactic acid microorganisms.
(3) A number of optimum technological parameters for drying fermented pregelatinized flour using convective drying have been developed: drying at a temperature of 40–45°C for 170–200 min and an air flow rate of 1.5–1.8 m/s above, 0.3–0.5 m/s below, 0.8 m/s on the right and 0.1–0.15 m/s on the left to provide stable biotechnological and microbiological properties.

It has been revealed that microorganisms are destroyed least during convective drying, the survival of microorganisms when using this method for concentrating is maximum, and the microbial titer in the samples is not less than 1 × 10^5 CFU/g.

(4) In terms of organoleptic indicators, the obtained dry fermented pregelatinized flour is polydisperse powder non-uniform in a particle size, with no foreign impurities, of a cream color, with a taste and odor peculiar to fermented pregelatinized flour. The microflora of the obtained dry fermented pregelatinized flour is mono-shaped and consists of the lactic acid bacteria Lactobacillus helveticus H10 adapted to high temperatures (40–45°C), the acidity is 58–60 deg., the moisture is 7.0–9.0% and the mass fraction of sugar is 34–36%.

(5) The technological capability and the optimal dosage of addition of dry fermented pregelatinized flour in the amount of 20–30% of the weight of flour in making rye-wheat breads have been established. The samples were characterized by the acidity, specific volume and porosity optimal for long-life rye-wheat bakery products.

(6) It has been established that the use of dry fermented pregelatinized flour in the optimal dosage for various methods for making dough did not have a significant effect on the physico-chemical indicators of the quality of rye-wheat bakery products. The obtained data allow us to recommend the quick dough method for making dough in the development of bakery products from a mixture of rye and wheat flour due to the higher acidity of crumb.

(7) It has been established that the combined use of Novamyl 3D and sorbic acid (in the amount of 0.001% and 0.2% of the weight of flour, respectively) in the technology of rye-wheat bakery products with dry fermented pregelatinized flour suppressed the microbiological spoilage and preserved the freshness of bakery products for 14 days.

REFERENCES


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PROTOTYPE OF VIETNAMESE TAMARIND FISH SAUCE FORTIFIED WITH IRON, ZINC AND VITAMIN A

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Abstract: Fortification with iron, zinc and vitamin A of Vietnamese tamarind fish sauce has been studied. The Vietnamese tamarind fish sauce formula consisted of 42% of slurry, 37% of sugar and 21% of fish sauce, and was fortified with 36 mg of iron (Fe), 15 mg of zinc (Zn) and 10000 IU of vitamin A palmitate per 30 g of sauce. The sauce is acidic food with pH of 3.21, the total titratable acidity of 29.63% and the water activity of 0.87. The viscosity of fortified sauce was 777 cP and it was characterized by non-Newtonian behavior. The color coordinates L*, a* and b* were 20.71, 11.46 and 23.42, respectively, which was lower than those of traditional sauce. After pasteurization, the iron, zinc and vitamin A contents were reduced to 30.73 mg, 14.21 mg and 7306 IU per 30 g, respectively, while otherwise the physical and chemical quality did not significantly change. The sensory scores of the pasteurized and fortified sauce were slightly lower than that of the sauce before pasteurization or of traditional sauce, but without a significant difference.

Keywords: Vietnamese tamarind fish sauce, fortification, iron, zinc, vitamin A palmitate, pasteurization

INTRODUCTION

Micronutrient deficiencies (MNDs) are the significant factors that cause health problems, even though some deficiencies are not clinically evident. They have socioeconomic impacts particularly in the developing and low-income countries. While several micronutrient deficiency problems affect all age groups, they tend to have a more serious impact on young children and women of reproductive age [1]. Besides obvious and direct health effects, MNDs have profound implications to economic development and productivity, particularly in terms of inflating public health costs and reducing the human capital. While somewhat less than in other Asian countries, micronutrient deficiencies remain prevalent in Vietnam due to the Vietnamese diet, disproportionately comprised of rice and vegetables as staple foods that are low in micronutrient contents. Even in this era, the Vietnamese government has tried to campaign for dietary quality and diversity, encouraging the consumption of foods rich in protein and minerals while discouraging the consumption of the staple food rice or noodles that contain mainly carbohydrates. However, up to now the micronutrient deficiencies of iron, zinc and vitamin A remain unresolved problems for the Vietnamese people [2].

Iron deficiency is a major of micronutrient problems significantly related to public health, and is of primary importance today. An iron deficiency investigation in Vietnam in 2014–2015 by the National Institute of Nutrition of Vietnam showed that iron deficiency was common in three types of regions, namely the urban, rural and mountainous regions of the country, with a 47.3% rate in pregnant women, 23.6% in non-pregnant women and 50.3% in children under 5 years of age. The study covered 9 provinces: Ha Noi, Hue, Ho Chi Minh, Nam Dinh, Nghe An, Bac Lieu, Bac Can, Quang Ngai, and Kon Tum [3].

Zinc is involved in the activities of various enzymes, and in the expression of genotype, cell division and body development, immune function, appetite, and appetite regulation. According to the survey on a nutritional status in 9 provinces mentioned previously, the overall fraction of pregnant women with zinc deficiency was 80.3%, of children – 69.4%, and of women of reproductive age – 63.6%. The rates of zinc deficiency currently remain quite high [3].

Moreover, NIN also reported that between 2014 and 2015 the vitamin A deficiency rate in Vietnamese children under 5 was 8.2% in the urban areas, 13.1% in the rural areas and 16.1% in the mountainous areas [3]. [4] also reported that about 14 % of women were deficient in vitamin A.

Tamarind fish sauce is the signature sauce consumed regularly with rice and some vegetables by most Vietnamese families, because it is cheap, easy to make and appetizing. This sauce is consumed with a wide range of foods, particularly fried or steamed seafoods. According to market surveys, personal communications and international food exchange, this sauce is quite similar with the dips found in Thailand and in other Asian countries. With a busy lifestyle as in a single or small family, and with little cooking...
To prevent or contribute a reduction in micronutrient deficiency in a lot of countries, the micronutrient fortification of staple foods has been proven as a simple and effective way to increase micronutrients in daily meals. Therefore, the aim of this study was to develop Vietnamese tamarind fish sauce fortified with iron, zinc and vitamin A, to address the deficiency problems with a vision of a commercialized product for wide-spread consumption in future.

STUDY OBJECTS AND METHODS

Materials. Tamarind pulp without seeds, fish sauce, white sugar, finger chili, and garlic were purchased from a supermarket located in Songkhla province, then brought back to run an experiment at the Department of Food Technology, the Faculty of Agro-Industry, Prince of Songkla University, Thailand. Iron sodium ethylenediaminotetraacetate (FeNaEDTA · 3H2O), zinc sulfate (ZnSO4 · 7H2O) and vitamin A palmitate were bought from Merck Chemical Company (Darmstadt, Germany), all of an analytical grade. Methanol obtained from Sigma-Aldrich Company (Steinheim, Germany), all of an HPLC grade. Ethanol (CH3OH), chloroform (CHCl3) and hexane (C6H14) were obtained from Sigma-Aldrich Company (Steinheim, Germany), all of an analytical grade.

Table 1. Experimental formulations of Vietnamese tamarind fish sauce obtained from a mixture design program

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tamarind slurry (%)</th>
<th>Fish sauce (%)</th>
<th>Sugar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFS1</td>
<td>40</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>TFS2</td>
<td>40</td>
<td>25</td>
<td>35</td>
</tr>
<tr>
<td>TFS3</td>
<td>35</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>TFS4</td>
<td>45</td>
<td>20</td>
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<tr>
<td>TFS5</td>
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<td>37</td>
</tr>
<tr>
<td>TFS6</td>
<td>37</td>
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<td>37</td>
</tr>
<tr>
<td>TFS7</td>
<td>36</td>
<td>22</td>
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</tr>
<tr>
<td>TFS8</td>
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<tr>
<td>TFS9</td>
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<tr>
<td>TFS10</td>
<td>35</td>
<td>28</td>
<td>37</td>
</tr>
<tr>
<td>TFS11</td>
<td>35</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>TFS12</td>
<td>45</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>TFS13</td>
<td>35</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>TFS14</td>
<td>35</td>
<td>25</td>
<td>40</td>
</tr>
</tbody>
</table>

Vietnamese tamarind fish sauce preparation. The tamarind pulp was mixed with water (1 : 4 w/w) and then homogenized using a blender (Philips HR-2068, Thailand) to obtain tamarind slurry. The tamarind slurry (TS), 35–45% by weight, fish sauce (FS) (10–30%) and sugar (SG) (35–55%) were mixed following the experimental design by Design-Expert Statistical package version 7.0 (Statease, Inc., Minneapolis, MN). The 14 experimental formulations of Vietnamese tamarind fish sauce are shown in the Table 1. The mixture of tamarind slurry, fish sauce and sugar accounted for 95% of the actual formulation, while the rest was garlic (2.5%) and chili (2.5%). All the treatments were characterized by pH, the total titratable acidity, water activity, color, viscosity and sensory evaluation to find out the proper formulation mainly based on a sensory score.

Fortified Vietnamese tamarind fish sauce preparation. The fortificants were iron sodium ethylenediaminotetraacetate (FeNaEDTA · 3H2O), zinc sulfate (ZnSO4 · 7H2O) and vitamin A palmitate. The selected Vietnamese tamarind fish sauce from the above experiment was added with 18 or 36 mg of iron and 15 or 30 mg of zinc, and vitamin A palmitate at 5,000 or 10,000 IU per serving size (30 g) of the sauce, following the factorial experimental design, and the homogenization was reached using a blender again (Philips HR-2068, Thailand). All the fortified sauces were characterized by pH, the total titratable acidity, water activity, color, viscosity and sensory evaluation to find the suitable levels of the fortificants based on the sensory score.

Pasteurization of fortified Vietnamese tamarind fish sauce. The Vietnamese tamarind fish sauce was placed after fortification in a glass bottle (120 g) then heated in a water bath at the controlled 90–95°C temperature for 5 min. After that, the product was cooled down before evaluating pH, the total titratable acidity, water activity, color, viscosity, sensory scoring and the iron, zinc and vitamin A contents.

Chemical analysis. The pH value of the sauces in this study was determined at 25°C using a pH meter (Metler 350, Singapore). Each sample was homogenized with sterilized distilled water in a 1 : 5 w/w sample:water ratio, and was allowed to stand for 2 min before measuring pH. The total titratable acidity of sauces as % tartaric acid was determined based on the method of [5]. The sample was homogenized with sterilized distilled water at a 1 : 10 w/w sample:water ratio, then filtered using Whatman No.4 filter paper. Subsequently, 3 drops of phenolphthalein were added and mixed in. The samples were titrated with 0.1M NaOH. The total titratable acidity as % tartaric acid (TA) was calculated as follows:

\[
\text{Total titratable acidity (\%) = \frac{\text{ml f NaOH} \times \text{Molarity of NaOH} \times \text{molecular mass of TA}}{\text{Weight of sample}} \times 100,}
\]

where the Molarity of NaOH = 0.1N; the molecular mass of TA = 150.087.
The water activity ($a_w$) of the samples was determined using Water Activity Meter Aqua Lab (Series, WA, USA).

The iron and zinc content obtained in the sauce were analyzed following AOAC, 2012. Samples of 0.5 gram were digested with 5 ml of concentrated nitric acid and 2 ml of 30% hydrogen peroxide under pressure in a closed vessel. Then heated in a microwave oven until the samples were digested. Afterwards, the samples were cooled down and filtered using Whatman No. 1 (110 nm pore size) filter paper. Each sample solution was transferred to a 25 ml volumetric flask and the volume was made up with distilled water [6]. The extract samples were used to determine iron and zinc by Inductively Couple Plasma Mass Spectrometry (ICP-MS) (Perkin-Elmer SCIEX, Shelton, CT, USA). The RF power was 1350 W, the plasma gas flow rate was 2 l/min, the carrier gas flow was 0.94 l/min and the make-up gas flow was 0.15 l/min. The sample take rate was 100 µl/min and the sample depth was 6.0 mm.

The vitamin A content was determined using high-performance liquid chromatography (HPLC) (Agilent Technologies 1200 series, SomaQualme, Washington, USA). A five-gram sample was mixed with 25 ml of CH$_3$OH:CHCl$_3$ (3 : 7, v/v) then the mixture was shaken for 2 hours. Afterwards, 0.5 M Na$_2$SO$_4$ was added up to 50 ml and centrifuged at 2500 rpm for 5 min. The bottom layer was evaporated at 40°C until dryness, then the residue was diluted with 1 ml of C$_6$H$_{14}$ and injected to the HPLC system using the column LiChrospher 100RP-18 (4.6 × 250 mm, 5 µm) at 40°C for 30 min, with the mobile phase a mixture of methanol and water was freshly prepared (98 : 2 v/v). The flow rate was 2 ml/min, the injection volume – 20 µl, and the absorbance was observed at 325 nm [7].

Physical analysis. The color coordinates of the samples were measured using a color meter (Hunter Lab, Model Color Quest XT, United States), which was calibrated using a white standard porcelain plate ($L^* = 93.6$, $a^* = -0.94$ and $b^* = 0.40$). The color is expressed as $L^*$, $a^*$ and $b^*$, where $L^*$ represents lightness within the range 0–100, $a^*$ means redness to greenness (+ is red, – is green), and $b^*$ means yellowness to blueness (+ is yellow, – is blue).

The viscosity was measured at rotation speeds of 20, 40, 60, 80, and 100 rpm at room temperature (30 ± 2°C) using a Brookfield Viscometer (model RTV, USA) fitted with a size 63 spindle. Thereafter, the viscosity (cP) versus speed (rpm) plots were assessed to classify a rheological behavior type.

Sensory evaluation. All of the treatments were evaluated by serving to fifty untrained panelists including the graduate students and technicians from the Department of Food Technology, Prince of Songkla University, Thailand. The panelists were asked to evaluate the likabilities of appearance, color, odor, texture, taste and overall for each sample using a 9-point hedonic scale, from “1 – dislike extremely” to “9 – like extremely”.

Statistical analysis. All the experiments were run in triplicates. The data were subjected to Analysis of Variance (ANOVA) and the differences between the means were assessed by Duncan’s Multiple Range Test [8] for statistical significance. The data analysis was performed using an SPSS package (SPSS 6.0 for Windows, SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Vietnamese tamarind fish sauce. The pH of food indicates the free hydrogen ions present therein [9]. It was found that the pH of all the samples was within the range of 3.34–3.68 (Fig. 1a), which was indicated as acidic sauce, mainly because of tamarind slurry. Therefore, the more tamarind slurry was added the more acid with a lower pH was found as shown in Fig. 1b. This result agrees with the study of [10], who reported that chili sauce with the highest vinegar content (16%) was the most acidic sauce.

Water activity ($a_w$) is an important factor related to the shelf life and quality of a food product. The water activity of Vietnamese tamarind fish sauce is shown in Fig. 1c. The TFS4 code had the highest $a_w$ of around 0.905, even though not significantly different from the code TFS12 (p > 0.05). In contrast, the lowest $a_w$ of the samples TFS3 and TFS8 was about 0.858. The significant differences in $a_w$ across the samples (p < 0.05) were due to the differences in salt concentration from fish sauce and in sugar used in the sauce. [11] reported that when the salt concentration of electro-dialysis treated fish sauce was reduced from 25% to 6%, the $a_w$ increased significantly.

Color is the most conspicuous characteristics of food perceived by consumers [12]. The color coordinates of Vietnamese tamarind fish sauces are shown in Fig. 2. The $L^*$ coordinate ranged from 26.56 to 29.22. Generally, the $L^*$ of the sauce depended on tamarind slurry and fish sauce, mainly due to the brownish-red color of tamarind pulp [13] and the amber color of fish sauce [14]. The $L^*$ increased when the amounts of tamarind slurry and fish sauce decreased (p < 0.05). The $L^*$ of TFS11 and TFS13 was the highest at 29.22, whereas TFS2 gave the least $L^*$ value at 26.56. The values of $a^*$ were within the range of 15.9–18.18. Significantly the highest $a^*$ was found for the samples TFS3 and TFS8 at 18.18, while the cases of TFS4 and TFS12 had $a^*$ at 15.9 which is below the others (p < 0.05). The $b^*$ values ranged from 33.25 to 34.88. In this parameter, the case TFS8 had the highest values at 34.88, whereas TFS11 gave the least $b^*$ at 33.25. This result suggests that the $a^*$ and $b^*$ color coordinates of the sauces also depended on tamarind slurry and fish sauce. Generally, the color of a food product depends on its raw materials or the compounds produced during processing, storage, etc. [15]. In this study, the color of tamarind fish sauce was affected by its ingredients such as tamarind slurry, fish sauce, sugar and red chili.

The viscosity of Vietnamese tamarind fish sauce is shown in Fig. 3a. The viscosities of the cases of TFS11 and TFS13 were the highest at about 563 cP, followed by TFS5, TFS7 and TFS14 as 512, 520 and 519 cP, respectively. In contrast, the cases of TFS4 and TFS12 had the lowest viscosities of about 420 cP due to their least amount of sugar. The tamarind fish sauces were not characterized by non-Newtonian behavior with shear thinning, with an increase in the shear rate the apparent viscosity decreased significantly (Fig. 3b). The viscosity and concentration of the solutions can be directly
proportional [16]. When a food product contains more dissolved particles, the higher friction between the particles leads to higher viscosity (p < 0.05). It pointed out that sugar is the major determinant of TFS11 and TFS13 with 45% sugar. [17] mentioned that the highest viscosities were found for the sauce and ketchup determined by the brown sugar addition and a CMC thickening agent.

Fig. 1. Chemical properties of Vietnamese tamarind fish sauces.

Fig. 2. Color of Vietnamese tamarind fish sauces.
The sensory scores of Vietnamese tamarind fish sauces from fifty non-trained panelists are summarized in Table 2. Each treatment was evaluated for appearance, color, odor, texture (smoothness and good blend), taste and overall liking. The four codes TFS1, TFS2, TFS5 and TFS7 exhibited comparatively high scores for all the attributes. The code TFS5 had the highest scores for all the attributes, significantly better than the others (p < 0.05). In addition, the data analysis gave the optimum blends suggesting two formulations. The first formulation has 41.8% TS, 20.4% FS and 37.7% SG; and the second formulation has 42.1% TS, 20% FS and 37.9% SG. These formulations were prepared and subjected to sensory tests, with TFS5 included for comparison. The sensory scores did not significantly differ between the three formulations. This indicates that the TFS5 formula was appropriate for further experiments.

**Vietnamese tamarind fish sauce fortified with iron, zinc and vitamin A.** The Vietnamese tamarind fish sauce consisted of 42% of tamarind slurry, 37% of sugar and 21% of fish sauce fortified with NaFeEDTA · 3H2O (18 mg and 36 mg of Fe/30 g), ZnSO4 · 7H2O (15 mg and 30 mg of Zn/30 g) and vitamin A palmitate (5000 IU and 10000 IU/30 g). Therefore, 8 treatments of fortified Vietnamese tamarind fish sauces and the control sample were made as indicated in Table 3.

**Table 3. Levels of iron, zinc and vitamin A fortification of Vietnamese tamarind fish sauce**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron (mg/30 g)</td>
</tr>
<tr>
<td>FFS1</td>
<td>18</td>
</tr>
<tr>
<td>FFS2</td>
<td>18</td>
</tr>
<tr>
<td>FFS3</td>
<td>36</td>
</tr>
<tr>
<td>FFS4</td>
<td>18</td>
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<tr>
<td>FFS5</td>
<td>18</td>
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<td>36</td>
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<tr>
<td>FFS7</td>
<td>36</td>
</tr>
<tr>
<td>FFS8</td>
<td>36</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

**pH of fortified tamarind fish sauce is shown in Fig. 4a. There was a significant difference in pH between the control sample and the fortified samples (p < 0.05) with the control sample that had pH at 3.41 while the fortified ones had 3.18–3.33.** [18] reported that the pH of the fortified pumpkin solution (containing 500 ppm of L-(+) ascorbic acid and 500 ppm FeSO4 · 7H2O with the addition of 2.49 g/l FeSO4 · 7H2O) was 3.60, while the control solution
containing 500 ppm of L-(+) ascorbic acid had pH 4.00 because of the acid hydrolysis of Fe$^{2+}$ ions \( [\text{Fe(H}_2\text{O)}_6^{2+} + + \text{H}_2\text{O} \rightleftharpoons \text{Fe(H}_2\text{O)}_5^{+} + (\text{OH})^{2+} + \text{H}_3\text{O}^-] \) and the exchange of iron ions and micellar bound H$^+$ [19]. In addition, this current study had higher titratable acidity for the fortified sauces than for the control sample (Fig. 4b). The titratable acidity of fortified sauces was between 29.12 and 29.83 while the control sample had 28.18.

The water activity (a$_w$) ranged within 0.865–0.881 as shown in Fig. 4c. Although there were no significant differences between the eight fortified tamarind fish sauces (p > 0.05), the a$_w$ of the fortified sauces was lower than that of the control ones, due to the added salts. [18] reported that the a$_w$ of the control pumpkin solution was 0.940 and it decreased to 0.930 with fortification. According to [20], the higher dissolved solid concentration in the solution had a lower water activity, which supported a decrease in a$_w$ in the fortified sauce affected by mineral salts fortification.

The color coordinates of the control sample were higher than those of the fortified sauces (Fig. 5). The $L^*$ of the control sample was 27.47 while the fortified sauces had values within the range of 19.24–20.71. The $a^*$ of the control sample was 20.2 while the fortified sauces were within the range of 9.54–11.46. The $b^*$ of the control sample was 34.9, and the fortified ones gave values between 21.59 and 23.42. The color can be strongly affected by iron addition [21]. It was observed that the added FeEDTA was pale-yellow in color [22] and affected on the color of sauce. [22] also mentioned that FeEDTA-fortified sugar added to tea gave blackish discoloration. While the food fortified with zinc and vitamin A does not in general lose its sensory acceptability.

The viscosity of the fortified Vietnamese tamarind fish sauce remained non-Newtonian (Fig. 6a). Fig. 6b, indicates that the viscosity of the control case was 622 cP while the fortified sauces were within the range of 731–796 cP. This result pointed out that the viscosity was increased by fortification with salts (p < 0.05). The higher amount of salts and higher viscosity may be due to the absorption of water by salts and increasing concentration of a solid in the sauce.

The organoleptic testing of the fortified Vietnamese tamarind fish sauces by fifty non-trained panelists for appearance, color, odor, texture (smoothness and good blend), taste, and overall likability is summarized in Table 4. There were no significant differences between the control and the fortified sauces in most attributes, except for the appearance and color of the FFS8 sample (p > 0.05). The statistical analysis of the sensorial score showed that the sauce FFS7 exhibited a higher score compared to the other fortified sauces and was close to the control sample (p < 0.05). Based on the Vietnamese Recommended Dietary Allowances, the levels of iron and zinc were acceptable in the FFS7 sauce. It is known that vitamin A is sensitive to light, oxygen, moisture and to some extent to heat [23], so it could be lost during processing. The FFS7 sauce could serve well as a fortified sauce.

![Fig. 4. Sensory acceptability scores of fortified Vietnamese tamarind fish sauces.](image)
Fig. 5. Color properties of the fortified Vietnamese tamarind fish sauces.

Fig. 6. Viscosity property of the fortified Vietnamese tamarind fish sauce.

Table 4. Sensory acceptability scores of fortified Vietnamese tamarind fish sauces

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Appearance</th>
<th>Color</th>
<th>Odor</th>
<th>Texture</th>
<th>Taste</th>
<th>Overall</th>
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</thead>
<tbody>
<tr>
<td>FFS1</td>
<td>7.59 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.65 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.91 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.91 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.71 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.97 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS2</td>
<td>7.56 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.26 ± 0.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.85 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.06 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.97 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.97 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS3</td>
<td>7.24 ± 0.43&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.18 ± 0.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.09 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.68 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.68 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS4</td>
<td>7.41 ± 0.50&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.35 ± 0.49&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.79 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.88 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.79 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS5</td>
<td>7.50 ± 0.51&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.71 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.29 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.91 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.24 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS6</td>
<td>7.35 ± 0.49&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.29 ± 0.46&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.85 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.09 ± 0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.68 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS7</td>
<td>7.15 ± 0.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.03 ± 0.39&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.09 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.12 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.21 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.03 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>FFS8</td>
<td>6.97 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.91 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.94 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.03 ± 0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.94 ± 0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.76 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>7.08 ± 0.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.29 ± 1.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.08 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.02 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.02 ± 1.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.06 ± 0.95&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Mean ± SD from 50 panalists; <sup>ab</sup>Means within a column with different superscripts are significantly different (p < 0.05).
Pasteurization of the fortified Vietnamese tamarind fish sauce. Pasteurization is a technology used to preserve foods, but it can affect some enzyme activities and the flavor and color of some food products [24]. The color coordinates of the PFTFS sauce (L* = 18.75, a* = 10.3, b* = 22.19) were below those of TFS (L* = 27.95, a* = 19.19, b* = 35.3) and FTFS (L* = 20.7, a* = 11.25, b* = 23.24) (Table 5). The color of the fortified tamarind fish sauce after pasteurization was darker than before the thermal processing due to the Maillard reaction that causes the non-enzymatic browning of food products [25]. In addition, the sauce fortified with transition metal, such as iron and zinc, was darker due to a catalyst function. PFTFS also showed increased viscosity at 826 cP, higher than the others (p < 0.05). [26] also reported that the rheological parameters of the tomato paste increased with an increase in the heating temperature (60, 80 and 90°C). Moreover, the solid content of the sauce increased during pasteurization due to water evaporation, and protein unfolding caused increased solvation.

TFS (Traditional tamarind fish sauce); FTFS (Fortified tamarind fish sauce); PFTFS (Pasteurized tamarind fish sauce). Mean ± SD from the triplicate measurements of 2 different lots. a-bMeans within a column with different superscripts are significantly different at p < 0.05.

The pH value of sauce changed between 3.21 and 3.41. pH seemed to slightly increase after pasteurization when not a significant difference (p < 0.05). [27] reported that the pH of apple juice was directly affected by the Maillard reaction that causes the non-enzymatic browning of food products [25]. In addition, the sauce fortified with transition metal, such as iron and zinc, was darker due to a catalyst function. PFTFS also showed increased viscosity at 826 cP, higher than the others (p < 0.05). [26] also reported that the rheological parameters of the tomato paste increased with an increase in the heating temperature (60, 80 and 90°C). Moreover, the solid content of the sauce increased during pasteurization due to water evaporation, and protein unfolding caused increased solvation.

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**CONCLUSION**

Iron, zinc and vitamin A are essential micronutrients for human health, but the deficiencies therein remain an unsolved problem in a lot of countries including Vietnam. To solve micronutrient deficiency, fortification is normally applied to a popular food ingredient, such as tamarind fish sauce. The basic formulation of Vietnamese tamarind fish sauce consisted of 42% of tamarind slurry, 37% of sugar and 21% of fish sauce. The appropriate fortification levels consist of 42% of tamarind slurry, 37% of sugar and 21% of fish sauce. The appropriate fortification levels are fortified into dairy products such as milk, iron bound with amino acids in casein micelles, while the zinc was associated with colloidal calcium phosphate in casein micelles. Thus, the content of available iron and zinc may be reduced. The reductions in the iron and zinc of tamarind fish sauce by pasteurization might be due to the interactions of the iron, zinc and sauce components, especially proteins in the fish sauce. The vitamin A palmitate content in the fortified sauce after pasteurization significantly decreased, from 10000 IU to 7306 IU (p < 0.05) (Table 6).

TFS (Traditional tamarind fish sauce); FTFS (Fortified tamarind fish sauce); PFTFS (Pasteurized tamarind fish sauce). Mean ± SD from the triplicate measurements of 2 different lots. a-bMeans within a column with different superscripts are significantly different at p < 0.05.

It is known that vitamin A is fat soluble [23] and with a low fat content in the tamarind fish sauce, vitamin A seemed to move up to the surface of the sauce where it was oxidized or easily degraded by thermal processing. Moreover, in a transparent bottle, light also causes loss of vitamin A. [33] reported that the content of vitamin A in milk was decreased by heating. Pasteurization not only reduced the trace elements and vitamin A content in the tamarind fish sauce but also degraded its organoleptic properties (Table 7). After pasteurization, the appearance was darker with a slight unpleasant smell, and the texture was no longer homogenous.

**Table 5.** Physical characteristics of fortified Vietnamese tamarind fish sauce before and after pasteurization

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Color coordinates</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>TFS</td>
<td>27.47 ± 0.23</td>
<td>17.06 ± 0.77</td>
</tr>
<tr>
<td>FTFS</td>
<td>20.70 ± 0.30</td>
<td>11.25 ± 0.33</td>
</tr>
<tr>
<td>PFTFS</td>
<td>18.75 ± 0.40</td>
<td>10.30 ± 0.30</td>
</tr>
</tbody>
</table>

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Table 6. Chemical qualities of fortified Vietnamese tamarind fish sauce before and after pasteurization

<table>
<thead>
<tr>
<th>Property</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TFS</td>
</tr>
<tr>
<td>pH</td>
<td>3.41 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total titratable acidity (%)</td>
<td>28.18 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aw</td>
<td>0.88 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (mg/30g)</td>
<td>0.13 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc (mg/30g)</td>
<td>0.042 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>0.00 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>FTFS</td>
</tr>
<tr>
<td>pH</td>
<td>3.21 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total titratable acidity (%)</td>
<td>29.63 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aw</td>
<td>0.87 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (mg/30g)</td>
<td>36.00 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc (mg/30g)</td>
<td>15.04 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>10.000 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>PFTFS</td>
</tr>
<tr>
<td>pH</td>
<td>3.23 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total titratable acidity (%)</td>
<td>29.07 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aw</td>
<td>0.854 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (mg/30g)</td>
<td>30.73 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zinc (mg/30g)</td>
<td>14.21 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>7.306 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 7. Sensory acceptability score of fortified Vietnamese tamarind fish sauce before and after pasteurization

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Attributes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance</td>
</tr>
<tr>
<td></td>
<td>TFS</td>
</tr>
<tr>
<td></td>
<td>FTFS</td>
</tr>
<tr>
<td></td>
<td>PFTFS</td>
</tr>
</tbody>
</table>

Note. TFS (Traditional tamarind fish sauce); FTFS (Fortified tamarind fish sauce); PFTFS (Pasteurized tamarind fish sauce). Mean ± SD from 50 panelists; <sup>a</sup>MMeans within a column with different superscripts are significantly different at p < 0.05.

ACKNOWLEDGMENTS

This study was supported by the Higher Education Research Promotion and the Thailand’s Education Hub for Southern Region of ASEAN Countries Project Office of the Higher Education Commission. The authors would like to thank Asst. Prof. Kongkarn Kijroongrojana for the Design-Expert Statistical package version 7.0, and the team of Research and Development Office (RDO) of Prince of Songkla University, Thailand for the assistance with English.

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EFFECT OF VARIOUS DOSES OF IONIZING RADIATION ON THE SAFETY OF MEAT SEMI-FINISHED PRODUCTS

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Abstract. The increase in the shelf life of perishable food products is one of the priority trends in the development of the food and processing industry. A study has been carried out to determine the effect of various doses of ionizing radiation on the safety of refrigerated semi-finished pork products stored in the modified atmosphere. The meat samples were processed singly by ionization at different doses – 8 kGy and 12 kGy with the help of a linear electron accelerator of a UELR-10-10S2 type with power of up to 10 MeV. The refrigerated pork neck on the bone, packed using modified atmosphere packaging (MAP) and processed by ionization at doses of 8 kGy and 12 kGy, meets the requirements of the technical regulations of the Customs Union "On Food Safety" (TR TS 021/2011) and "On safety of meat and meat products" (TR TS 034/2013) for the entire storage period. The irradiation dose of 12 kGy leads to a slight color change, an increase in the acid and peroxide numbers and volatile fatty acids, but within the limits of the norm; a slight decrease in the moisture content with a high degree of correlation of the studied indicators. The indicators of microbiological safety of the meat semi-finished products processed by different irradiation doses are within the normal range. It has been established that the higher the irradiation dose, the lower the values of microbiological indicators. The organoleptic indicators are confirmed by histological studies. The radiation processing of meat semi-finished products allows us to prolong the shelf life of the meat semi-finished products packed using MAP more than 3 times. Based on the results of the studies, it is possible to recommend the processing of meat semi-finished products by ionization at a dose of 8 kGy to increase their shelf life.

Keywords: Pork, processing by ionization, storage time, packaging, modified atmosphere, radiation processing


INTRODUCTION

The increase in the shelf life of perishable food products is one of the priority trends in the development of the food and processing industry. To provide the quality of refrigerated meat semi-finished products, an ESL (Extended shelf life) technology is used in the process of storage, which consists in packaging products into a vacuum skin pack (Vacuum Skin Packaging) and the modified atmosphere (MAP) [1, 2, 3]. For red meat, MAP is used with a high oxygen content [4, 5], since at an oxygen concentration of 70–80% oxymyoglobin is in the surface layers of meat and makes it look fresh [6, 7]. But at the same time, the high oxygen content in the package activates the oxidative processes of lipids in meat (the oxidation of polyunsaturated fatty acids), which have a negative effect on such organoleptic indicators as consistency, taste and color [4, 8]. In addition, the oxygen of the medium promotes the conversion of bright red oxymyoglobin into gray-brown metmyoglobin during storage [9]. The carbon dioxide of the medium prevents bacteria from multiplying in meat: at a concentration of 25%, the maximum suppression of aerobic microflora is achieved [3]. The authors of [2, 3] found that when storing pork in a vacuum skin pack and in MAP, the number of populations of psychotropic bacteria is comparable. At the same time, the lowest content of lactic acid bacteria was recorded in the pork packed using MAP. But the packaging of meat in a vacuum contributes to the oozing of meat juice, which accumulates in the folds of the surface of the packaging film, which worsens the consumer properties of food products [10] and is a medium for microbiological spoilage. It should be noted that refrigerated meat has a high water activity index. The Food Codex (USA) reflects recommendations for determining food storage conditions depending on pH and the water activity level [11]. As a result of the use of such technological methods as drying, freezing and adding preserving agents (during adsorption and osmosis), the water activity decreases, the enzymatic processes of lipid oxidation and the growth of microorganism colonies

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slow down. The author of [12] notes the significant role of water activity (AW) in ensuring the safety and quality of food.

But at the same time, the current technologies, despite the fact that the ESL technology is most often used in the storage of refrigerated meat semi-finished products and meat products, cannot be called perfect. Today, Russia actively introduces the technology of processing raw meat materials (except for poultry, rabbit and horse meat) by ionization. This technology can be used to process meat raw materials both in carcasses and in packaging. The processing of food products by ionization helps to reduce its microbiological contamination, which, accordingly, allows us to extend the shelf life [13–16]. It follows from the literature [17–19] that various doses of irradiation of food products may negatively affect their chemical composition. At the same time, the irradiation of food products with a significant fat content can lead to the deterioration of organoleptic indicators, since the free radicals formed during irradiation accelerate lipid oxidation [20, 21].

In this regard, the studies have been carried out of the effect of different irradiation doses on the shelf life of the refrigerated meat raw materials packed using MAP and other quality indicators during storage.

STUDY OBJECTS AND METHODS

To carry out the studies, three groups of meat semi-finished products – pork meat on the bone from pork collar butt, were formed in accordance with the established butt cut boundaries: the anterior boundary was along the line of the head cut, the posterior boundary was between the 4th and 5th thoracic vertebrae and the lower boundary was along the ventral margin of the cervical and thoracic vertebrae (TU-10.11.10-008-57668367 “Meat. Beef and pork cut for retail. Specifications” with a shelf life of up to 10 days at a temperature of 0 ± 4.0°C). The first group is the control group, the second and the third are the experimental ones. The domestic source raw materials were in compliance with the requirements of GOST 31476-2012 “Meat and meat products. General requirements and methods of microbiological testing”, GOST 31747-2012 (ISO 4831:2006, ISO 4832:2006) “Food products. Methods of detection and quantity determination of coliforms", GOST R 54346-2011 "Meat and meat products. Methods of determination of fat", GOST 19496-2013 "Meat and meat products. Methods for determination of peroxide number", GOST 23042-2015 "Meat and meat products. Method for determination of peroxide number", GOST 21807-2015 "Microbiology of food and animal feeding stuffs. Determination of water activity", GOST 9793-2016 "Meat and meat products. Method for determination of moisture content", GOST 55480-2013 "Meat and meat products. Method for determination of acid value", the peroxide number of fat – according to GOST R 54346-2011 "Meat and meat products. Method for determination of peroxide number", water activity – according to GOST ISO 21807-2015 "Microbiology of food and animal feeding stuffs. Determination of water activity", meat histology – according to GOST 19496-2013 "Meat and meat products. The method of histological investigation", volatile fatty acids – according to GOST 233819-2016 "Meat and meat products. Determination of volatile fatty acids by gas chromatography". The physicochemical parameters of meat semi-finished products were determined by infrared spectroscopy using a Food Scan Meatanalyser (Denmark production) express analyzer.

The quality of meat was studied at the Joint-Stock Company Food Combine "Good taste" and on the basis of the Department of Food Engineering of USE (Ekaterinburg). The results of the studies were statistically processed using such standard computer programs as Microsoft Excel XP and Statistica 8.0.

RESULTS AND DISCUSSION

In accordance with GOST R 52529-2006 "Meat and meat products. Method of electron paramagnetic resonance for indication of radiation-treated meat and meat products containing bones", the source meat raw materials were studied using electron paramagnetic resonance. EPR signals were not detected in the samples studied, therefore, the products were not previously processed by ionization (Fig. 1).
The samples of meat of the control group can be referred to fresh meat in organoleptic indicators after 10 days of storage: the meat is moistened in places, red; the muscles on the cut are slightly moist and leave a moist spot on the filter paper; the consistency is elastic, dense; the dimple formed when pressing with a finger is quickly leveled; the odor is specific, characteristic of fresh pork; the lard color is white, the consistency is characteristic of pork fat – soft, elastic; the tendons are elastic, dense; the surface of the vertebrae is smooth, shiny; the broth is fragrant, transparent. After 20 days of storage, the meat of the control group is of questionable freshness: the meat is gray-red, when in contact with the film – gray; the muscles on the cut are moist, leave a spot on the filter paper, slightly sticky; the dimple is leveled slowly when pressing with a finger; after 30 days of storage, the meat of the control group is stale: covered with mucus, the color is gray with the presence of green areas; there are signs of bacterial microflora; the muscles on the cut are swollen, loosely adhere to each other, sticky; the consistency is flabby; the fat has a grayish-mat shade, the slimy consistency; the connective tissue is loose with the signs of destruction; the smell of meat and odor of broth is unpleasant, harsh; the broth is muddy, with a lot of flakes resulting from the aggregation of proteins.

The meat irradiated at a dose of 8 kGy (the second experimental group) and 12 kGy (the third experimental group) after 10, 20 and 30 days of storage refers to fresh meat in organoleptic indicators. A slight change in color to brownish-red and the appearance of the smell of light oxidation plume not characteristic of fresh meat should be noted in the samples of the third group.

The analysis of the obtained histological study data by revealing changes in the microstructure of the muscle tissue corresponds to the results of the organoleptic estimation of meat. It has been established that the samples of meat of the control group after 10 days of storage and the meat of the second and third experimental groups for the whole storage period are fresh: the structure of the nuclei of muscle fibers is distinct, the color is uniform; the striation of muscle fibers is clear and distinct (Fig. 2–4).

After 20 days of storage, the structure of the nuclei of muscle fibers in the meat of the control group is indistinct, the color is non-uniform, the striation of the muscle fibers is indistinct, the muscles loosely adhere to each other, and there is a microflora in the form of weak patches. Such meat refers to the meat of questionable freshness (Fig. 5).
After 30 days of storage, the meat of the control group showed the almost complete disappearance of nuclei, the almost complete disappearance of the striation of muscle fibers, the mucilaginous areas of the surface of meat have a dark reddish-brown color; the muscle fibers are swollen, deformed, loosely adhere to each other; muscle ruptures can be noted with the formation of a fine-grained protein mass; there is a microflora in the form of multiple patches (Fig. 6).

The results of the studies of organoleptic indicators and the histology of meat are confirmed by the microbiology of the control and experimental samples of pork neck on the bone packed using MAP (Table 1).

It has been established that the microbiological parameters of pork neck on the bone of all the three groups after 10 days of storage meet the requirements of TR TS 034/2013 "On safety of meat and meat products". The irradiation processing of the refrigerated meat semi-finished products packed using MAP has a positive effect on their shelf life. Thus, in the samples of meat of the second and third groups, QMAFAANM and coliforms were not detected after 20 days of storage, and after 30 days of storage all the microbiological indicators under study did not exceed the maximum allowable level. It has been established that the higher the radiation dose, the lower the values of microbiological indicators.

The microbiological parameters of pork neck on the bone of the control group after 20 and 30 days of storage do not meet the requirements of TR TS 034/2013 "On safety of meat and meat products" in the indicators of QMAFAANM and coliforms, the yeast content has the maximum allowable value of $1.0 \times 10^7$ CFU/g.

Table 1. Microbiological indicators of the control and experimental samples of the refrigerated pork neck on the bone packed using MAP during storage (n = 5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1 – control (the non-irradiated pork)</td>
</tr>
<tr>
<td></td>
<td>After 10 days of storage</td>
</tr>
<tr>
<td>Total viable count, CFU/g</td>
<td>$0.9 \times 10^2$</td>
</tr>
<tr>
<td>Coliforms (g), not allowed in 0.01 g of product</td>
<td>Not detected</td>
</tr>
<tr>
<td>Pathogenic microorganisms (g), including salmonella and listeria</td>
<td>Not detected</td>
</tr>
<tr>
<td>Yeast, CFU/g</td>
<td>$0.9 \times 10$</td>
</tr>
<tr>
<td>Sulfite-reducing clostridia in 0.01 g</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>After 20 days of storage</td>
</tr>
<tr>
<td>Total viable count, CFU/g</td>
<td>$0.8 \times 10^4$</td>
</tr>
<tr>
<td>Coliforms (g), not allowed in 0.01 g of product</td>
<td>Not detected</td>
</tr>
<tr>
<td>Pathogenic microorganisms (g), including salmonella and listeria</td>
<td>Not detected</td>
</tr>
<tr>
<td>Yeast, CFU/g</td>
<td>$6.0 \times 10^2$</td>
</tr>
<tr>
<td>Sulfite-reducing clostridia in 0.01 g</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Fig. 6. Striated muscle tissue of the control samples of the refrigerated pork neck on the bone packed using MAP after 30 days of storage.
Table 2. Parameters of purity of fats in the control and experimental samples of the refrigerated pork neck on the bone packed using MAP during storage ($M \pm m; n = 5$)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>No. 1 – control (the non-irradiated pork)</th>
<th>No. 2 – experimental (the pork irradiated with a dose of 8 kGy)</th>
<th>No. 3 – experimental (the pork irradiated with a dose of 12 kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 10 days of storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid number, mg KOH/g of fat</td>
<td></td>
<td>0.81 $\pm$ 0.03</td>
<td>0.80 $\pm$ 0.02</td>
<td>0.95 $\pm$ 0.03</td>
</tr>
<tr>
<td>Peroxide number, mmol of active oxygen/kg of fat</td>
<td></td>
<td>1.72 $\pm$ 0.03</td>
<td>1.71 $\pm$ 0.08</td>
<td>1.95 $\pm$ 0.07</td>
</tr>
<tr>
<td></td>
<td>After 20 days of storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid number, mg KOH/g of fat</td>
<td></td>
<td>2.09 $\pm$ 0.20</td>
<td>0.96 $\pm$ 0.10</td>
<td>1.10 $\pm$ 0.20</td>
</tr>
<tr>
<td>Peroxide number, mmol of active oxygen/kg of fat</td>
<td></td>
<td>4.76 $\pm$ 0.20</td>
<td>1.86 $\pm$ 0.20</td>
<td>2.27 $\pm$ 0.10</td>
</tr>
<tr>
<td></td>
<td>After 30 days of storage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid number, mg KOH/g of fat</td>
<td></td>
<td>4.92 $\pm$ 0.30</td>
<td>1.09 $\pm$ 0.20</td>
<td>1.27 $\pm$ 0.20</td>
</tr>
<tr>
<td>Peroxide number, mmol of active oxygen/kg of fat</td>
<td></td>
<td>10.33 $\pm$ 0.60</td>
<td>2.03 $\pm$ 0.10</td>
<td>2.94 $\pm$ 0.30</td>
</tr>
</tbody>
</table>

The indicators of fat purity have been studied (Table 2). It has been established that the acid (AN) and peroxide (PN) numbers of the fat extracted from the meat samples of the second group have lower values after 10, 20 and 30 days of storage compared to the meat samples of the control and third experimental group. Consequently, the higher the dose of irradiation, the more active the oxidation processes in meat systems, which is consistent with the literature data [20, 21]. It should be noted that AN and PN of fat in the first group after 30 days of storage is at a level of 4.92 mg KOH/g of fat and 10.33 mmol of active oxygen/kg of fat, respectively, which exceeds the norm (4.0 mg KOH/g of fat and 10.0 mmol of active oxygen/kg of fat) for fresh fat.

Figure 7 shows the dynamics of volatile fatty acids (VFA) during the storage of the control and experimental samples of the refrigerated pork neck on the bone packed using MAP irradiated at different doses.

The indicators of VFA in the experimental samples of meat irradiated at different doses correspond to fresh meat. Thus, after 10, 20 and 30 days of storage in the second experimental group, they were 0.6 mg, 1.5 mg and 2.2 mg KOH in 25 g, which is below the control sample by 60.0%, 73.2% and 76.1%, respectively. Similar results on a change in the amount of fatty acids during storage have been obtained in the samples of meat of the third experimental group: the indicators of VFA after 10, 20 and 30 days of storage are below control by 40.0%, 46.4% and 56.5%, respectively. In the samples of meat of the control group after 20 days of storage, the content of VFA is 5.6 mg KOH in 25 g, which corresponds to the meat of questionable freshness; after 30 days of storage – 9.2 mg KOH in 25 g, which corresponds to stale meat.

The studies of the chemical composition of meat raw materials during storage (Fig. 8–10) have been carried out. The high degree of correlation of a change (decrease) in the water content depending on the radiation dose, which is within the range from 0.891 immediately after irradiation to 0.995 on the 30th day of storage has been experimentally revealed. A decrease in the moisture content in all the samples studied and a decrease in the moisture content in the meat semi-finished products during storage can be noted (Fig. 8). Thus, after 30 days of storage in the first
control group, the amount of moisture decreased by 1.3%, in the second and third experimental groups – by 2.1% and 2.3%, respectively. It has been established that the processing of meat by ionization contributes to the reduction of moisture in a food product. Thus, after irradiating the samples of meat of the second experimental group at a dose of 8 kGy, there is a decrease in the water content by 0.3%, after irradiating the meat samples of the third experimental group at a dose of 12 kGy – by 1.2% compared to the unirradiated samples of the meat of the first control group. Similar results were obtained during the storage of meat raw materials. Thus, after 10 days of storage, the amount of moisture decreased by 0.5% and 1.5%, respectively, after 20 days – by 0.8% and 1.9%, respectively, after 30 days – by 1.1% and 2.2% %, respectively. The decrease in the water content is due to the radiolysis of water as a result of irradiation with the appearance of free hydroxyl groups (-OH), the atoms of hydrogen molecules and hydrogen peroxides (H₂O₂).

Consequently, the processing of meat by ionization increases the losses of moisture during storage, which, possibly, creates unfavorable conditions for the development of microflora.

The activity of water, along with the total content of water in the refrigerated meat, is important for the shelf life of meat. Water activity is the indicator of the presence of free water, which is a medium for the acceleration of enzymatic processes (in particular, lipid oxidation) and the vital activity of microorganisms. The activity of water after irradiation in the meat samples of the second experimental group is 0.934 units, in the third experimental group – 0.921 units, which is 0.025 units (or 2.6%) and 0.038 units (or 4.0%), respectively, lower than the meat samples of the first control group. It should be noted that the obtained results are below the mid-values of water activity for meat (0.95–1.00 units). As a result of the studies, an insignificant decrease in the value of water activity during storage has been established: in the samples of meat of the second experimental group by 0.003 units and the third group – by 0.005 units. The results obtained are consistent with a change in the microbiological indicators in the studied meat samples (Table 1). The reduction of water activity in the meat samples of the experimental groups contributes to a slowdown in the processes of microbial spoilage. It has been established that the higher the dose of irradiation, the lower the water activity indexes and the microbiological indicators. Consequently, when processing by ionization, the development of microorganisms slows down as a result of a decrease in water activity. The revealed tendency is consistent with the studies of a number of authors [23, 24].

The study of the dynamics of fat during storage has been carried out (Fig. 9).

During storage, there is a decrease in the fat content in the studied samples of meat products. Thus, in the control samples – by 4.2%, in the second and third experimental groups – by 4.3% and 4.8%, respectively, after 30 days of storage (Fig. 6). An increase in the irradiation dose of up to 12 kGy enhances the lipid oxidation processes due to the destruction of ether bonds in triglycerides with the presence of water, which is followed by the accumulation of free fatty acids, as well as peroxide compounds. The data obtained are consistent with the studies [25].

An increase in the proportion of protein in the dry residue in all the meat samples has been noted. Thus, in the samples of meat of the first, second and third groups – by 0.9%, 1.5% and 1.9%, respectively (Fig. 10). A high degree of correlation dependence of an increase in the proportion of proteins in the dry residue during storage has been established: for the unirradiated pork – 0.973, for the pork irradiated at a dose of 8 kGy – 0.997, for the pork irradiated at a dose of 12 kGy – 0.997. The protein to fat ratio in the dry residue in all the groups of samples of pork neck on the bone increases during storage: for the first group of samples at the beginning of the experiment from 1.33 : 1.00 to 1.51 : 1.00 by the end of the experiment; for the second group of samples – from 1.35 : 1.00 to 1.55 : 1.00, respectively; for the third group of samples – from 1.43 : 1.00 to 1.69 : 1.00, respectively. The protein to fat content ratio has the higher values of indicators with an increase in the radiation dose, which is in agreement with the opinion of researchers [26].
Thus, based on the results of complex studies it has been established that the refrigerated pork neck on the bone packed using MAP and processed by ionization at doses of 8 kGy and 12 kGy, corresponds to the requirements of the technical regulations of the Customs Union "On Food Safety" (TR TS 021/2011) and "On safety of meat and meat products" (TR TS 034/2013) for the entire storage period. Consequently, the radiation processing of meat semi-finished products at doses of 8 kGy and 12 kGy allows us to prolong the shelf life of the meat semi-finished products packed using MAP more than 3 times from 10 days to 30 days and more. The radiation dose of 12 kGy leads to an insignificant color change and the appearance of a slight oxidation plume; an increase in the acid and peroxide numbers of the fat extracted from meat raw materials, but within the limits of the norm; an increase in the content of volatile fatty acids; an insignificant decrease in the moisture content, water activity, the fat content and an increase in the proportion of protein in the dry residue of the meat semi-finished product packed using MAP in comparison with the meat packed using MAP and processed at a radiation dose of 8 kGy with a high degree of correlation of the studied parameters. The indicators of microbiological safety of the meat semi-finished products processed at different doses of irradiation are within the limits of the norm: the higher the dose of irradiation, the lower the values of microbiological indicators. The organoleptic quality indicators are confirmed by the histological studies of muscle tissue in accordance with the requirements of GOST 19496-2013. According to the results of the studies, it is possible to recommend the processing of the refrigerated meat semi-finished products packed using MAP by ionization at a dose of 8 kGy to increase their shelf life.

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POSSIBILITIES OF ENRICHING FOOD PRODUCTS WITH ANTHOCYANINS BY USING NEW FORMS OF CEREALS

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Abstract: At present, there are a number of high-tech trends in the development of food raw materials in the world market, the future nutritional value and composition of which can be laid even at the stage of breeding a variety, as a result of production of which the raw materials will be obtained containing the specified components — vitamins, minerals and biologically active compounds. It is possible to refer to such kinds of food raw materials common wheat of a purple color which has a high content of anthocyanins. Despite the antioxidant capacity and the related health benefits, the studies on the use of raw materials with a high level of anthocyanins as an ingredient for food products are extremely rare. This article presents the results of the use of food products of new forms of cereals in production, namely, the isogenic wheat lines specially created for comparative studies at the Institute of Cytology and Genetics of the SB RAS with the help of molecular genetic methods, which only differ in a small segment of the genome containing the regulatory anthocyanin biosynthesis gene \textit{Pp3/TaMyc1}. Flour and wheat bran with anthocyanins (PG) and the control (RG) group were used for the production of flour confectionery products in order to obtain products with a high level of anthocyanins as an ingredient for food products are extremely rare. This article presents the results of the use of food products of new forms of cereals in production, namely, the isogenic wheat lines specially created for comparative studies at the Institute of Cytology and Genetics of the SB RAS with the help of molecular genetic methods, which only differ in a small segment of the genome containing the regulatory anthocyanin biosynthesis gene \textit{Pp3/TaMyc1}. Flour and wheat bran with anthocyanins (PG) and the control (RG) group were used for the production of flour confectionery products in order to obtain products with a high level of anthocyanins beneficial for human health. The share of anthocyanins in the products obtained from PG wheat was 2.5–2.6 times higher than in the similar products obtained on the basis of the control line. The revealed differences between PG and RG in the end-use products testify to the resistance of anthocyanins to technological processing. It has been estimated that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of anthocyanins will be up to 0.83 mg. Thus, a high content of anthocyanins in PG allows to produce the enriched confectionery products with a high nutritional value.

Keywords: Enriched food products, bioflavonoids, anthocyanins, food market, cereals, flour confectionery, nutritional value


INTRODUCTION

The main trends in the development of the industry in the field of healthy nutrition in Russia include the development of technologies for the production of qualitatively new common and special food products with the specified properties based on the new types of raw materials obtained using new technological methods. Scientific researches of A.A. Pokrovsky, V.A. Tutelyan, M.M. Gaporov, V.B. Spirichev and V.M. Poznyakovskiy and other scientists in our country made a great contribution to solving the problems of the development and manufacturing of nutrient-rich food products.

The basis of the modern food production strategy is to find the new resources of essential food components, to use the non-traditional types of raw materials and create the new advanced technologies that increase the nutritional and biological value of a product and give it the desired properties and extend its shelf life. Food enrichment is an effective way to eliminate the deficit of essential nutrients in human nutrition (Pokrovsky, 2002) [1].

The creation of consumer good products with a high food and biological value requires the expansion and improvement of the raw materials base of the food industry. The modern molecular genetics offers some approaches that, when combined with traditional breeding methods, make it possible to accelerate the creation of new varieties. At present, there are a number of high-tech trends in the development of food raw materials in the world market, the future nutritional

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value and composition of which can be laid even at the stage of breeding a variety, as a result of production of which the raw materials will be obtained containing the specified components – vitamins, minerals and biologically active compounds. For example, it has been shown that it is possible to regulate the content of amyllose in bread (to increase the proportion of resistant starch for a diet for celiac disease) or biologically active polyphenolic compounds (anthocyanins) by creating plants with the specified characteristics even at the stage of wheat selection (Schönhofer et al., 2017; Khlestkina et al., 2017) [2, 3].

Plants with the specified content of protein, carbohydrates, microelements, vitamins and the secondary metabolites useful for human health (for example, the polyphenolic compounds which are plenty in red grapes, tea, wine, pomegranate, blueberry and cranberry, but which can also be synthesized by some varieties of cereals) can be created by using the natural variability of wheat and other cereals.

This article describes the possibility of increasing the content of anthocyanins in flour confectionery production using a new type of raw materials – common wheat of a purple color, development of which can be essentially accelerated under control of diagnostic molecular genetic markers. The choice of such a format as flour confectionery products for the creation of fortified food products with a high content of anthocyanins was determined, first of all, by the global production.

Flour confectionery products are the largest segment of the confectionery market in terms of sales, and the biscuit market, in turn, constitutes a significant part of the group of flour confectionery products. In 2010–2015, the production of biscuit and other flour confectionery products for long-term storage in the Russian Federation, according to Rosstat [Russian Statistics Committee], showed a steady positive trend, its growth was 126.6% over the period (from 1,097 thousand tons to 1389 thousand tons). At the same time, it should be noted that there is a significant proportion of the products in this group that do not meet the principles of healthy nutrition and, therefore, the developments of such product formulations are needed that preserve the usual taste properties and have a high nutritional value.

STUDY OBJECTS AND METHODS

**Plant material.** Spring bread wheat near-isogenic lines (red-grained, RG (i:S29Pp-A1Pp-D1pp3² with an uncoloured pericarp) and a purple-grained, PG ((i:S29Pp-A1Pp-D1pp3²)), differing in a small segment of 2A chromosome (between the markers Xgwm339 and Xgwm817) containing the regulatory anthocyanin biosynthesis gene Pp3/TaMyc1 (Tereshchenko et al., 2012; Shoeva et al., 2014; Gordeeva et al., 2015) [4, 5, 6] were used for accurate comparative estimation.

The RG and PG wheats were grown in the Novosibirsk region at the experimental site of the selection and genetic complex of the Institute of Cytology and Genetics of SB RAS (55°02'N, 82°56'E) in the summer of 2016.

**Estimation of the technological properties of flour, bran and the quality of the finished products.** In the development of products from wheat that synthesizes bioflavonoid pigments anthocyanins, the flour was used from the grain of the RG and PG lines, as well as the bran obtained by milling these samples of grain. For the purposes of a comparative analysis, wheat flour of the premium grade was used (GOST R 52189-2003).

To estimate the quality of raw materials, standard methods were used (GOST 27676-88; GOST 27839-2013): the humidity was determined using an accelerated method for drying in a drying cabinet, acidity – using the method for the acid-base titration of water-flour dough; whiteness – using a R3-TBMS-M whiteness meter; the falling number – using ChP-1; the mass fraction of crude gluten was obtained by washing with water followed by weighing; the quality of crude gluten was characterized by color, elastic properties (using IDK-3M) and its hydration ability; the gas-forming ability of flour – using the volumetric method using Yago-Ostrovsky's device.

To estimate the quality of the end-use products, their humidity and absorption ability were determined using standard methods (GOST 5900-14, GOST 10114-80). To obtain the objective estimation of organoleptic indicators, closed tasting estimation was performed, expressed in points according to a 30-point scale. Table 1 provides product variants.

The studied products were enriched by modifying the regulatory trade formulation of Ovsyanochka biscuit, namely by replacing and / or supplementing separate components with experimental raw materials with a high content of anthocyanins.

In the first two variants (1 and 2), the formulation replaced oatmeal with the bran from RG and PG flour. The third and fourth variant envisaged not only the addition of bran instead of oatmeal, but also the replacement of wheat bread flour with PG and RG flour. Thus, the fourth variant provided for the maximum use of raw materials with a high content of anthocyanins.

Table 1. Variants of products made and studied in the present paper

<table>
<thead>
<tr>
<th>No.</th>
<th>Flour as per GOST R 52189–2003</th>
<th>Flour from the grain of the red grain strain</th>
<th>Used flour and bran</th>
<th>Flour from the grain of the purple grain strain</th>
<th>Bran from the grain of the red grain strain</th>
<th>Bran from the grain of the purple grain strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>
Analysis of the content of anthocyanins and antioxidants in bran and finished products. The extracts were obtained by adding 10 ml of a 1% aqueous solution of HCI to 1 g of the ground sample, mixing and incubating for 1 hour at 37°C. The supernatant obtained by centrifugation for 15 min at 4°C at 5000 rpm was used for the analysis.

The anthocyanin content was estimated using a SmartSpec™ Plus spectrophotometer (BioRad, www.bio-rad.com). The recalculation from OD530-700 into the mass concentration was carried out using the method described above (Abdel-Aal, Hucl, 1999) [7] using cyanidin-3-glucoside as a standard. The antioxidant activity was estimated using a Blizar antioxidant activity analyzer (Interlab, Russia) according to the manufacturer's instructions. Gallic acid (mg/l) was used as a reference substance.

The average of the three successive measurements was used for the analysis. The significance of differences between the samples, in terms of the studied parameters, was estimated using the Mann-Whitney test.

RESULTS AND DISCUSSION

Tables 2 and 3 present the results of the comprehensive analysis of the used flour and bran, respectively. The mass fraction of the crude gluten of flour from the grain of the red and purple grain strains was practically the same and very high, much higher than that of wheat flour of the premium grade. At the same time, the gluten was poor in quality and long in elasticity, but had a good hydration ability. On the basis of the "falling number" indicator, the low autolytic activity of flour from the grain of RG and PG strains was detected, which may be due to the low activity of its own enzymes and/or a starch state.

Four biscuit variants were made and analyzed in the course of trial baking: two variants with the replacement of oatmeal with bran from RG and PG flour, two variants with the addition of bran instead of oatmeal and the replacement of wheat bread flour with PG and RG flour (see Table 1).

The following parameters were used to estimate the quality of products: shape, surface, color, structure, a shape in the fracture, taste and water absorption (Table 4).

To obtain the objective estimation of organoleptic indicators, closed tasting estimation was performed, expressed in points according to a 30-point scale.

The wheat flour products with the addition of PG bran had the highest scores, according to the organoleptic estimation, due to the high estimate of taste and aroma, texture and consistency (Table 5). Biscuit from PG flour and bran was looser with a distinct porous structure. Water absorption is higher in the samples with the addition of PG bran.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Round</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface</td>
<td>Rough with interspersions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Gold brown</td>
<td>Brown</td>
<td>Gold brown</td>
<td>Brown</td>
</tr>
<tr>
<td>Structure</td>
<td>Loose</td>
<td>Loose</td>
<td>Gold brown</td>
<td>Loose with a porous structure</td>
</tr>
<tr>
<td>Shape in the fracture</td>
<td>Loosened</td>
<td>More loosened</td>
<td>Loosened</td>
<td>More loosened</td>
</tr>
<tr>
<td>Taste</td>
<td>Natural, without a foreign taste</td>
<td>Distinct slightly nutty</td>
<td>DISTINCT</td>
<td>Distinct, slightly nutty</td>
</tr>
<tr>
<td>Smell</td>
<td>Natural, without a foreign taste</td>
<td>Natural, without a foreign taste</td>
<td>Distinct</td>
<td>Distinct</td>
</tr>
<tr>
<td>Water absorption, %</td>
<td>192.0</td>
<td>209.0</td>
<td>180.0</td>
<td>211.0</td>
</tr>
</tbody>
</table>

Note. GDI is a gluten deformation index.
The unusual color of products with the addition of bran and/or flour from PG was not a negative factor in estimating the quality of products. The addition of bran was estimated well thanks to a "crispy taste" – a smoothly pronounced crispy taste.

Fig. 1 presents the results of anthocyanin content in bran and the finished products in terms of cyanidin-3-glucoside (numbers 1–6 of products are according Table 1). Calculated equivalent to gallic acid.

The content of anthocyanins in the bran of the PG strain was about 4.5 times more than that in the bran of the RG strain. The share of anthocyanins in the products obtained from flour and bran of the PG strain was 2.6 times higher than in the case of a similar product obtained on the basis of RG.

In the case of using GOST flour, the addition of PG bran resulted in an anthocyanin content of 2.5 times higher than when using RG bran. The revealed differences between PG and RG not only in bran, but also in the finished products testify to the resistance of anthocyanins to technological processing. The calculations show that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of available anthocyanins can reach up to 0.83 mg.

Fig. 2 presents the results of the estimation of the content of antioxidants in bran and the finished products. Bran has a high mass fraction of antioxidant compounds. This indicator does not depend on the origin of bran (RG or PG).

The comparison with the earlier obtained data shows that the amount of the antioxidants consumed with biscuit, which is made with the addition of bran (Figure 2), is not lower than that when consuming bread (Khlestkina et al., 2017) [3].

There are continuous changes in the structure of a variety of confectionery products, since innovations are immanently inherent in the food industry and are the strategic parameter of its development. At present, product developments are aimed not only at providing an attractive appearance and taste variety, but also at taking into account the interests of the consumers who evaluate the useful properties of new products closely.

The new properties of products that provide an increase in their nutritional and biological value, meet the modern world trends in the field of healthy nutrition and allow us to form new niches in the field of various specialized functional products. At the same time, the characteristic feature of modern food products is the complexity of their formulations, and the manufacturing of a food product of a certain nutritional value is provided by the content of a large number of food ingredients of a different chemical nature (Usenko, 2016) [8].

The enrichment of food products with natural ingredients has an advantage over chemical preparations and premixes, and is as an alternative to the processes of chemicalization in the food industry. The composition of these products, in addition to vitamins and minerals, includes protein substances, dietary fiber and other valuable food components, which are in the form of natural compounds and in a form that is better absorbed by the body.

In terms of the orientation to manufacture natural products, researchers are increasingly focusing on the creation of new types of raw materials, the future nutritional value and composition of which can be laid even at the stage of creating a variety. A special place in these studies is held by the products with a high content of flavonoids. Polyphenols are mainly represented by flavonoid compounds, which include both a number of colorless compounds, for example, rutin, and colored molecules (for example, anthocyanins, which give a pinkish, blue and red color to plant tissues). It is not only customary berries that it is not only customary berries that can be brightly colored, but also cereal grain. In this case, anthocyanins are only synthesized in the aleurone layer and the pericarp giving the grain a bluish or purple color, respectively (Adzhieva et al., 2015) [9].

Since anthocyanins perform protective functions in plants, in particular, protect their tissues from excessive UV radiation, donor plants of an anthocyanin color of grain were found in the areas with a high level of insolation, for example, in Ethiopia. The "collectors" of

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**Table 5. Quality parameters of organoleptic indices (numbers 1–4 of products are according Table 1)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average score</td>
<td>22.5</td>
<td>23.2</td>
<td>20.2</td>
<td>21.0</td>
</tr>
</tbody>
</table>

**Fig. 2. Content of antioxidants (mg/g) in biscuit made with the addition of the bran of the control strain and the strain of wheat with purple grain and the content of anthocyanins in bran (numbers 1–6 of products are according Table 1).**

The composition of these products, in addition to flavonoids, represented by flavonoid compounds, which include both a number of colorless compounds, for example, rutin, and colored molecules (for example, anthocyanins, which give a pinkish, blue and red color to plant tissues). It is not only customary berries that can be brightly colored, but also cereal grain. In this case, anthocyanins are only synthesized in the aleurone layer and the pericarp giving the grain a bluish or purple color, respectively (Adzhieva et al., 2015) [9].

Since anthocyanins perform protective functions in plants, in particular, protect their tissues from excessive UV radiation, donor plants of an anthocyanin color of grain were found in the areas with a high level of insolation, for example, in Ethiopia. The "collectors" of
plant resources, such as N.I. Vavilov, have long been interested in the dark-colored southern forms of cereals, and some breeders have begun to "transfer" the color from donors to the cultivated "northern" varieties. However, in the northern latitudes, with insufficient insolation, the anthocyanin color of a stem and other parts of a plant that provides a color for grain (due to pleiotropy) is non-uniform, which can become an obstacle to the registration of the variety, so the breeders tried to get rid of the signs of the anthocyanin color.

At the same time, by the end of the 20th century, there was already quite a lot of evidence about the benefits of anthocyanins for human health, in particular, the use of anthocyanins prevents cardiovascular diseases, vision pathologies, diabetes, arthritis and has an anticancer and anti-inflammatory effect (Howard and Kritchevsky 1997; Wang et al., 1999; Tsuda et al., 2003; Lila 2004; de Pascual-Teresa et al., 2010; Hui et al., 2010; Cassidy et al., 2011; Sancho and Pastore, 2012) [10, 11, 12, 13, 14, 15, 16, 17].

Therefore, since the beginning of the 21st century, the creation and production of varieties of cereals (rice, corn, wheat) with colored (purple) grain (Garg et al., 2016) [18] is becoming increasingly popular [18], as well as the unconventional forms of vegetable crops (for example, the tomatoes and peppers colored with anthocyanins) and potatoes with a purple flesh (Stryginova, Khlestkina, 2017) [19]. The value of such products, although the number of anthocyanins contained therein is lower than, for example, in blueberry or red grapes, is that the products from cereals and potatoes are included in the daily diet. In addition, these products are a more effective way of storing nutrients than in such perishable food products as berries and fruits.

Since 2000s, the breeding and production of cereals (rice, wheat, maize) enriched with anthocyanins became broadly adopted. The grain that produces these compounds has a purple or black color. Among cereals, rice as an important crop and also as a model species, became broadly adopted. The grain that produces these compounds has a purple or black color. Among cereals, rice as an important crop and also as a model species, has left ahead other crops in genetic studies, breeding and the production of purple (black)-grained rice. Among cereals, rice as an important crop and also as a model species, has left ahead other crops in genetic studies, breeding and the production of purple (black)-grained rice.

China is the leading rice producer in the world and the production of purple (black)-grained rice. Among cereals, rice as an important crop and also as a model species, has left ahead other crops in genetic studies, breeding and the production of purple (black)-grained rice.

In addition, Chaudhary (2003) reported an upcoming demand of black rice as an organic food coloring agent in the EU and the USA. In 2015, the size of the global market of natural food color was estimated as 1.32 billion USD and continued to grow. The demand for natural food color in the Asia Pacific region expected to gain the highest growth rate over 7% by revenue from 2016 to 2025 due to the rapid growth of the food industry and health awareness related to synthetic color (Vayupharp and Laksanalamai, 2015; Grand View Research, 2017) [24, 25]. Anthocyanins are the water-soluble pigments found in black rice grain and bran. The shade of anthocyanins can be varied from orange to red to blue depending on the pH of a food matrix (Vayupharp and Laksanalamai, 2015) [24]. The main concern of food industry toward the stability of natural colour and extraction yield drove a lot of researchers to determine appropriate pretreatment and extraction methods (Ngamwonglumleit et al., 2015) [26].

The conventional extraction methods including soxhlet extraction, maceration and hydrodistillation are commonly used but they require a long extraction time and a large amount of a solvent. The recent development of the advanced extraction methods such as supercritical fluid extraction, pressurized liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed-electric field extraction and enzyme-assisted extraction have emerged as an alternative choice since they consume less of a solvent, need a shorter extraction time and are more environmentally friendly (Cheok et al., 2014) [27].

### Table 6. Rice Varieties in Thailand

<table>
<thead>
<tr>
<th>Market Classification</th>
<th>Approximate Varieties</th>
<th>Areas or Province</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fragrant rice</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Jasmine or Kao Horn Mali</td>
<td>Kao Hom Doc Mali 105 (KDML105 and Gor-Kor)</td>
<td>Thung Kula plain (2 million hectares), Surin, Buri Rum</td>
</tr>
<tr>
<td>2. Jungwad</td>
<td>The same variety as Hom Mali 105 Khao’ Jow Hawm Suphan Buri Khao’ Jow Hawm Khlong Luang 1</td>
<td>A province other than in (1) Non-photosensitive, irrigated Non-photosensitive, irrigated, mostly planted in the Central Plain</td>
</tr>
<tr>
<td>3. Pathumthani</td>
<td>Pathumthani 1</td>
<td>Non-photosensitive, irrigated</td>
</tr>
<tr>
<td>Non-fragrant white rice</td>
<td>A lot of varieties, photosensitive and non-photosensitive</td>
<td>Most provinces</td>
</tr>
<tr>
<td>Glutinous rice</td>
<td>Niaw Ubon, Niaw San-pah-tawng</td>
<td>Northeastern and Northern</td>
</tr>
</tbody>
</table>

*Source: Titapiwatanakun, 2012 [23].*
The natural question was whether these compounds can be destroyed during processing. A number of studies have shown that potato and wheat anthocyanins are fairly resistant to processing (Mulinacci et al., 2008; Lemos et al., 2015; Khlestkina et al., 2017 [28, 29, 3]). Another problem is to "teach" plants to produce anthocyanins not only in the hulls of grain, but also in the endosperm. So far, geneticists have managed to achieve this only with the help of methods of genetic engineering (Zhu et al., 2017) [30], but GMO-plants, as is known, cannot be grown in many countries, including ours. However, the potential for using the anthocyanins contained in grain hulls remained underestimated.

Taking into account the growing interest in the products that contain natural anthocyanids, at the initiative of the Research Center for Food Safety of Novosibirsk State University, the possibilities of using the development of Russian geneticists for the purpose of creating products from the new types of wheat grains were analyzed for the first time within the framework of this interdisciplinary study. The Center was created at Novosibirsk State University (NSU) within the framework of Project 5–100 in August 2015 as a result of the competition of the joint laboratories of NSU and the Siberian Branch of the Russian Academy of Sciences. The priority areas of the Center are the scientific studies in the field of the modern technologies in the food market that affect the consumer and technological characteristics of food raw materials and food products and joint interdisciplinary studies in the development of innovative food products as part of the implementation of interdisciplinary scientific projects to ensure global food security. At a time when the food market is dominated by the technological solutions that break food chains, the fundamentally new approaches are needed based on the interdisciplinary and systemic interaction of scientists from different fields of science, taking into account state and public interests. Thus, the problem of ensuring food security requires the fundamentally new, nonstandard solutions based on the interdisciplinary and systematic interaction of scientists from different fields of science in order to improve the quality of life of the population and the rational use of natural resources.

One of the developments of the Center was the study of the antioxidant activity of anthocyanins of purple wheat in flour confectionery products. Using the isogenic lines of wheat specially designed for comparative studies, which differ only in a small segment of the genome that has the regulatory gene of biosynthesis of anthocyanins Pp3/TaMye1, it was shown that in terms of the baking and organoleptic properties of the product, purple wheat is not inferior to and in some cases manifests itself better than the control strain (Khlestkina et al., 2017) [3]. In addition, the presence of anthocyanins increases the shelf life of bakery products and their stability in the provocative conditions that promote the development of mold fungi. It was found that when eating 100 grams of bread made from flour with the addition of bran of purple wheat grain, more than 1 mg of anthocyanins will get into the body (Khlestkina et al., 2017) [3]. It is worth noting that, regardless of pigmentation, the addition of bran increases the antioxidant potential of the product by itself, which can also be of value to human health.

In this paper, we used the flour and bran from the same isogenic wheat lines for confectionery production. Note that the conditions were simulated for the extraction of anthocyanins closest to those in digestion in the digestive tract in order to estimate the amount of anthocyanins available for consuming. It has been shown that when eating 100 grams of biscuit made from flour with the addition of bran of purple wheat grain, the consumption of anthocyanins will be up to 0.83 mg.

Thus, our interdisciplinary study demonstrates a way to manufacture enriched confectionery products with a high antioxidant potential. The further developments of confectionery products based on the use of raw materials (wheat and bran) with a high content of anthocyanins can form a whole family of products with health-promoting properties. The uniqueness of these products is evidenced by the fact that at the present time none of bread wheat varieties have been registered in the State Register, from the grain of which the products containing anthocyanins can be produced.

Although a lot of researchers have reported that purple (or black) rice contains a high amount of an antioxidant agent, especially anthocyanins in pericarp (Yawadio et al., 2007; Chotimarkorn et al., 2008; Leardkamolkarn et al., 2012) [31, 32, 33] and contains a higher amount of proteins, vitamins and minerals than common white rice (Suzuki et al., 2004) [34], it is not well accepted among main rice consumers since it is difficult to cook. Moreover, purple (or black) rice contains some undesirable characteristics, such as, a distinct taste, a dark appearance and a hard cooked rice texture (Ujjawal, 2016) [22]. Thus, an alternative to the use of black rice have been studied, such as, wheat replacement in bakery products (Jung et al., 2002; Joo and Choi, 2012) [35, 36], noodles (Sirichokworrakit et al., 2015) [37] and ice cream (Chuaykarn et al., 2013) [38]. In addition, the results of the studies testify to the fact that the potential consumer who has received information about the health benefits of anthocyanins is interested in purchasing such cereals at a price higher than that of similar products (Bruschi et al., 2015) [39].

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest.
REFERENCES


STUDY OF THE BIOFUNCTIONAL PROPERTIES OF CEDAR PINE OIL WITH THE USE OF IN VITRO TESTING CULTURES


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Abstract: Cedar pine (Pinus sibirica) nuts are an environmentally friendly natural product that contains a unique set of useful biologically active substances. Due to its composition, pine nuts and their derivative products are widely used in a comprehensive therapy and prevention program for a lot of diseases. The objects of the study were cedar oil and oil emulsions (the cedar oil concentration was 1.0, 5.0 and 10.0%). The antimicrobial properties were determined using the diffusion method and by measuring the optical density. The antioxidant properties were estimated according to the ability to stimulate the bifidobacteria growth. The antioxidant activity was determined using the fluorescent ORAC method. The antimicrobial activity was estimated according to the ability to inhibit the angiotensin-1-converting enzyme. All studied experimental oil emulsion samples, regardless of a pressing method (cold or heat), showed high antimicrobial characteristics without suppressing only Candida albicans EMTC 34 and Proteus vulgaris ATCC 63 from the studied 10 strains of the main testing cultures. The prebiotic properties of the emulsions obtained with the addition of cedar oil have been determined. The number of cells of the bifidobacteria cultivated in nutrient media with the addition of cedar oil are usually reported about [10, 11]. Cedar nut oil is a real natural storehouse of useful biologically active substances. Its composition includes proteins, carbohydrates, saturated and unsaturated fatty acids, phosphatides, vitamins A, B1, B2, B3 (PP), E, D, lecithin, essential oils, amino acids and minerals [16–21]. The vitamin E content in pine nut oil is several times more than that in olive oil. The use of cedar oil in the diet makes it possible to restore working capacity and cope with psychoemotional disorders. The beneficial effect of cedar oil in the complex of both therapeutic and preventive measures is well known [22–28]. The limited consumption of oil, as part of dietary nutrition, is recommended for faster satiation [29].

Keywords: Cedar pine nuts (Pinus sibirica), Oil, Antimicrobial, prebiotic, antioxidant properties


INTRODUCTION

Cedar pine has always been highly demanded by the local population in any place of its growth thanks to extremely useful qualities. The most consumed pine nuts are the fruits of Korean pine (Pinus koraiensis), cedar pine (Pinus sibirica), umbrella pine (Pinus pinea) and chilgoza pine (Pinus gerardiana) [1, 2]. As raw materials, cedar is very convenient for a non-waste product manufacturing process [3–6]. The more northern the latitudes of cedar pine habitats, the higher the value of cedar products. The high demand for pine nuts has resulted in an increase in their world output, according to FAO, China, Korea, Pakistan and Russia (Siberia and the Far East) are the main exporting countries [7].

Traditionally, it is believed that the most valuable cedar product is cedar oil, which is produced from cedar kernels [8, 9]. The oil yield depends on a pressing method (cold and heat pressing, solvent extraction), but about 45 to 65 g of butter per 100 g of nuts is usually reported about [10, 11]. Cedar nut oil is a high-quality natural and ecologically friendly food supplement [12, 13] that is necessary for the normal functioning of the human body [14, 15].

Cedar oil is a real natural storehouse of useful biologically active substances. Its composition includes proteins, carbohydrates, saturated and unsaturated fatty acids, phosphatides, vitamins A, B1, B2, B3 (PP), E, D, lecithin, essential oils, amino acids and minerals [16–21]. The vitamin E content in pine nut oil is several times more than that in olive oil. The use of cedar oil in the diet makes it possible to restore working capacity and cope with psychoemotional disorders. The beneficial effect of cedar oil in the complex of both therapeutic and preventive measures is well known [22–28]. The limited consumption of oil, as part of dietary nutrition, is recommended for faster satiation [29].

The clear antioxidant effect of both nuts themselves and their derivative products has been noted. The study [12] of rats has found an increase in the antioxidant capacity in the blood of the animals that used Far-Eastern pine oil. Lin et al. [30] derived four peptides from deodor cedar nuts with pronounced
antioxidant properties that were enhanced to 90% by an impulse action and an electric field. The ability of cedar oil to reduce cellular activity is planned to be used in the inhibition of cancer metastases [1, 31].

Cedar needles, which are widely represented in traditional Chinese medicine, primarily with their antimicrobial properties, as well as their biologically active characteristics, are of particular interest to researchers [32]. A lot of types of cedar needles are added into functional foods and food supplements to enhance a nutritional and / or pharmaceutical effect [33, 34]. If needles, with their antimicrobial properties, are also noted for their biologically active characteristics, nuts are probably noted for the similar effect, too, i.e., in addition to their nutritional and functional properties, they also have antimicrobial characteristics.

The study was aimed at the in vitro biofunctional properties (antibacterial, antioxidant, prebiotic, etc.) of the oil derived from the nuts of cedar pine that grows in the Kemerovo region as the basis of biologically active ingredients in 96-well culture plates.

**STUDY OBJECTS AND METHODS**

The experimental studies were carried out at the Research Institute of Biotechnology of the Kemerovo State University.

The objects of the study were the samples of the oil made with the use of two methods (cold and heat pressing) from nut kernels of cedar pine that grows on the territory of the Kemerovo Region (Tashtagol District, the crop of 2016 and 2017) and 1.0%, 5.0% and 10.0% O/W emulsions of this cedar oil.

Testing cultures: opportunistic and pathogenic strains of microorganisms: *Pseudomonas aeruginosa* ATCC 9027 is an opportunistic bacterium that induces nosocomial infections in humans; *Candida albicans* EMTC 34 is a microscopic fungus, the causative agent of opportunistic human infections; *Acaligenes faecalis* EMTC 1882 is an opportunistic bacterium that induces intraabdominal infections, septicaemia and meningitis in humans; *Leuconostoc mesenteroides* EMTC 1865 is an opportunistic bacterium that induces infectious diseases in humans; *Escherichia coli* ATCC 25922 is an opportunistic bacterium that induces gastroenteritis in humans; *Enterococcus casseliflavus* EMTC 1866 is a pathogenic bacterium that induces sepsis in humans; *Salmonella enterica* ATCC 14028 is a pathogenic bacterium that induces gastroenteritis in humans; *Staphylococcus aureus* ATCC 25923 is a pathogenic bacterium that induces pneumonia, meningitis, osteomyelitis, endocarditis, infectious toxic shock and sepsis in humans; *Bacillus mycoides* EMTC 9 are permanent contaminating agents of food raw materials and food products that induce food toxic infections in humans; *Proteus vulgaris* ATCC 63 is an opportunistic bacterium that induces intestinal infections in humans; as well as the *Bifidobacterium adolescentis* MC 42 bifidobacteria strain and the MDCK1 canine kidney epithelial cell line.

**Sample preparation.** The shell was preliminarily separated from the pine nut kernel, Sample No. 1 is from the crop of 2016, Sample No. 2 is from the crop of 2017. To prepare the samples of seeds from the cedar pine layers different in height (upper, middle and lower), 3 single samples weighing 100 g each were taken from each batch.

To obtain particles of 2 mm in size, the pine nut kernel was grinded in an electric mill for each of the pressing methods (cold or heat). In the case of cold pressing, the pine nut kernel was put under a UP-20 hydraulic press (Nizhny Novgorod, Russia) and the oil was pressed out by means of a gradual increase in the load. Heat pressing is washing the heated pine nut kernels with hot water and then the heat pressing thereof.

The 1.0%, 5.0% and 10.0% cedar O/W emulsions were prepared by adding cedar oil (1 g, 5 g or 10 g) slowly to water (100 g) in the presence of an emulsifier (0.01 g, 0.05 and 0.10 g of soya lecithin, respectively) stirring continuously (the stirring rate is 500 rpm).

The antibacterial (antimicrobial) properties of the in vitro oil obtained from pine nuts were determined in terms of the growth of the opportunistic and pathogenic test strains of microorganisms using the diffusion method and a method based on the measurement of optical density.

**Diffusion method** [35, 36]. The testing culture was inoculated on a dense nutrient medium as lawn. The microorganisms were grown at the values of pH and temperature optimal for each test strain for 24 hours. The culture liquid was centrifuged at 7000 rpm for 10 minutes and the supernatant was separated. To separate the cells, the supernatant was filtered through Milllex-GV filters (0.22 μm, Nihon "Millipore", USA). The antimicrobial activity was estimated by measuring the inhibition zones with respect to the testing culture of a microorganism [37]. The paper discs were dipped in the emulsions (cedar oil) / suspensions (a protein-vitamin complex, a carbohydrate-mineral complex) containing the tested food ingredients squeezing out the excesses. The discs were put on the agar with a testing culture observing the rules of asepsis. The disks were arranged so that the distance between their centers was not less than 24 mm. After placing the discs on the agar, they were pressed with a sterile needle or pincers until they completely contacted with the surface of the medium.

After 15 minutes after placing the discs, the cups were inverted and incubated at the pH and temperature optimal for each test strain for 24 hours. After incubation, the diameter of the zones of complete incubation was measured (according to the observation with the naked eye), including the diameter of the disk, to the nearest whole millimeter using a caliper, ruler or stencil designed for these purposes.

**Optical density measurement.** The method for determining antimicrobial activity based on the measurement of optical density is as follows. To estimate the antibacterial effect of the food ingredients, the testing cultures were co-incubated with the studied ingredients in 96-well culture plates.
Incubated in a thermostat for 12...24 h to allow the experimental wells of the plate. The plate was provided with the same moisture conditions in all the plates. Cells were cultured in 100 μl of saline solution without the addition of food ingredients. The plate was incubated in a thermostat for 2 hours, then the contents of the wells were aspirated and 100 μl of a working DCFH-DA solution (30 min) was added, after which the solution was aspirated and the peroxide oxidation was induced by adding 100 μl of a working 2,2′-azobis (2-amidino-propano) dihydrochloride (AAPH) solution into the wells.

The fluorescence intensity was determined at an excitation wavelength of 485 nm and an emission wavelength of 528 nm using a Synergy 2 spectrophotometer-fluorometer immediately after the addition of AAPH (“0”) in 30 minutes, 60 minutes and 90 minutes.

The anti-hypertensive (hypotensive) activity of the in vitro food ingredients derived from pine nuts was determined in vitro according to their ability to inhibit the angiotensin-1-converting enzyme (ACE), a key link in the renin-angiotensin system that regulates human blood pressure. The most sensitive method for determining the ACE inhibitory activity of a substance is a method with the use of internally-quenched ACE substrates. As an internally-quenched substrate, o-aminobenzoyle-phenylalanyl-arginyl-lysyl(dinitrophenyl)-proline was used, the measurements were carried out using a BioTek Synergy 2 microplate photometer-fluorometer (USA). The reaction time was 30 minutes at 37°C.

Statistical analysis. All the experiments were carried out n-fold, n = 5. The data were processed using mathematical statistics standard methods. The homogeneity of the sampling effects was checked using Student's t-test. The differences between the averages were considered significant if the confidence interval was less than 5% (p ≤ 0.05).

RESULTS AND DISCUSSION

The previous studies of the nut kernels of the crop of 2016–2017 of the cedar pines that grow in the Kemerovo region confirmed the nutritional value of the samples studied [39, 40]. Oil was derived from the cedar kernels by heat and cold pressing as well as emulsions with the addition thereof.

Table 1 shows the results of the determination of antimicrobial properties of the examples of cedar oil emulsions using the diffusion method. All the experimental samples of cedar oil obtained both by cold and heat pressing are characterized by high antimicrobial activity against all the tested strains except for Candida albicans EMTC 34 and Proteus vulgaris ATCC 63. It should be noted that the antimicrobial properties are determined by cedar oil concentration (up to a concentration of 5.0%). The further increase in the content of cedar oil is not followed by a significant increase in the diameter of inhibition zones of pathogenic test strains.
Table 1. Results of the determination of antimicrobial properties of the examples of cedar oil emulsions using the diffusion method

<table>
<thead>
<tr>
<th>Samples</th>
<th>TC1</th>
<th>TC2</th>
<th>TC3</th>
<th>TC4</th>
<th>TC5</th>
<th>TC6</th>
<th>TC7</th>
<th>TC8</th>
<th>TC9</th>
<th>TC10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0% emulsion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>8.0 ± 1.6</td>
<td>0.0 ± 0.0</td>
<td>10.0 ± 1.0</td>
<td>12.2 ± 0.8</td>
<td>7.4 ± 1.1</td>
<td>7.2 ± 0.8</td>
<td>14.0 ± 1.0</td>
<td>11.4 ± 1.1</td>
<td>8.2 ± 1.3</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>No. 2</td>
<td>8.4 ± 1.1</td>
<td>0.0 ± 0.0</td>
<td>11.2 ± 1.3</td>
<td>10.4 ± 1.1</td>
<td>10.4 ± 1.1</td>
<td>15.0 ± 0.7</td>
<td>14.6 ± 0.9</td>
<td>7.6 ± 1.1</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>5.0% emulsion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>16.6 ± 1.1</td>
<td>4.2 ± 0.8</td>
<td>23.0 ± 1.6</td>
<td>22.4 ± 1.1</td>
<td>19.6 ± 0.9</td>
<td>19.8 ± 0.8</td>
<td>24.8 ± 0.8</td>
<td>18.8 ± 1.3</td>
<td>21.6 ± 1.1</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>No. 2</td>
<td>22.6 ± 1.1</td>
<td>5.6 ± 1.5</td>
<td>24.8 ± 0.8</td>
<td>20.6 ± 1.1</td>
<td>22.6 ± 1.1</td>
<td>20.6 ± 1.1</td>
<td>24.8 ± 0.8</td>
<td>20.2 ± 0.8</td>
<td>22.0 ± 1.0</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>10.0% emulsion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>16.6 ± 1.1</td>
<td>4.4 ± 1.1</td>
<td>23.4 ± 1.8</td>
<td>22.6 ± 1.1</td>
<td>19.8 ± 0.8</td>
<td>20.0 ± 1.0</td>
<td>25.0 ± 0.7</td>
<td>19.0 ± 1.2</td>
<td>21.6 ± 1.1</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>No. 2</td>
<td>23.0 ± 1.4</td>
<td>5.8 ± 1.3</td>
<td>24.8 ± 0.8</td>
<td>21.0 ± 1.6</td>
<td>23.0 ± 1.4</td>
<td>21.0 ± 1.4</td>
<td>25.0 ± 0.7</td>
<td>20.8 ± 0.8</td>
<td>22.2 ± 0.8</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>Control 1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Control 2</td>
<td>22.0 ± 1.1</td>
<td>24.0 ± 1.2</td>
<td>21.0 ± 1.1</td>
<td>23.0 ± 1.2</td>
<td>20.0 ± 1.0</td>
<td>19.0 ± 1.0</td>
<td>23.0 ± 1.2</td>
<td>26.0 ± 1.3</td>
<td>24.0 ± 1.2</td>
<td>22.0 ± 1.1</td>
</tr>
</tbody>
</table>

Note. TC1 is Pseudomonas aeruginosa ATCC 9027, TC2 is Candida albicans EMTC 34, TC3 is Alcaligenes faecalis EMTC 1882, TC4 is Leuconostoc mesenteroides EMTC 1865, TC5 is Escherichia coli ATCC 25922, TC6 is Enterococcus casseliflavus EMCC 1866, TC7 is Salmonella enterica ATCC 14028, TC8 is Staphylococcus aureus ATCC 25923, TC9 is Bacillus mycoides EMTC 9, TC10 is Proteus vulgaris ATCC 63; Control 1 is the antibiotic ciprofloxacin; Control 2 is a nutrient medium without oil.

Fig. 1. Results of the determination of antimicrobial properties of cedar oil in a liquid nutrient medium: (1) oil (cold pressing); (2) oil (heat pressing); (3) Control 1 (the antibiotic ciprofloxacin); (4) Control 2 (a nutrient medium without oil). The data are expressed as a mean ± standard deviation (n = 5). TC1 is Pseudomonas aeruginosa ATCC 9027, TC2 is Candida albicans EMTC 34, TC3 is Alcaligenes faecalis EMTC 1882, TC4 is Leuconostoc mesenteroides EMTC 1865, TC5 is Escherichia coli ATCC 25922, TC6 is Enterococcus casseliflavus EMTC 1866, TC7 is Salmonella enterica ATCC 14028, TC8 is Staphylococcus aureus ATCC 25923, TC9 is Bacillus mycoides EMTC 9, TC10 is Proteus vulgaris ATCC 63.
The antimicrobial activity of cedar oil in a liquid nutrient medium was determined for the samples of 5.0% O/W cedar emulsions. The results are shown in Fig. 1.

The analysis of the data (Fig. 1) shows that the results of determining the antimicrobial activity of cedar oil in a liquid nutrient medium are consistent with the data obtained using the diffusion method (Table 1). Cedar oil shows high antimicrobial activity with regard to the test strains of *Pseudomonas aeruginosa* ATCC 9027, *Alcaligenes faecalis* EMTC 1882, *Leuconostoc mesenteroides* EMTC 1865, *Escherichia coli* ATCC 25922, *Enterococcus casseliflavus* EMTC 1866, *Salmonella enterica* ATCC 14028, *Staphylococcus aureus* ATCC 25923 and *Bacillus mycoides* EMTC 9.

Figures 2–4 show the diagrams of a change in the number of bifidobacteria cells in the nutrient media that contain the oil derived from pine nuts.

The oil emulsions obtained with the addition of cedar nut oil have prebiotic properties since the number of cells of the bifidobacteria cultivated in nutrient media with oil (Fig. 2–4) increases compared to the amount of *Bifidobacterium adolescentis* cultivated in a nutrient medium with no addition of cedar oil. When cultivating bifidobacteria for 48 hours in a nutrient medium that contains cedar oil (5.0 and 10.0% emulsions in water), the number of cells increases up to 3.0 times compared to the control.

Figures 5–7 show the results of the determination of the antioxidant properties of the test cedar oil samples. Cedar oil shows pronounced antioxidant properties; there is a decrease for all the test samples in the intensity of fluorescence after 90 minutes of cell incubation (from 100% to 81.8%). Antioxidant activity grows with an increase in the concentration of ingredients up to 5.0%. The further increase in the concentration of cedar oil is followed by a slight increase in antioxidant activity.

Table 2 shows the results of the determination of the antihypertensive activity of the *in vitro* oil obtained from pine nuts. All the test emulsion samples with oil obtained from pine nuts have shown hypotensive properties. An oil emulsion, at a concentration of 5.0% already, has the highest ability to inhibit the angiotensin-1-converting enzyme, approximately by 70%, the further increase in the oil content practically does not change the inhibitory characteristics. The hypotensive properties of cedar oil are probably due to the presence of vitamins E and PP in its composition.
Fig. 5. Results of in vitro determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 30 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

Fig. 6. Results of in vitro determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 60 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

Fig. 7. Results of in vitro determination of the antioxidant effect of cedar oil on the cultured MDCK1 cells for 90 min: (1) oil (cold pressing); (2) oil (heat pressing). The data are expressed as a mean ± standard deviation (n = 5).

Table 2. Results of the determination of the antihypertensive activity of the in vitro oil obtained from pine nut kernels

<table>
<thead>
<tr>
<th>Test sample</th>
<th>ACE inhibition degree, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0 %</td>
</tr>
<tr>
<td>Oil (cold pressing)</td>
<td>29.2 ± 0.9</td>
</tr>
<tr>
<td>Oil (heat pressing)</td>
<td>28.9 ± 1.2</td>
</tr>
</tbody>
</table>

CONCLUSIONS

According to the results of the study of the oil produced from the nuts of cedar pine that grows in the Kemerovo region, all the test samples obtained by both cold and heat pressing are characterized by high antimicrobial activity in relation to all the test strains, except for Candida albicans EMTC 34 and Proteus vulgaris ATCC 63. The antimicrobial characteristics depend on the concentration of cedar oil and can be observed at a concentration of up to 5.0%.

Pine cedar oil, regardless of a production method, has a pronounced prebiotic effect. The number of the Bifidobacterium adolescentis cells, cultivated in nutrient media with cedar oil exceeds the amount when cultivated in a nutrient medium without cedar oil from 1.7 to 3.1 times. The highest value (the difference is about 3 times) of the yield of bifidobacterial cells was observed during the cultivation in a nutrient medium supplemented with cedar oil for 48 hours (a 5.0% and 10.0% emulsion in water). The in vitro study of the antioxidant effect of cedar oil using the cultured MDCK1 cells has shown that cedar oil is characterized by pronounced antioxidant properties after 90 minutes of cell incubation for all the test samples (a decrease in fluorescence intensity). There was a decrease in antioxidant activity with an increase in the concentration of ingredients up to 5.0% for all the samples. The further increase in the concentration of food ingredients results in a slight increase in antioxidant activity.

The determination of antihypertensive activity of in vitro cedar oil shows that all the test samples have revealed antihypertensive characteristics. Cedar oil in a 5.0% emulsion inhibits angiotensin-1 by 68.9%, in a 10.0% emulsion – up to 70.2%, which is explained by the presence of vitamins E and PP therein.

The confirmation in vitro of the availability of biofunctional properties of the oil obtained from the nuts of cedar pine that grows in the Kemerovo region allows it to be used as the basis of not only biologically active food supplements, but also of dietary, therapeutic, preventive and sports nutrition.

ACKNOWLEDGMENTS

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REFERENCES


STUDY OF THE MICROBIOLOGICAL COMPOSITION OF DAIRY PRODUCTS AND MAYONNAISE USING DNA BARCODING AND METABARCODING

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Abstract: Prokaryotic and eukaryotic microorganisms cause spoilage of produced dairy and fat-and-oil products. In addition, these products can be contaminated with pathogenic microorganisms. The standard practice of detecting bacterial pathogens is based on the cultivation of microorganisms due to which the analysis lasts from 5 to 7 days. Molecular genetic methods can reduce the analysis time to 1–2 days. In this paper, the ready-made commercial products of the dairy and fat-and-oil industry have been analyzed for the microbiological composition using classical DNA barcoding and DNA metabarcoding. During the study, representatives of the genera Pseudomonas, Bacillus, Lactococcus, Kocuria, Staphylococcus, Moraxella, Paucalisalbacillus, Acinetobacter, Klebsiella, Paenibacillus, Lysinibacillus, Enterobacter, Acetobacter and Massilia have been defined. When analyzing the quantitative ratio of microorganisms, it was revealed that dairy and fat-and-oil products are most often seeded with Bacillus sp., among which Bacillus licheniformis (16.67% of colonies) and Bacillus subtilis (11.4% of colonies) can be distinguished. Among Pseudomonas sp., Pseudomonas fluorescens (19.3% of colonies) are the most numerous. Lactococcus lactis, Acetobacter indonesiensis and Moraxella osloensis bacteria also significantly contaminate dairy and fat-and-oil products. Mayonnaise is contaminated with yeast of the Pichia genus. The analysis revealed opportunistic pathogenic species: Staphylococcus warneri, Staphylococcus epidermidis, Klebsiella pneumoniae, Bacillus cereus, Vibrio sp. The presented method for detecting microbial contamination using an Ion torrent PGM platform seems promising for the rapid testing of the produced dairy and fat-and-oil products.

Keywords: Bacteria, prokaryotic microorganisms, food products, seeding, spoilage, DNA barcoding, DNA metabarcoding


INTRODUCTION

The manufacturers of dairy products, mayonnaise and other food ingredients are currently faced with the problem of contaminating products with prokaryotic and eukaryotic microorganisms that cause product damage, as well as pathogenic microorganisms. Water, industrial equipment, workers, inward raw materials, etc. may be the source for microorganisms to get into products [1]. All groups of the population are exposed to the risk of contamination by pathogenic microorganisms due to the consumption of contaminated food products, however, infants, elderly people and people who have a weakened immune system tend to the most severe consequences [2]. The list of foodborne diseases is getting constantly updated, until the 1960s, the most common food pathogens causing disease were Salmonella spp., Shigella spp., Escherichia coli, Clostridium botulinum and Staphylococcus aureus. In the 1980s and 1990s, new pathogenic species were added to this list, such as Campylobacter spp., Yersinia spp., Listeria monocytogenes, Vibrio cholera, Enterococcus faecalis and also the enterohemorrhagic strain O157 : H7 Escherichia coli [3]. The infections caused by these bacterial pathogens are now endemic in a lot of countries and cause a wide range of diseases [4]. Foodborne diseases are the result of taking the food products contaminated with pathogenic microorganisms and/or their toxins [5]. New trends in nutrition that consist in the consumption of raw and fresh food products, dry food products that have not been processed, and exotic ingredients cause a significant increase in foodborne diseases. The globalization of the food market additionally affects the outbreaks of foodborne diseases, which makes food safety a universal problem [6].

In Russia, according to Rospotrebnadzor, among other types of poisoning 147301 cases of acute intestinal infections and toxic infections caused by the defined pathogens were registered in 2016; still in 438019 cases it was not possible to define causative agents. The problem of contamination of food products by pathogenic and opportunistic microorganisms is relevant not only in the Russian Federation, but also in the world. Thus, in the...
USA 76000000 cases of food toxic infections have been registered for the last two decades, from which 5000 have ended with a lethal outcome. The data collection and processing system – FoodNet in the USA collects data for nine types of foodborne infections from nine states [7]. The number of confirmed cases for nine different diseases in 2002 was: salmonellosis – 6028, campylobacteriosis – 5006, shigellosis – 3875, escherichiosis – 647, cryptospordiosis – 541, yersiniosis – 166, vibriosis – 103, listeriosis – 101, cyclosporidiosis – 43. In 1996, 11,826 cases of food poisoning caused by the consumption of raw milk were reported in the United States. Of 1733 cases, significant sources of foodborne diseases. For example, bacteria were detected worldwide [8].

Of 219 samples of raw milk tested in Brazil, 16.9% contained Listeria spp. and 32.4% contained Yersinia enterocolitica [13]. The spoilage of dairy products is caused by Streptococcus spp. and Bacillus spp., which can survive even after ultra-pasteurization [14]. In cheeses the diphertheria bacteria of the Brachybacterium genus [15], as well as L. monocytogenes [16] were identified. C. tyrobutyricum is well known as the cause of amylc fermentation of cheeses [17].

The standard practice of detecting bacterial pathogens is based on the cultivation of microorganisms, their microscopic observation and a biochemical analysis [21]. Although seeding with the subsequent phylogenetic analysis is the gold standard for estimating pathogenic microorganisms, the whole procedure has several limits, such as labor intensity, the duration of the analysis (5–7 days) and the complexity of the quantitative analysis. In addition, these methods are not able to detect several pathogens at the same time, so there is a need for the rapid identification of the bacterial pathogens passed through food products using high sensitivity methods [22].

A lot of authors have recently developed some methods of molecular-genetic identification based on real-time PCR using Taqman probes to identify the key pathogenic bacteria capable of seeding food products: Salmonella spp., L. monocytogenes, E. coli, thermotolerant Campylobacter spp., Yersinia enterocolitica, pathogenic Vibrio spp., Staphylococcus aureus and pathogenic Clostridia spp. [23].

DNA barcoding has recently become increasingly popular. DNA barcoding is used as an instrument of taxonomic identification of organisms [24–26]. This approach consists in sequencing the gene site and comparing the obtained sequence with those that are already available in international genetic databases, such as Boldsystem and GenBank. The gene of subunit 1 of cytochrome oxidase of mitochondrial DNA for animals [27], the gene of the internal transcriptional spacer (ITS) of nuclear DNA for fungi [28] and the genes of rbcL and matK for plants are most often used as such a gene [29]. The limit of this method is complexity in the analysis of a substrate that has the mixtures of DNA of the organisms of different taxonomic groups. A more advanced approach is the so-called DNA metabarcoding, which is performed using next generation sequencers (NGS sequencing). In this case, the analysis of complex biological mixtures is possible. The next-generation sequencing revolutionized food microbiology by developing new high-performance technologies, such as 16S rRNA microbiological profiling and shotgun sequencing, which were used to study the microbiota composition of various food products [30].

At present, the manufacturers of dairy and fat-and-oil products of Russia are obliged to comply with the requirements of the Customs Union for food safety, in particular, the products should not contain pathogenic microorganisms. There is also a limitation of the number of yeast and mold microorganisms and the total number of bacteria. The purpose of this study was to analyze the ready-to-eat commercial products of the dairy and fat-and-oil industry produced in Russia, for their microbiological composition, using classical DNA barcoding, as well as DNA metabarcoding.

**STUDY OBJECTS AND METHODS**

**Study objects.** As the study object, the food dairy and fat-and-oil products produced in Russia were used (Table 1). 1 g of a solid or 1 ml of a liquid solution was taken for the study.

**Microbiological inoculation.** To check the bacterial content of food products for different physiological groups of microorganisms, seeding was carried out in the nutrient media of the following composition:

1. FPA (fish-peptone agar) (to determine the total microbial number of mesophilic, aerobic and facultative anaerobic microorganisms): pancreatic fishmeal hydrolyzate – 12 g/l; enzymatic peptone – 12 g/l; NaCl – 6 g/l; microbiological agar – 10 g/l; pH 7.1–7.5.
2. Giss-GRM medium (to identify enterobacteria): pancreatic fishmeal hydrolyzate – 6 g/l; NaCl – 3.5 g/l; Na2HPO4 – 0.2 g/l; mannitol – 3.5 g/l; blue thymol bromine – 0.04 g/l; microbiological agar – 3.5 g/l; pH 7.4.
3. GRM nutrient medium No.2 (Saburo) (to detect yeast and mold): pancreatic fishmeal hydrolyzate – 10 g/l; pancreatic casein hydrolyzate – 10 g/l; yeast extract – 2 g/l; NaH2PO4 – 2 g/l; D-glucose – 40 g/l; microbiological agar – 10 g/l; pH 6.0. To suppress the growth of the extraneous microflora, 10 ml of 1% chloramphenicol solution per 1 liter of the medium were added to the medium before seeding.
Table 1. Characteristics of the studied food products

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of the product</th>
<th>Fat per 100 g of product, g</th>
<th>Protein per 100 g of product, g</th>
<th>Carbohydrates per 100 g of product, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk &quot;Manufacturer No.1&quot;</td>
<td>3.2</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>2</td>
<td>Milk &quot;Manufacturer No.2&quot;</td>
<td>2.5</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>3</td>
<td>Milk &quot;Manufacturer No.3&quot;</td>
<td>4.0</td>
<td>2.8</td>
<td>4.7</td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise sauce &quot;Manufacturer No.1&quot;</td>
<td>25.0</td>
<td>0.8</td>
<td>12.6</td>
</tr>
<tr>
<td>6</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>67.0</td>
<td>0.4</td>
<td>2.0</td>
</tr>
<tr>
<td>8</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>15.0</td>
<td>–</td>
<td>3.8</td>
</tr>
<tr>
<td>12</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td>0.1</td>
<td>10.0</td>
<td>3.5</td>
</tr>
<tr>
<td>14</td>
<td>Yoghurt &quot;Manufacturer No.1&quot;</td>
<td>7.8</td>
<td>3.5</td>
<td>16.0</td>
</tr>
<tr>
<td>15</td>
<td>Yoghurt &quot;Manufacturer No.3&quot;</td>
<td>2.9</td>
<td>3.9</td>
<td>14.8</td>
</tr>
</tbody>
</table>

CFU (Colony forming units: the indicator of the number of viable microorganisms per volume unit) were counted using the method of 10-time limiting dilutions in three biological repetitions. In accordance with the requirements of ASTM D5465-93 (2012) (GOST 26670-91) Petri dishes with the amount of CFU from 30 to 300 were counted.

DNA barcoding. DNA was isolated from the colonies of microorganisms using the Probe-GS kit (DNA technology, Russia) according to the attached instructions. The polymerase chain reaction was carried out using Taq polymerase with a Mastercycler personal device (Eppendorf, Germany). The following components were mixed in a 0.25 ml tube: a 10X reaction buffer – 2.5 μl; 10 mM dNTP – 1 μl; a 10 μmol primer – 1 μl; a 10 μmol reverse primer – 1 μl; 25 mM Mg2+ – 3 μl; a matrix – 1 μg; thermostable Taq-polymerase – 2.5 units; deionized water – up to 25 μl. The following temperature cycles were used: 3 min at 94°C, 35 cycles, 30 sec at 94°C, 30 sec at 54°C, 45 sec at 72°C and the final elongation for 10 min at 72°C. The following were used as primers: to amplify bacterial DNA: direct 785F GGATTAGATACGCCCTGGTA, reverse 1492R TACGGAATCTTTGTTAGCAGTT; for the amplification of fungal DNA: direct ITS1 TCCGTAGGTGAACCTGCGG, reverse ITS2 TCCTCCGCTTATTGATATGC. The PCR products were visualized by means of 2% agarose gel. Ethidium bromide was used as nucleic acid dye. The size of the products was determined by comparison with the DNA markers of the known length (Evrogen, Russia).

The extraction from agarose gel and amplicon purification was performed using Taq polymerase with a Mastercycler personal device (Eppendorf, Germany). The following components were mixed in a 0.25 ml tube: a 10X reaction buffer – 2.5 μl; 10 mM dNTP – 1 μl; a 10 μmol primer – 1 μl; a 10 μmol reverse primer – 1 μl; 25 mM Mg2+ – 3 μl; a matrix – 1 μg; thermostable Taq-polymerase – 2.5 units; deionized water – up to 25 μl. The following temperature cycles were used: 3 min at 94°C, 35 cycles, 30 sec at 94°C, 30 sec at 54°C, 45 sec at 72°C and the final elongation for 10 min at 72°C. The following were used as primers: to amplify bacterial DNA: direct 785F GGATTAGATACGCCCTGGTA, reverse 1492R TACGGAATCTTTGTTAGCAGTT; for the amplification of fungal DNA: direct ITS1 TCCGTAGGTGAACCTGCGG, reverse ITS2 TCCTCCGCTTATTGATATGC. The PCR products were visualized by means of 2% agarose gel. Ethidium bromide was used as nucleic acid dye. The size of the products was determined by comparison with the DNA markers of the known length (Evrogen, Russia).

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High-performance sequencing. The DNA of microorganisms was isolated using a Probe-GS kit (DNA technology, Russia) according to the attached instructions. The multiplex PCR was performed using the primers 785F/1492R and ITS1/ITS2 (see above). The PCR products were purified by magnetic particles AMPure XP Beads (Beckman Coulter, USA). The purified product was used to prepare sequencing libraries using the Ion AmpliSeq Library Kit 2.0 according to the kit protocol. The Ion Xpress Barcode Adapters (Thermo Fisher Scientific, USA) was used to barcode the samples. To determine the concentration of libraries, a real-time PCR kit (The Library Quantification Kit Ion Torrent Platforms (Kapa Biosystems)) was used.

The sequencing was performed using an IonTorrent PGM platform with an Ion PGM Hi-Q View Sequencing Kit together with the system Ion OneTouch 2 and an Ion PGM Hi-Q View OT2 Kit (Thermo Fisher Scientific, USA).

The source material for the bioinformatic analysis was bam files containing the source information about the reads obtained during DNA sequencing. The bam files containing nucleotide sequences in the binary format were converted to the FASTQ format using a samtools Version 1.2 package [31]. The demultiplexing was performed using the application fastq-multx, which is part of the software package ea-utils, Version 1.3 [32]. For this purpose, a file was prepared containing the sequences of primers that are part of the amplicons of different origin and the following command was executed:

fastq-multx -B barcodes.fa <имя_файла.fastq> -m 5 -o %fastq.
The -m 5 argument resolves up to 5 discrepancies between the primer sequence and the initial amplicon sequence in a FASTQ file. This parameter has been obtained empirically and does not affect the accuracy of the further analysis, making it possible at the same time to reduce the number of reads discarded as unidentified. The fitting of reads was performed for each sample using the command:

```
usearch -fastx_truncate <имя_файла.fastq> -
trunclength <длина_рида> -fastqout
<имя_файла_длина.fastq>.
```

Further on, the reads were filtered according to reading quality based on the expected number of errors [33]:

```
usearch -fastq_filter <имя_файла_длин. fastq> -
fastq_maxee 1.0 -fastqout <имя_файла_filtered. fasta>.
```

Before searching for OTU (Operational taxonomic unit), unique sequences were identified:

```
usearch -fastx_uniques <имя_файла_filtered.fasta> -
fastaout <имя_файла_uniques.fasta>
```

Two different approaches were used to search for OTU. The former is based on the UNOISE2 algorithm [34], the task of which is to reduce the noise level in the sample by correcting errors. The result of its application is the isolation of all biologically correct sequences in a set of reads:

```
usearch -unoise3 <имя_файла_uniques.fasta> -
-zotus <имя_файла_zotus.fasta>.
```

The read filtering, searching for unique sequences and clustering to obtain OTU were performed using a USEARCH software package version 10.0.240 [35]. The species of microorganisms contained in the sample was identified using a SILVA database (https://www.arb-silva.de/) in the case of bacterial DNA, and a BOLD Systems database (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) in the case of DNA of eukaryotic microorganisms.

**RESULTS AND DISCUSSION**

In the course of microbiological analysis, the amount of the colonies grown in nutrient media was initially estimated. Tables 2 present the number of the colonies grown in an FPA nutrient medium (CFU/ml) for the studied products.

It was impossible to calculate the number of colonies due to their high concentration in the sample of yoghurt "Manufacturer No.1" in its initial dilution. Table 3 presents the number of the colonies grown in a Giss nutrient medium (CFU/ml) for the studied products.

In general, the number of the colonies of microorganisms grown in the Giss medium was less than that in the FPA medium. To determine the number of eukaryotic microorganisms, the Saburo medium was used with the addition of an antibiotic (see materials and methods). Tables 4 present the number of the colonies grown in the Saburo nutrient medium (CFU/ml) for the studied products.

Filamentous fungi were not detected in any of the samples. Contaminating with yeast was found in mayonnaise "Manufacturer No.1".

In aseptic conditions, the grown colonies were selected, DNA was isolated from them and the amplification of the 16S rRNA gene in bacteria or the amplification of the DNA segment involving the genes 18S rRNA, 5.8S rRNA and 28S rRNA and the intergenic segments ITS1 and ITS2 in eukaryotic microorganisms were carried out. The amplicons were then isolated and sequenced using the Sanger method. A total of 117 nucleotide sequences were obtained. The obtained nucleotide sequences were compared with those already available in the international GenBank database. Table 5 presents the identified taxa of microorganisms.

**Table 2.** Number of microorganisms from the studied food products in the FPA nutrient medium

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of the product</th>
<th>CFU/ml of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk &quot;Manufacturer No.1&quot;</td>
<td>$2.7 \times 10^2$</td>
</tr>
<tr>
<td>2</td>
<td>Milk &quot;Manufacturer No.2&quot;</td>
<td>$1.3 \times 10^2$</td>
</tr>
<tr>
<td>3</td>
<td>Milk &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Mayonnaise sauce &quot;Manufacturer No.1&quot;</td>
<td>(4.0 \times 10^2)</td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Mayonnaise &quot;Manufacturer No.1&quot;</td>
<td>$7.5 \times 10^2$</td>
</tr>
<tr>
<td>7</td>
<td>Mayonnaise &quot;Manufacturer No.2&quot;</td>
<td>$7.5 \times 10^2$</td>
</tr>
<tr>
<td>8</td>
<td>Mayonnaise &quot;Manufacturer No.3&quot;</td>
<td>$2.0 \times 10^2$</td>
</tr>
<tr>
<td>9</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td>$9.0 \times 10^2$</td>
</tr>
<tr>
<td>10</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td>$1.2 \times 10^2$</td>
</tr>
<tr>
<td>11</td>
<td>Yoghurt &quot;Manufacturer No.1&quot;</td>
<td>$1.3 \times 10^5$</td>
</tr>
<tr>
<td>12</td>
<td>Yoghurt &quot;Manufacturer No.2&quot;</td>
<td>$1.1 \times 10^5$</td>
</tr>
<tr>
<td>13</td>
<td>Yoghurt &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3.** Number of microorganisms from the studied products in the Giss nutrient medium

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of the product</th>
<th>CFU/ml of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk &quot;Manufacturer No.1&quot;</td>
<td>$2.8 \times 10^2$</td>
</tr>
<tr>
<td>2</td>
<td>Milk &quot;Manufacturer No.2&quot;</td>
<td>$9.5 \times 10^2$</td>
</tr>
<tr>
<td>3</td>
<td>Milk &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Mayonnaise sauce &quot;Manufacturer No.1&quot;</td>
<td>$2.4 \times 10^2$</td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>$1.3 \times 10^2$</td>
</tr>
<tr>
<td>6</td>
<td>Mayonnaise &quot;Manufacturer No.1&quot;</td>
<td>$2.0 \times 10^3$</td>
</tr>
<tr>
<td>7</td>
<td>Mayonnaise &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Mayonnaise &quot;Manufacturer No.3&quot;</td>
<td>$2.2 \times 10^3$</td>
</tr>
<tr>
<td>9</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td>$1.7 \times 10^2$</td>
</tr>
<tr>
<td>10</td>
<td>Cottage cheese &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Yoghurt &quot;Manufacturer No.1&quot;</td>
<td>$2.2 \times 10^4$</td>
</tr>
<tr>
<td>12</td>
<td>Yoghurt &quot;Manufacturer No.2&quot;</td>
<td>$2.5 \times 10^4$</td>
</tr>
<tr>
<td>13</td>
<td>Yoghurt &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 4. Number of microorganisms from the studied products in the Saburo nutrient medium contaminated with yeast and filamentous fungi

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of the product</th>
<th>CFU/ml of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk &quot;Manufacturer No.1&quot;</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Milk &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Milk &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Mayonnaise &quot;Manufacturer No.1&quot;</td>
<td>(5.0 \times 10^3)</td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Mayonnaise &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Mayonnaise sauce &quot;Manufacturer No.1&quot;</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Cottage cheese &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Yoghurt &quot;Manufacturer No.1&quot;</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Yoghurt &quot;Manufacturer No.2&quot;</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>Yoghurt &quot;Manufacturer No.3&quot;</td>
<td>0</td>
</tr>
</tbody>
</table>

The data on the number of microorganisms in the FPA medium are often in agreement with the data obtained in the Giss medium. In some samples, a difference in the number of bacteria in the FPA medium and in the Giss medium was revealed. Thus, in the FPA medium, when seeding the samples of mayonnaise sauce "Manufacturer No.2" and yoghurt "Manufacturer No.3" CFU is 0, and in the Giss medium with mannitol CFU/ml is from \(10^2\) to \(10^3\). In the Giss medium, when seeding the samples of mayonnaise "Manufacturer No.2", yoghurt "Manufacturer No.1" and cottage cheese "Manufacturer No.2" CFU/ml is 0, and in the FPA medium CFU/ml is from \(10^3\) to \(10^6\) for a number of samples.

It should be noted that the representatives of the genera *Pseudomonas*, *Bacillus* and *Lactococcus* have been identified in both media. The seeding in the FPA nutrient media and the Giss medium made it possible to identify the differences at the genus level: the seeding in the FPA medium made it possible to identify the representatives of the genera *Kocuria*, *Staphylococcus*, *Moraxella*, *Pausalisbacillus*, *Acinetobacter* and *Klebsiella*, while the representatives of the genera *Paenibacillus*, *Lysinibacillus*, *Enterobacter*, *Acetobacter* and *Massilia* were identified in the Giss medium.

### Table 5. Comparative microbiological analysis when seeding in the FPA and Giss nutrient media

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of the product</th>
<th>Identified bacteria taxa in the FPA medium</th>
<th>Identified bacteria taxa in the Giss medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Milk &quot;Manufacturer No.1&quot;</td>
<td><em>Pseudomonas</em> sp.</td>
<td><em>Pseudomonas</em> fluorescens</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus</em> sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acinetobacter</em> sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> fluorescens</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Milk &quot;Manufacturer No.2&quot;</td>
<td><em>Bacillus</em> sp.</td>
<td><em>Acetobacter</em> sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> sp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lactococcus</em> lactis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Moraxella</em> osloensis</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Milk &quot;Manufacturer No.3&quot;</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>4</td>
<td>Mayonnaise &quot;Manufacturer No.1&quot;</td>
<td><em>Pseudomonas</em> sp.</td>
<td>Not identified</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> fluorescens</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise &quot;Manufacturer No.2&quot;</td>
<td><em>Pseudomonas</em> fluorescens</td>
<td>Not identified</td>
</tr>
<tr>
<td>6</td>
<td>Mayonnaise &quot;Manufacturer No.3&quot;</td>
<td><em>Bacillus</em> sp.</td>
<td>Not identified</td>
</tr>
<tr>
<td>7</td>
<td>Mayonnaise sauce &quot;Manufacturer No.1&quot;</td>
<td><em>Bacillus</em> sp.</td>
<td><em>Enterobacter</em> cloacae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Bacillus</em> sp.</td>
</tr>
<tr>
<td>8</td>
<td>Mayonnaise sauce &quot;Manufacturer No.2&quot;</td>
<td>Not identified</td>
<td><em>Bacillus</em> sp.</td>
</tr>
<tr>
<td>9</td>
<td>Yoghurt &quot;Manufacturer No.1&quot;</td>
<td><em>Kocuria</em> rosea</td>
<td><em>Lactococcus</em> lactis</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lactococcus</em> lactis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> fluorescens</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> sp.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Yoghurt &quot;Manufacturer No.2&quot;</td>
<td><em>Bacillus</em> sp.</td>
<td><em>Pseudomonas</em> fluorescens</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Enterobacter</em> cloacae</td>
</tr>
<tr>
<td>11</td>
<td>Yoghurt &quot;Manufacturer No.3&quot;</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>12</td>
<td>Cottage cheese &quot;Manufacturer No.1&quot;</td>
<td><em>Pseudomonas</em> fluorescens</td>
<td><em>Lysinibacillus</em> sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Staphylococcus</em> warneri</td>
<td><em>Acetobacter</em> sp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus</em> sp., <em>Moraxella</em> osloensis</td>
<td><em>Massilia</em> varians</td>
</tr>
<tr>
<td>13</td>
<td>Cottage cheese &quot;Manufacturer No.2&quot;</td>
<td><em>Klebsiella</em> pneumonia, <em>Bacillus</em> sp.</td>
<td>Not identified</td>
</tr>
</tbody>
</table>
Table 6. Percentage of the grown colonies of microorganisms in the FPA and Giss medium

<table>
<thead>
<tr>
<th>No.</th>
<th>Identified taxon</th>
<th>Total frequency of occurrence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacillus sp.</td>
<td>32.5</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas sp.</td>
<td>23.7</td>
</tr>
<tr>
<td>3</td>
<td>Lactococcus lactis</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>Acetobacter indonesiensis</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>Moraxella osloensis</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Filamentous fungi and yeast were not detected in any of the samples except for the mayonnaise sample of Manufacturer No.1. According to the sequence of the DNA segment that includes the genes 18S rRNA, 5.8S rRNA and 28S rRNA and the intergenic segments ITS1 and ITS2, this yeast has been identified as the representatives of Saccharomycetes of the Saccharomycetaceae family of the Pichia genus.

Table 6 presents the quantitative ratios of the identified microorganisms.

When analyzing the quantitative ratio of microorganisms, it was revealed that Bacillus sp., among which it is possible to distinguish conditionally Bacillus licheniformis (16.67% of colonies) (the taxon closest to DNA in the international GenBank database) and Bacillus subtilis (11.4% of colonies) most often contaminate dairy and fat-and-oil products. Among Pseudomonas sp., Pseudomonas fluorescens (19.3% of colonies) are the most numerous.

To study the method of high-performance sequencing in order to identify microbiological contamination of fat-and-oil products, we analyzed 3 samples of milk, 3 samples of mayonnaise, 2 samples of mayonnaise sauce and 2 samples of cottage cheese. After the preliminary enrichment of the studied samples of food products, 100 μl of broth was selected (or the precipitate was resuspended in 100 μl of broth, if the isolation was made from mayonnaise or mayonnaise sauce). Then DNA was isolated and PCR was performed. Since one sample that corresponds to one barcode of the sequencer, contained several types of the amplicons obtained from multiplex PCR (the fragments of bacterial 16S rRNA and a segment that includes the genes 18S rRNA, 5.8S rRNA and 28S rRNA, and also the intergenic segment ITS1 in fungi), the demultiplexing of reads was carried out before the further analysis (see materials and methods). As a result, two new reads that contain the DNA sequences of bacteria and fungi were obtained, respectively, for each source file.

Table 7 presents the sequence coincidences obtained during high-performance sequencing with the sequences of prokaryotic and eukaryotic microorganisms available in the international databases.

When analyzing the taxa revealed by means of high-performance sequencing, it turned out that the list of microorganisms in dairy products is less extensive than in the case of identification by classical DNA barcoding. While there was a different situation in high-fat products (mayonnaise and mayonnaise sauces). This effect is most likely due to the additional concentration of microorganisms when enriching mayonnaise and mayonnaise sauces, since when studying these products using classical DNA barcoding there was no concentration and the products were immediately introduced into nutrient media. Besides, the additional optimization is probably required when preparing DNA libraries from dairy products, as well as, possibly, the targeted amplification of target microorganism groups. The high-performance sequencing has also better revealed the presence of eukaryotic microorganisms in the studied food products.

Table 7. List of microorganisms detected by high-performance sequencing

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Product</th>
<th>Prokaryotic microorganisms</th>
<th>Eukaryotic microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mayonnaise &quot;manufacturer no.1&quot;</td>
<td>Not identified</td>
<td>Radulidium subulatum, Pichia kudriazevii, Cryptococcus sp., Epicoccum nigrum</td>
</tr>
<tr>
<td>2</td>
<td>Mayonnaise &quot;manufacturer no.2&quot;</td>
<td>Not identified</td>
<td>Pichia kudriazevii</td>
</tr>
<tr>
<td>3</td>
<td>Mayonnaise &quot;manufacturer no.3&quot;</td>
<td>Lactobacillus sp., Vibrio sp., Acinetobacter sp., Bacillus sp., Streptococcus sp., Klebsiella sp., Leuconostoc sp.</td>
<td>Not identified</td>
</tr>
<tr>
<td>4</td>
<td>Mayonnaise sauce &quot;manufacturer no.1&quot;</td>
<td>Acinetobacter sp., Lactobacillus sp., Bacillus sp., Escherichia sp., Aeromonas sp.</td>
<td>Pichia kudriazevii</td>
</tr>
<tr>
<td>5</td>
<td>Mayonnaise sauce &quot;manufacturer no.2&quot;</td>
<td>Bacillus sp.</td>
<td>Not identified</td>
</tr>
<tr>
<td>6</td>
<td>Milk &quot;manufacturer no.1&quot;</td>
<td>Bacillus sp., Acinetobacter sp.</td>
<td>Pichia sp.</td>
</tr>
<tr>
<td>7</td>
<td>Milk &quot;manufacturer no.2&quot;</td>
<td>Bacillus sp., Lactococcus lactis</td>
<td>Cryptococcus sp., Pichia sp.</td>
</tr>
<tr>
<td>8</td>
<td>Milk &quot;manufacturer no.3&quot;</td>
<td>Not identified</td>
<td>Not identified</td>
</tr>
<tr>
<td>9</td>
<td>Cottage cheese &quot;manufacturer no.1&quot;</td>
<td>Lactococcus lactis, Bacillus sp.</td>
<td>Pichia manshurica, Pichia kluveri</td>
</tr>
<tr>
<td>10</td>
<td>Cottage cheese &quot;manufacturer no.2&quot;</td>
<td>Lactococcus lactis, Bacillus sp.</td>
<td>Pichia sp.</td>
</tr>
</tbody>
</table>
The analysis of species composition of microorganisms using classical DNA barcoding, as well as metabarcoding, allowed us to identify opportunistic pathogenic species from the number of products: *Staphylococcus warneri*, *Staphylococcus epidermidis*, *Klebsiella pneumonia*, *Bacillus cereus*, *Vibrio sp.* *Klebsiella pneumoniae* is one of the causative agents of pneumonia, as well as some urogenital infectious diseases and purulent abscesses of the spleen and liver. This microorganism can also be pathogenic to some animals. Some strains are multiresistant to antibiotics, the capsule is a virulence factor [36–38].

*Staphylococcus warneri*, like other staphylococci, belongs to the 4th group of pathogenicity, it rarely causes human and animal diseases, it is mainly characteristic of the patients with a blunt immunity. There are cases of conjunctivitis, the infections of the urogenital tract and septicemia associated with *S. warneri* [39, 40].

*Staphylococcus epidermidis* occurs on the mucous membranes and human skin, can cause endocarditis, sepsis, the purulent infection of wounds and urinary tract infections [39].

*Bacillus cereus* is a dangerous pathogen that causes foodborne toxic infections in human (including diarrhea and the emetic syndrome). The diarrheal syndrome is caused by a high molecular weight peptide toxin, whereas the emetic syndrome is caused by a low molecular weight thermostable toxin [36, 41].

*Vibrio sp.* identified by high-performance sequencing, also poses a risk to human health, at the same time, some species of this genus are even related to the 2nd and 3rd group of pathogenicity [41, 43].

In addition to pathogenic microorganisms, the microorganisms that spoil products were detected. The microbiological spoilage of products – the development of harmful microorganisms in food products with the subsequent accumulation of their waste products – can also be dangerous because of the evolved toxins and the development of pathogenic microbiota. The key cause of microbiological spoilage of products is fermentation. Most often, the spoilage of products is caused by acetic and amylitic fermentation, as well as rotting [44]. Acetic fermentation forms the *Acetobacter* bacteria genus, which we detected in milk and cottage cheese. These bacteria are able to oxidize ethanol to acetic acid, acetate and lactate. *Acetobacter* is of particular importance for the food industry, since these bacteria spoil products, producing acetic acid or ethyl acetate. The substrates that contain sugar or fruit are particularly susceptible to acetic acid fermentation [45].

In the course of the analyzes made, we found the presence a large amount of bacteria of the *Bacillus* genus the reproduction of which can lead to the decay of food substrates. The rotting process is caused by putrefactive microorganisms that are widespread in all habitats, including animals and plant organisms. The deepest breakdown of proteins is caused by the representatives of the *Enterobacteriaceae* family (for example, the genera *Proteus* and *Escherichia*) and the spore-forming bacteria of the genus *Bacillus* and *Clostridium* [46].

With the help of high-performance sequencing, we managed to identify eukaritic microorganisms in several samples, while with the help of microbiological seeding, eukarytic microorganisms were only detected in one of the samples. This can be explained by the higher sensitivity of new generation sequencing when identifying microorganisms compared to microbiological seeding or dead eukarytic microorganisms were possibly identified, since their DNA can be retained for a long time in food substrates.

In our study we used the method of classical DNA barcoding (the sequencing of marker genes of prokaryotic and eukarytic microorganisms), as well as the method of DNA metabarcoding based on high-performance sequencing on an Ion torrent PGM platform. The main advantage of the method of classical DNA barcoding is the lower cost of analysis in relation to metabarcoding. The main disadvantage of the method of classical DNA barcoding is the requirement for the homogeneity of biological material, which is achieved either by the preliminary separation of the studied microorganisms or by the molecular cloning of PCR fragments. In our case this requirement was achieved by means of the preliminary seeding of microorganisms in solid nutrient media. When performing metabarcoding based on high-performance sequencing, the preliminary seeding is not required.

The next-generation sequencing (NGS) collectively describes several technologies that provide the mass parallel sequencing of heterogeneous DNA fragments. With regard to the monitoring of the microbial community, these fragments consist of the short segments amplified using the universal primers targeted at the known marker genes, predominantly the prokaryotic 16S rRNA and fungal ITS genes. At present, two NGS systems are basically used for profiling microbial communities; these are the sequencing platforms 454 Life Sciences pyrosequencing [47] and Illumina [48]. We have shown that the Ion torrent PGM platform is also capable to identify effectively the complex mixtures of microorganisms. The presented detection method using an Ion torrent PGM platform seems promising for rapid microbial characteristics in multiple food samples with the unknown composition of microorganisms by determining numerous nucleic sequences simultaneously without the need for cloning PCR products and microbiological seeding. However, it should be noted that high-performance sequencing is an expensive and time-consuming method yet [49].

The microorganisms that we have identified, which belong to the pathogenic group in ready-made dairy and fat-and-oil products, are a wake-up call. The production control at the enterprises that produced the studied products was probably carried out using only the microorganisms of the 3rd group of pathogenicity, such as *Shigella spp.* and *Listeria monocytogenes*. However, the microorganisms of the 4th pathogenicity group (in particular, *Bacillus cereus*, *Staphylococcus sp.*, *Klebsiella pneumoniae* and *Vibrio sp.*) that we have detected, are not equally allowed in ready-made dairy and fat-and-oil products, as, for example, *Shigella spp.* and *Listeria monocytogenes* (TR TS 033/2013 "On safety of milk and dairy products" and TR TS 024/2011 "Technical Regulations on the safety of fat-and-oil products"), since they also belong to pathogenic microorganisms, although belong to the last
4 group of the list of pathogenic microorganisms (SP 1.3.2322-08 "Safety of work with microorganisms"). The technical regulations of the customs union do not allow the presence of pathogenic microorganisms in any food products, the presence of coliforms is also not allowed or is sharply limited, the limitations of the amount of yeast and mold are introduced. Thus, for example, TR TS 033/2013 "On safety of milk and dairy products" separately prescribes the non-admissibility of the presence of the coliforms S.aureus, L. monocytogenes and Salmonella spp., while for a number of dairy products the counting of eukaryotic microorganisms is not regulated, but for other products, the amount of yeast and mold should not exceed 50 per 1 cm³ (g). In the international documents that regulate the microbiological standards of food products, for example, European Hygiene and Food Safety Criteria 2073/2005, there is also a ban on the content of pathogenic microorganisms, especially L. monocytogenes, Cronobacter spp. (Enterobacter sakazakii) and Salmonella spp.

In general, the methods that we have presented on the basis of DNA metabarcoding can become an alternative to standard microbiological seeding during production control at the food enterprises of the dairy and fat-and-oil industry, since, despite the high cost of high-performance sequencing, the analysis time is significantly reduced, which will allow to faster load up store shelves with the products with a short shelf life.

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TECHNOLOGICAL PROPERTIES OF MILK OF COWS WITH DIFFERENT GENOTYPES OF KAPPA-CASEIN AND BETA-LACTOGLOBULIN

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Abstract: The presence of the desirable alleles and genotypes of casein and whey protein genes in the genome of cows affects the milk protein content, quality and technological properties of their milk. Two important properties of milk its producibility are judged on are cheeseability and heat resistance. The present studies aimed at estimating the technological properties of milk of black-motley × Holstein and Kholmogorskaya breeds cows of the Tatarstan type with different kappa-casein (CSN3) and beta-lactoglobulin (BLG) genotypes. The study was carried out using a sampling of the first-calf cows of 5 cattle-breeding farms of the Republic of Tatarstan. In animals, the CSN3 and BLG genotypes have been determined by a PCR-RFLP analysis. The cheeseability, heat resistance and thermostability of milk have been estimated using standard methods. The studies have established that the CSN3 and BLG genotypes of cows affected the condition of a casein clot and duration of milk clotting time. The best cheese-making properties of milk were inherent in the animals with the BB and AB genotypes of the CSN3 and BLG genes. They were superior to the coevals with the AA genotype in terms of the highest yield of the desired dense casein clot and the shortest duration of milk clotting time. The first-calf cows, which are the carriers of an A allele of the CSN3 gene, were superior to the animals with the BB genotype of the CSN3 gene on the thermostability of milk including that on the proportion of animals with this milk characteristic. The BLG genotype of the studied animals did not significantly affect the thermostability of milk. Moreover, the highest thermostability of milk was characteristic of black-motley × Holstein cows with the AA genotype.

Keywords: Cow, milk, cheeseability, thermostability, allele, genotype, CSN3, BLG, PCR, RFLP


INTRODUCTION

The manufacture of dairy products is impossible if dairy raw materials do not meet the requirements for their development. In this context, attention should be paid to two important properties of milk its producibility, namely, the cheeseability and heat resistance are judged on.

The cheeseability of milk is a set of indicators of technological, physical and chemical and hygienic properties, as well as the chemical composition of milk [1]. To produce cheese and cottage cheese, only milk, which can coagulate with the formation of a dense casein clot, can be used when affected by a rennet enzyme [2, 3].

The heat resistance of milk is the technological property of milk to resist high temperatures without protein coagulation [4]. This property of milk is an important condition for the development of sterilized products that are in high consumer demand due to their long shelf life. To manufacture such products, milk is treated at high temperatures (110–160°C) [2, 3].

Therefore, high requirements are imposed to milk as the raw materials used for the manufacture of such dairy products as cottage cheese, cheese, yogurt,
canned food, including gerodietic and functional foods [5–11].

The studies on the technological properties of milk with the involvement of the modern molecular genetic methods of diagnostics in cattle breeding are of particular interest. A lot of countries currently use genetic markers that are related to the qualitative features of dairy productivity [12].

The evidence has been presented that the presence of the "desirable" alleles and genotypes of casein (alpha-casein [13], beta-casein [14] and kappa-casein [15–17]) and whey (beta-lactoglobulin [15, 19] and alpha-lactalbumin [20]) milk proteins in the genome of cows have an effect on milk protein content, quality and technologival properties of their milk [3, 21, 22].

In this regard, the present studies aimed at estimating the technological properties of milk of cows of black-motley × Holstein and Kholmogorskaya breeds of the Tatarstan type with different kappa-casein (CSN3) and beta-lactoglobulin (BLG) genotypes.

In accordance with the aim of the study the following tasks were being solved:
- to genotype the studied sampling of first-calf cows in several farms of the Republic of Tatarstan on the A and B alleles of the CSN3 and BLG genes by a PCR-RFLP analysis;
- to determine the cheeseability and thermostability of the milk of the studied sampling of first-calf cows depending on their genotype of the CSN3 and BLG genes.

STUDY OBJECTS AND METHODS

The studies were carried out in Agricultural production cooperative named after Lenin and Dusym, LLC of Atkinsky District, the LLC named after Tukay of Baltasinsky District, Biryulinskiy Stud Farm, OJSC and Hammer and sickle, LLC in Vysokogorsky District of the Republic of Tatarstan with 608 first-calf cows of the black-motley × Holstein breed and 265 first-calf cows of the Kholmogorskaya breed of the Tatarstan type, respectively.

To carry out molecular genetic studies in animal were collected blood samples from the jugular vein. DNA was extracted from the samples of whole preserved (10 mM of EDTA) blood using a combined alkaline method. DNA extraction procedure. 100 μl of blood is mixed with 1 ml of dH2O and centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant is discarded, and 50 μl of 0.2 M NaOH is added to the precipitate and the mixture is thoroughly vortexed until the suspension is completely clarified. The resulting homogenate is thermostated at 60°C for 10 minutes. A proportional volume of 1 M Tris-HCl (pH 8.0) was added to the lysate followed by the careful vortexing of the mixture. 500 μl of 96% ethanol are added to the resulting homogenate followed by holding the mixture in a freezer (–20°C) for 30 minutes. The nucleoprotein complex is precipitated by centrifugation at 12,000 rpm for 10 minutes. The supernatant is discarded, and the residue is dried at 60°C for 12 minutes by opening the lid of the tube. 100 μl of 10% ammonia are added to the dried precipitate, the mixture is vortexed carefully and thermostated at 60°C for 10 minutes, then vortexed again and held in a thermostat at 60°C for 10 minutes. The resulting homogenate is held in a thermostat at 95°C for 15 minutes with the lid of the tube open.

In animals, the CSN3 and BLG genotypes have been determined by a PCR-RFLP analysis.

The CSN3 gene was amplified using a Tertzik thermocycler (Russia) in volumes of reaction mixtures (20 μl) containing the appropriate buffer (60 mM Tris-HCl (pH 8.5), 1.5 mM MgCl2, 25 mM KCl, 10 mM 2-Mercaptoethanol and 0.1 mM Triton X-100) 0.2 mM dNTPs, 1 U Taq DNA polymerase (SibEnzyme, Russia), 0.5 mM of the oligonucleotide primers AB1 and AB2 [23] and 1 μl of a DNA sample as follows:

× 1 : 94°C – 4 min;
× 40 : 94°C – 10 sec, 63°C – 10 sec, 72°C – 10 sec;
× 1 : 72°C – 5 min; storage: 4°C [24].

The RFLP-identification of genotypes on the allelic variants A and B of the CSN3 gene was performed by treating 20 μl of a PCR sample of 10 μl of the restriction enzyme HinfI in the 1 × buffer "O" (SibEnzyme, Russia) at 37°C overnight.

The BLG gene was amplified using a Tertzik thermocycler (Russia) in volumes of reaction mixtures (20 μl) containing the appropriate buffer (60 mM Tris-HCl (pH 8.5), 1.5 mM MgCl2, 25 mM KCl, 10 mM 2-Mercaptoethanol; 0.1 mM Triton X-100), 0.2 mM dNTPs, 1 U of Taq DNA polymerase (SibEnzyme, Russia), 0.5 mM of the oligonucleotide primers BLGP3 and BLGP4 [25] and 1 μl of a DNA sample as follows:

× 1 : 94°C – 4 min;
× 38 : 94°C – 10 sec, 60°C – 10 sec, 72°C – 10 sec;
× 1 : 72°C – 5 min; storage: 4°C [15].

The RFLP-identification of genotypes on the allelic variants A and B of the BLG gene was performed by treating 20 μl of a PCR sample of 5 μl of the restriction enzyme HaeIII in the 1 × buffer "C" (SibEnzyme, Russia) at 37°C overnight.

Table 1 presents the spectrum of the genotype-specific RFLP fragments generated during the reaction. The cheeseability of milk was determined with the help of a rennet and rennet fermentation sample. Preparation of a rennet enzyme solution. 1 g of rennet powder with an activity of 100 thousand units is dissolved in a mixture of distilled water and glycerol of an equal volume. After 24 hours, the solution is well mixed, filtered through a paper filter, poured into dark dishes and stored in a fridge for no more than 5 days. Immediately before use, the solution is diluted 25 times with distilled water. Then, 10 ml of the same sample of the mixed milk is added into each of three tubes. The tubes with milk are put in a water bath at 35°C, a thermometer is placed in one tube to monitor the water temperature. The milk temperature is brought to 35°C, then 1 ml of the diluted rennet enzyme solution of the same temperature is added into two tubes. The content of the two tubes is quickly mixed and placed in the water bath fixing the time. The temperature is maintained at 35°C. The duration of milk clotting time is determined in minutes, taking into account the time interval from the addition of the rennet to the formation of a dense clot.
Table 1. Primers for genotyping *Bostaurus* on the allelic variants *A* and *B* of the *CSN3* and *BLG* genes, generated PCR products and RFLP fragments

<table>
<thead>
<tr>
<th>Oligonucleotide primers</th>
<th>PCR-product (bp)</th>
<th>Genotype-specific RFLP fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AB1: 5'-TGTGCTGAGTGATCCTAGTTATGG-3'</td>
<td>453</td>
<td></td>
</tr>
<tr>
<td>AB2: 5'-GCGTTGTCTTTTGTGTTCTCTTAG-3'</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>BLGP3: 5'-GTCCTTGTGCTGGACACCGACTACA-3'</td>
<td>262</td>
<td></td>
</tr>
<tr>
<td>BLGP4: 5'-CAGGACACCCGCTCCCCGTTATGA-3'</td>
<td></td>
<td>109</td>
</tr>
</tbody>
</table>

The heat resistance of milk was determined with the help of a thermal (crucible) sample. Setting a crucible sample. 2 ml of milk is added into each of molybdenum glass tubes. The tubes with milk are put in an ultrathermostat and heated to a temperature of 135°C fixing the time. If the consistency of milk does not change within 5 minutes, then it is considered heat-resistant.

The thermostability of milk was also determined taking into account the time interval from the moment the tubes were placed in the ultrathermostat until the first signs of protein coagulation.

The variational statistical analysis of the results of the studies was carried out using the biometric method [26]. The reliability of the obtained results of the studies was confirmed by the tabular data of Student's criterion.

RESULTS AND DISCUSSION

The results of cattle genotyping on the *A* and *B* alleles of the *CSN3* and *BLG* genes with the used sets of primers and restriction endonucleases for a PCR-RFLP analysis are satisfactory in terms of the reproducibility and identification of genotypes.

Thus, the primers AB1 and AB2 initiate the amplification of the *CSN3* gene locus of cattle with a length of 453 bp, and the *HinfI*-RFLP analysis of the generated genotype-specific fragments (*AA* = 326/100/27 bp, *BB* = 426/27 bp and *AB* = 426/326/100/27) provides a correct genotyping procedure (Fig. 1).

The primers BLGP3 and BLGP4 initiate the amplification of the *BLG* gene locus of cattle with a length of 262 bp, and the *HaeIII*-RFLP analysis of the generated genotype-specific fragments (*AA* = 153/109 bp, *BB* = 109/79/74 bp and *AB* = 153/109/79/74 bp) provides a correct genotyping procedure (Fig. 2).

The rationality of the use of whole milk for manufacturing protein-milk products, including cheese, is affected by its technological properties, such as coagulability under the influence of a rennet enzyme, the density of the formed casein clot and duration of milk clotting time.
The study has determined that the kappa-casein (CSN3) genotype of cows is associated both with the condition of a casein clot and with duration of milk clotting time. In all three samples, the milk from the cows of the Kholmogorskaya breed of the Tatarstan type with the AA genotype of the kappa-casein gene had the worst cheeseability properties. Both friable and flabby casein clots (Tables 2, 3, 4) were obtained from the milk of the cows (46.8–48.6%) of this genotype.

The presence of the allele B of the kappa-casein gene in the animal genome significantly affected the improvement of the condition of a casein clot. The proportion of milk with the condition of a casein clot characterized as dense in the cows of the homozygous genotype BB was 100%, and in the cows with the heterozygous genotype AB – 81.8–84.1%.

The most desirable in cheese-making is milk the clotting time of which when treated with a rennet enzyme is within the range of 15–40 minutes. If the milk clotting time is more than 40 minutes, there is a large loss of raw materials with a low yield of cheese due to a disruption in the manufacturing process. The best indicators on duration of milk clotting time have been noted in the first-calf cows with the genotype BB of the kappa-casein gene. The milk of these animals coagulated in the period with the lowest time interval – 16.9–18.2 min. The milk clotting time in the animals with the AA genotype turned out to be longer and was 30.4–31.3 minutes (P < 0.001).

The similar studies carried out using a single sampling of black-motley × Holstein cows with different genotypes of the kappa-casein gene also showed that there are intergroup differences in the cheese-making properties of milk. The groups of the cows carrying the allele A of the kappa-casein gene in their genotype had a higher proportion of animals with the worst condition of a casein clot. Both friable and flabby casein clots were obtained from the milk of 50.0% of the cows with the AA genotype (Table 5).

The presence of the allele B of the kappa-casein gene in the animal genome had a significant effect on the condition of a casein clot. The proportion of milk with the condition of a casein clot characterized as dense in the cows with the heterozygous genotype AB was 80.6%, and in the cows with the homozygous genotype BB was equal to 100.0% (Table 5).

**Table 2.** Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their CSN3 genotype in Hammer and Sickle, LLC

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a CSN3 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n %</td>
<td>AA %</td>
</tr>
<tr>
<td>n = 225</td>
<td></td>
<td></td>
<td>AA n %</td>
</tr>
<tr>
<td>dense</td>
<td></td>
<td>141 62.7</td>
<td>82 52.6</td>
</tr>
<tr>
<td>friable</td>
<td></td>
<td>73 32.4</td>
<td>66 42.3</td>
</tr>
<tr>
<td>flabby</td>
<td></td>
<td>11 4.9</td>
<td>8 5.1</td>
</tr>
<tr>
<td>time, min</td>
<td></td>
<td>28.5 ± 0.84</td>
<td>30.6 ± 0.99</td>
</tr>
</tbody>
</table>

**Note.** Difference between BB, AB and AA genotypes: *** P < 0.001.

**Table 3.** Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their CSN3 genotype in Agricultural Production Cooperative Society named after Lenin

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a CSN3 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n %</td>
<td>AA %</td>
</tr>
<tr>
<td>n = 219</td>
<td></td>
<td></td>
<td>AA n %</td>
</tr>
<tr>
<td>dense</td>
<td></td>
<td>147 67.1</td>
<td>57 51.4</td>
</tr>
<tr>
<td>friable</td>
<td></td>
<td>56 25.6</td>
<td>43 38.7</td>
</tr>
<tr>
<td>flabby</td>
<td></td>
<td>16 7.3</td>
<td>11 9.9</td>
</tr>
<tr>
<td>time, min</td>
<td></td>
<td>27.2 ± 0.34</td>
<td>31.3 ± 0.46</td>
</tr>
</tbody>
</table>

**Note.** Difference between BB, AB and AA genotypes: *** P < 0.001.

**Table 4.** Cheeseability of milk of the first-calf cows of the Kholmogorskaya breed of the Tatarstan type depending on their CSN3 genotype in Biryulinskiy Stud Farm, OJSC

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a CSN3 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n %</td>
<td>AA %</td>
</tr>
<tr>
<td>n = 164</td>
<td></td>
<td></td>
<td>AA n %</td>
</tr>
<tr>
<td>dense</td>
<td></td>
<td>104 63.4</td>
<td>58 53.2</td>
</tr>
<tr>
<td>friable</td>
<td></td>
<td>50 30.5</td>
<td>44 40.4</td>
</tr>
<tr>
<td>flabby</td>
<td></td>
<td>10 6.1</td>
<td>7 6.4</td>
</tr>
<tr>
<td>time, min</td>
<td></td>
<td>27.8 ± 0.59</td>
<td>30.4 ± 0.68</td>
</tr>
</tbody>
</table>

**Note.** Difference between BB, AB and AA genotypes: *** P < 0.001.
Table 5. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their CSN3 genotype in the LLC named after Tukay

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a CSN3 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>n = 107</td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>dense</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>friable</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>flabby</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>time, min</td>
<td></td>
<td>29.2 ± 0.67</td>
</tr>
</tbody>
</table>

Note. Difference between BB, AB and AA genotypes: *** P < 0.001.

Table 6. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their BLG genotype in the LLC named after Tukay

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and the duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a BLG genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>n = 107</td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>dense</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>friable</td>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>flabby</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>time, min</td>
<td></td>
<td>29.2 ± 0.67</td>
</tr>
</tbody>
</table>

Note. Difference between BB, AB and AA genotypes: ** P < 0.01.

Table 7. Cheeseability of milk of the black-motley × Holstein first-calf cows depending on their BLG genotype in Dusym, LLC

<table>
<thead>
<tr>
<th>Total of cows</th>
<th>Condition of a casein clot and the duration of milk clotting time</th>
<th>Distribution of cows</th>
<th>Including that with a BLG genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AA</td>
</tr>
<tr>
<td>n = 158</td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>dense</td>
<td></td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>friable</td>
<td></td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>flabby</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>time, min</td>
<td></td>
<td>28.5 ± 0.59</td>
</tr>
</tbody>
</table>

The best indicators on duration of milk clotting time were characteristic of the first-calf cows with the genotype BB of the CSN3 gene. The clotting time of their milk was the shortest – 18.9 min. The longest clotting time was noted for the milk of the cows with the AA genotype and was equal to 31.7 minutes. In this case, the milk from the animals with the heterozygous genotype AB was at the intermediate level of the analyzed indicator – 25.4 min. The first-calf cows carrying the allele B of the CSN3 gene in their genome were favorably inferior to their coevals with the AA genotype by 6.3–12.8 min (Table 5).

Similar results were obtained when carrying out a rennet test of the milk of the cows with different CSN3 genotypes in the studies of animals of the Yaroslavl breed [27], of the holsteinized Kholmogorsksaya breed of the "Tsentralny" type [28], the Samara type of black-motley cattle [29], of the Ural black-motley breed [17], the red-motley breed of the created Volga type [30], the Volga type of the red-motley breed [31], the Simmental and red-motley breeds [16], the Italian Holstein breed [32], the Danish Jersey and Holstein breeds [33], the dairy breeds of different ecological zones of the Siberia, Sakha (Yakutia) and Macedonia, namely black-motley, Holstein, red steppe and Simmental [34], the Sicilian Cinisara breed [35], Estonian Holstein, red-motley Holstein, Estonian red, the Estonian native breed [36] and the Macedonian Holstein breed [37]. In their studies, the milk from the cows with the AB and BB genotypes of the CSN3 gene compared to the milk from the animals with the AA genotype when affected by the enzyme had shorter coagulation periods. However, the studies of Norwegian red cattle have provided some other results. Thus, the duration of milk clotting time when affected by a rennet enzyme from the animals with different genotypes of the kappa-casein gene was in the following order: AB<AA<BE<BB [38].

It is believed that the whey protein beta-lactoglobulin, like the other protein fractions of whey, does not lend itself to rennet coagulation, and therefore they are absent in cheese mass. Nevertheless, the genetic types of this protein can affect the process of isolating whey from a casein clot and thereby improve the quality of cheese mass [3].

The study revealed that of 2 sampling of black-motley × Holstein first-calf cows with different beta-lactoglobulin (BLG) genotypes, the milk of the first-calf cows with the BB genotype had the best cheese-making properties. When affected by a rennet enzyme,
The long-term storage of milk and dairy products is impossible without high-temperature treatment (63–150°C) which is used for pasteurization, sterilization, thickening and drying. When treated by high temperatures, the product often undergoes irreversible protein coagulation and rapid milk coagulation. Therefore, the solution to the problem related to an increase in the heat resistance of milk is of high practical importance. In this regard, we have studied the heat resistance of milk of the cows with different genotypes of the kappa-casein gene.

The study has determined that the milk of three sampling of first-calf cows of the Tatarstan type with the BB genotype of the CSN3 gene had a lower thermostability (33.1–35.2 min), with the AA genotype – a high thermostability (60.1–65.8 min), and that with the genotype AB showed an intermediate value (57.5–62.9 min) (Table 8).

It has been determined in the studies of the thermostability of milk of a single sampling of black-motley × Holstein first-calf cows with different CSN3 genotypes that the milk of cows with the BB genotype had a lower thermostability (39.3 min), with the AA genotype – increased thermostability (57.2 min), and with the genotype AB – an intermediate value (56.5 min). The first-calf cows with the genotype AA of the CSN3 gene were superior to the coevals with the genotypes BB and AB by 17.9 min and 0.7 min, respectively (Table 9).

Similar results on the heat resistance of milk from the cows with different CSN3 genotypes were obtained in the studies of Holstein, Ayrshire, Kholmogorskaia and Kholmogor × Holstein cross-breeds of domestic lineage [3], the red-motley breed and the created Volga type of Russian breeding [30]. In their studies, the milk from the cows with the genotypes AA and AB of the CSN3 gene had a higher thermostability compared with the milk from the animals with the BB genotype. However, the studies of the Bestuzhev breed and domestic Bestuzhev × Ayrshire cross-breeds [3] gave some other results. Thus, the milk from the cows with the genotypes AB and BB of the CSN3 gene was more heat resistant compared to the milk from the animals with the AA genotype.

**Table 8.** Thermostability of milk of the first-calf cows of the Kholmogorskaia breed of the Tatarstan type with different CSN3 genotypes

<table>
<thead>
<tr>
<th>Farm</th>
<th>Thermostability of milk (min) of cows (head) with different CSN3 genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>AA</strong></td>
</tr>
<tr>
<td>Hammer and Sickle, LLC</td>
<td>156 head</td>
</tr>
<tr>
<td></td>
<td><strong>65.8 ± 0.72 min</strong></td>
</tr>
<tr>
<td>Agricultural Production</td>
<td>111 head</td>
</tr>
<tr>
<td>Cooperative Society named after Lenin</td>
<td><strong>63.7 ± 1.10 min</strong></td>
</tr>
<tr>
<td>Biryulinskiy Stud Farm, OJSC</td>
<td>109 head</td>
</tr>
<tr>
<td></td>
<td><strong>60.1 ± 1.82 min</strong></td>
</tr>
</tbody>
</table>

Note: Difference between **BB**, **AB** and **AA** genotypes: * P < 0.05; *** P < 0.001.
We also carried out a study of the thermostability of milk of two sampling of black-motley × Holstein cows with different BLG genotypes. The study showed that the thermostability of milk of first-calf cows with different BLG genotypes was within the ranges of 52.7–56.0 min (the genotype BB) and 58.5–58.9 min (the genotype AA). The animals carrying the B allele of the BLG gene were inferior in this indicator to the coevals with the genotype AA by 1.5–6.2 min (Table 10).

Similar results on the thermostability of milk of cows with different BLG genotypes were obtained in the studies of animals of the Russian black-motley breed [19], the Ukrainian black-motley breed [39] and the domestic black-motley and Bestuzhev breeds [22]. In their studies, the milk from the cows with the genotypes AA and AB of the BLG gene had a higher heat resistance compared to the milk from the animals with the genotype BB. However, the studies of the Holstein, Ayrshire and Kholmogorskaia breeds and Bestuzhev × Ayrshire cross-breeds of domestic selection [3] gave some other results. Thus, the thermostability of milk with different BLG genotypes was expressed in the following order: AA<AB<BB, while the order for the Bestuzhev breed and domestic Kholmogor × Holstein cross-breeds was AB<AA<BB, respectively.

**CONCLUSIONS**

The selected systems of cattle genotyping on the A and B alleles of the CSN3 and BLG genes by a PCR-RFLP analysis allowed us to genotype correctly the sampling of first-calf cows in several cattle-breeding farms in the Republic of Tatarstan.

The study of first-calf cows of the Kholmogorskaia breed of the Tatarstan type and black-motley × Holstein cows has shown that the best cheese-making properties of milk are inherent in the animals with the genotype BB and AB of the CSN3 gene. Their milk had the highest yield of the desired dense casein clot, as well as the shortest duration of milk clotting time, and they were significantly superior to their analogs with the AA genotype on these indicators. As for the BLG gene, the first-calf cows with the genotype BB and AB had the best cheese-making properties of milk. These animals were superior to their coevals with the AA genotype in terms of the highest yield of the desired dense casein clot and the shortest duration of milk clotting time.

The first-calf cows of the Kholmogorskaia breed of the Tatarstan type and black-motley × Holstein cows, which are the carriers of an A allele of the CSN3 gene, were superior to the animals with the BB genotype of the CSN3 gene on the thermostability of milk including that on the proportion of animals with this milk characteristic. The BLG genotype of the studied animals did not significantly affect the thermostability of milk. Moreover, the highest thermostability of milk was characteristic of the black-motley × Holstein cows with the AA genotype.

It is advisable to use the milk from the cows with the genotypes BB and AB of the CSN3 and BLG genes that has the best cheese-making properties for manufacturing cheeses and products of lactic acid fermentation. When processing the milk from the cows with the AA and AB genotypes of the CSN3 gene as the most heat-resistant, it is advisable to use it to produce drinking pasteurized and sterilized milk with a long shelf life and canned milk. It is ineffective to differentiate cow milk on heat resistance with considering of the BLG genotype.

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**REFERENCES**


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INTRODUCTION

The progressive development of processing lines is due to the calculation of the level of integrity of the existing technological systems by experimentally determining the stability of the specific subsystems that have been established at a certain level of stability [1, 2, 6, 8]. The concept of stability of a subsystem (the level of its organization or integrity) has a broader meaning in comparison with the stability that characterizes the quality of functioning of a system.

The known methods for analyzing complex technological flows are only related to the use of information entropy to estimate the efficiency of the existing processing lines in order to modernize them and do not allow us to use system approaches in their design and layout, which leads to the subjectivity of estimation of technologies and their hardware design. It is possible to solve the situation by predicting the stability of operation of technical and technological subsystems and systems as a whole on the basis of material and technical flows. The study objects are the integrity of technological and technical systems, the stability of processes, operations and equipment operation. As a result of the analysis of technological flows and a change in the entropy of the technical system, the mechanism of accumulation of structural information entropy has been studied. The carried out analytical and experimental studies have confirmed the possibility of predicting the stability of the operation of technical and technological systems, as well as the expediency of determining the ranges of variation in the parameters of operation of the lines, technological limits and the quality indicators of the finished and semi-finished products. Thus, this method is recommended for use in the food industry.

Keywords: Theory of technological flow, system analysis, entropy, stability of technological systems, energy and resource saving

quality indicators of the finished and semi-finished products. At the same time, the systemic complex analysis of technological flows can be carried out on the basis of studying the process of a change in the entropy of a technical system (the growth of system stability) by describing the processes of accumulation of the structural information calculated as the difference between the maximum and the real value of fuzzy entropy.

**STUDY OBJECTS AND METHODS**

The objects of the study are the integrity of technological and technical systems in general and their subsystems and the stability of their operation, that is, specific technologies, processes, operations and equipment items. The studies were carried out using the physico-mathematical methods of entropy estimation of the stability of a technological flow as a system and its subsystems in their interconnection at a certain level of stability.

An original method for assessing the integrity and stability of technological systems has been used based on the concept of fuzzy entropy [3, 7, 9, 10, 11] in analyzing material and technical flows for energy and resource saving purposes.

**RESULTS AND DISCUSSION**

One of the characteristics of the stability of subsystems is an entropy information function:

\[ H = \sum \mu_i \log \mu_i , \]

where \( \mu_i \) is a quantitative measure of states of a system (\( i = 1,2,... \)).

In the information theory, entropy is formed by a known amount of data about a certain phenomenon. Let \( P \) be the preliminary probability of the result of the experiment, that is, the a priori probability of an event, \( P_i \) is the obtained probability of the occurred event and let us assume \( P_i = 1 \). According to Shannon, information can be accurately a posteriori quantitatively estimated using the value \( I = K \log P \).

For example, there are two possible answers when playing chuck-farthing, i.e. \( P = 2 \). Rolling the dice, there will be 1 out of 6 possible variants, i.e. \( P = 6 \). With the base of logarithm 2 and \( K = 1 \) we have some information for the first case: \( I = \log_2 2 \) which is equal to one bit.

In fact, \( I \), which is called information entropy, is the measure of ignorance or the information obtained when solving a task. This suggests that the quantitative estimation of information and entropy \( S \) is analogous according to Boltzmann, because by equating the factor \( K \) and the Boltzmann constant \( k_B \), a natural logarithm can be used.

For the existing technological flows, the method for calculating the integrity level is recommended [4, 5, 7, 10], which is based on the analogy of determination of information and thermodynamic entropies due to their additivity and the properties characteristic only thereof [8, 9]. The definitions of entropy function and information entropy are often identical, i.e. \( \mu_t \) is characterized by a probability measure defined as a set \( (P_t, i = 1,2,...) \). The entropy function is determined by the equation:

\[ H = \sum_{i=1}^{\text{set}} P_t \log P_t, \text{ where } \sum_{i=1}^{\text{set}} P_t = 1. \]

The state of the system with the maximum entropy function \( H_{\text{max}} \) is represented by the "structureless" set of elements of a certain aggregate, as a result of which the measure of systemic organization is equal to the difference between the maximum entropy of the system and the entropy of the set of elements of a certain structure, i.e. \( \Delta H = H_{\text{max}} - H \). Normalizing \( H \), i.e. referring it to \( H_{\text{max}} \) we have \( \eta = 1 - H / H_{\text{max}} \) where: \( \eta \) is process stability, \( H \) is the entropy that corresponds to the distribution of the values of the qualitative indicators of the intermediate material, \( H_{\text{max}} \) is the maximum entropy according to the law of uniform distribution.

Then the entropy function is determined from the characteristic:

\[ H = - \sum P(x_i) \log P(x_i). \]

For 2 possible results, the a priori probability of an event is determined using the equation:

\[ P = - P \log_2 P - (1 - P) \log_2 (1 - P). \]

This indicates that entropy varies from zero to maximum, moreover, there is a zero value with \( P = 0 \) and \( P = 1 \), when the distribution is insignificant and there is no uncertainty in the system. The entropy becomes maximum in the case of the equal probability of separate observations \( (P = 0.5) \), and the distribution is totally uncertain:

\[ H_{\text{max}} = -0.5 \log_2 0.5 - 0.5 \log_2 0.5 = 1 \text{bit}. \]

To calculate the entropy function for the process, the control parameters that affect the subsequent subsystem and the entire system should be distinguished in each of the subsystems using expert estimates. In subsystems, along with a number of control parameters, the weight coefficient of each of them is determined using expert estimates.

The basic, as well as the allowable values of the parameters that correspond to their requirements are chosen from standards, technical specifications, process instructions, etc. Then, the standard value of the complex quality indicator is calculated within the range from 0 to 1. Within a certain time interval, testing is carried out fixing the values of the parameters for a given interval in the stationary mode of operation of the equipment.

The concept of process stability is applicable to study the qualitative and quantitative variability of the study object. With the system analysis of a quality change, the task is simplified, because the boundary of two intervals is determined, the properties of the entire.
set of samples that satisfy and do not satisfy the standard requirements can be split into. The study of the quantitative estimation of the variability of products requires the determination of the range boundaries, if they are not provided in the regulatory and technical documentation.

Summarizing, we note that the stability of the subsystem can be estimated using the indicator \( \eta_i = 1 - \frac{H_i}{H_{\text{max}}} \) where \( \eta_i \) and \( H_i \) are, respectively, the stability and entropy of the \( i \)-th subsystem, which in the case of a binary subsystem with 2 possible regime process states related to certain parameters is significant for the subsequent subsystem. This method is applicable for the growth of stability of a lot of technologies of animal and vegetable products. The analysis of the existing technologies of yogurt products and preparation of tobacco is given below as an example [7, 10].

In the 1st case, the processing chain was divided into 4 subsystems: \( A \) – milk standardization and compounding a mixture; \( B \) – processing a mixture and preparing a starter; \( C \) – forming a ready clot; \( D \) – packaging and cooling the finished product. As a result, the characteristics of the subsystems presented in Table 1 (\( P_i \) is the probability of yield of a product of a standard complex quality level from the subsystem) were obtained.

In this case, the integrity level is \(-0.8882\). The characteristic curves that connect the averaged stability of subsystems, their number in the system and the level of its integrity have been plotted in [7]. It has been established based on the characteristics obtained that the integrity level of the technological flow considered in the example is low and is in the area of cumulative systems, which leads to the need to change the existing technologies and their hardware.

In the second case [10], the technology was divided into three subsystems: \( A \) – the preparation of cut tobacco for the production of cigarettes; \( B \) – cutting tobacco; \( C \) – the formation of leaf tobacco of various commercial varieties. As a result of the calculations, the characteristics of the subsystems presented in Table 2 (\( P_i \) is the probability of yield of a product of a standard complex quality level from the subsystem) were also obtained.

The integrity level for the given production system is 0.22; 0.12; 0.09. As it can be seen from the calculations, the levels of integrity of the technological system under study in all time intervals have positive values and range from 0.09 to 0.22, and therefore one can conclude about the integrity of its nature.

It should be noted that the above method is related to the narrow use of only information entropy for the system analysis of complex technological flows, which leads to the possibility of estimating the efficiency of the existing processing lines for their modernization and does not allow us to use system approaches in their development, arrangement and final designing. This disadvantage that leads to the subjectivity of estimation of the existing technological flows can be eliminated by predicting the stability of subsystems and the system as a whole based on making out material and energy balances to determine energy costs and material losses. In addition, it is reasonable to determine the ranges of variation in the parameters of the operation of the lines, technological limits and the quality indicators of the finished and semi-finished products that can be obtained by comparing the calculated and empirical estimates to correlate the obtained data. Here, the process of a change in the entropy of a technical system (the growth of system stability) can be described by the process of accumulation of the structural information calculated as the difference between the maximum and the real value of fuzzy entropy.

**Table 1. Calculation of the values of stability of technology subsystems and the levels of its integrity**

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>( P_i )</th>
<th>( 1 - P_i )</th>
<th>(-P_i \log_2 P_i)</th>
<th>(-1(1 - P_i) \log_2(1 - P_i))</th>
<th>( H_i )</th>
<th>( \eta_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.84</td>
<td>0.16</td>
<td>0.2266</td>
<td>0.4245</td>
<td>0.6511</td>
<td>0.3489</td>
</tr>
<tr>
<td>B</td>
<td>0.88</td>
<td>0.12</td>
<td>0.1629</td>
<td>0.0442</td>
<td>0.2071</td>
<td>0.7929</td>
</tr>
<tr>
<td>C</td>
<td>0.87</td>
<td>0.13</td>
<td>0.1754</td>
<td>0.3840</td>
<td>0.5594</td>
<td>0.4406</td>
</tr>
<tr>
<td>D</td>
<td>0.90</td>
<td>0.10</td>
<td>0.1373</td>
<td>0.3333</td>
<td>0.4706</td>
<td>0.5294</td>
</tr>
</tbody>
</table>

**Table 2. Calculation of the values of stability of technology subsystems and the levels of its integrity**

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>( P_i )</th>
<th>( 1 - P_i )</th>
<th>(-P_i \log_2 P_i)</th>
<th>(-1(1 - P_i) \log_2(1 - P_i))</th>
<th>( H_i )</th>
<th>( \eta_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.96</td>
<td>0.04</td>
<td>0.06</td>
<td>0.19</td>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>0.94</td>
<td>0.06</td>
<td>0.08</td>
<td>0.24</td>
<td>0.32</td>
<td>0.68</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<td></td>
<td>1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.77</td>
<td>0.23</td>
<td>0.29</td>
<td>0.49</td>
<td>0.78</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>0.84</td>
<td>0.16</td>
<td>0.21</td>
<td>0.42</td>
<td>0.63</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>0.86</td>
<td>0.14</td>
<td>0.19</td>
<td>0.40</td>
<td>0.59</td>
<td>0.41</td>
</tr>
</tbody>
</table>
The structure of the flow is determined by connections between its separate operations. Any technological flow can be referred to one of three types: with a rigid, semi-rigid and with a non-rigid connection [1, 8]. In the flows with a semi-rigid and especially a non-rigid (flexible) connection, a "softer" production organization is allowed, which is due to imperfect technologies, the unstable properties of raw materials and semi-finished products, lack of machines with the required technical characteristics, various technological reliability of operations and constructive reliability of machines, etc. Taking into account the fact that there are mainly flows with a semi-rigid connection organized in the food enterprises of the most diverse sectors [7, 10, 13–18], let us describe for clarity a technological system that consists of three subsystems \( A, B \) and \( C \) with a semi-rigid connection (Fig.1).

The concept of process stability can be used to study the qualitative and quantitative variability of a product obtained as a result of a process. When studying the qualitative variability of the entire set of samples, the problem is simplified, since it is enough to determine the boundaries of two intervals that satisfy and do not satisfy the requirements of a standard. In this case, the system analysis of the aggregate of material and energy losses when the product goes through subsystems \( A, B \) and \( C \) is of interest.

The theoretical and experimental studies of technology subsystems are related to the determination of the adjustable operating parameters of equipment and are aimed at the objective justification of their rational values. Let us assume that the adjustable operating parameters for subsystems \( A, B \) and \( C \) are, for example, the parameters given in Table 3.

Material \( M_p \) and energy \( Q_p \) losses when the product goes through subsystems \( A, B \) and \( C \) are functionally dependent on the operating parameters \( \pi_1, \pi_2, ..., \pi_n \) introduced into the technology, then:

\[
M_p(A) = f_A(\pi_1, \pi_2, ..., \pi_n); Q_p(A) = f_A(\pi_1, \pi_2, ..., \pi_n); M_p(B) = f_B(\pi_1, \pi_2, ..., \pi_n); Q_p(B) = f_B(\pi_1, \pi_2, ..., \pi_n);
\]

\[
M_p(C) = f_C(f_A(\pi_1, \pi_2, ..., \pi_n), \pi_1, \pi_2, ..., \pi_n), f_A(\pi_1, \pi_2, ..., \pi_n); Q_p(C) = f_C(f_B(f_A(\pi_1, \pi_2, ..., \pi_n), \pi_1, \pi_2, ..., \pi_n), f_A(\pi_1, \pi_2, ..., \pi_n), \pi_1, \pi_2, ..., \pi_n).
\]

If the actual operating parameters or equipment condition indicators do not give an opportunity to provide the expected or prescribed losses, it destabilizes the technology. In this case, for the integrity of the system, it is necessary to set and solve a management task that requires decisions at the level of introducing corrected operating parameters \( \pi_1, \pi_2, ..., \pi_n \) into the technological flow.

This task is understood as the arrangement of a sequence of software and (or) hardware procedures that provide additional information. Decisions are made on the basis of the analysis of possible options for the operating parameters of process equipment that provide an output for the technological process from an uncontrolled state. At the same time, the technical personnel as a component of the system formalize the thinking, and it can behave programmed in accordance with the chosen rational mode of a technological process, which does not require high qualification.

Figure 2 presents the block diagram of the algorithm for solving the management task for minimizing losses by calculating the necessary options for the operating parameters of process equipment. Based on the system analysis and the implementation of the presented algorithm, it is possible to optimize a complex parameter that takes into account material and energy losses depending on the range of variable factors in each subsystem on the basis of their interrelation. An indicator \( K_{st} \) equal to the product of \( Q_{relative common} \) and \( M_{P common} \) or their sum can be taken as a complex parameter.

**Table 3. Adjustable operating parameters for subsystems \( A, B \) and \( C \)**

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>Operating parameters</th>
<th>Adjustable range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>( \pi_1, \pi_2, ..., \pi_n )</td>
<td>( \pi_{n_{min}} \leq \pi_n \leq \pi_{n_{max}} )</td>
</tr>
<tr>
<td>B</td>
<td>( \pi_1, \pi_2, ..., \pi_n )</td>
<td>( \pi_{n_{min}} \leq \pi_n \leq \pi_{n_{max}} )</td>
</tr>
<tr>
<td>C</td>
<td>( \pi_1, \pi_2, ..., \pi_n )</td>
<td>( \pi_{n_{min}} \leq \pi_n \leq \pi_{n_{max}} )</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic structure of a technological flow with a semi-rigid connection.
Let us define \( M_p \) and \( Q_p \) versus adjustable operating parameters functional characteristics for each of the subsystems.

Let us set the range of the adjustable operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) for each of the subsystems of technology.

Let us find such values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) in the subsystem A for which \( M_p(A) \) is minimal and denote this value as \( M_p(A_1) \).

Let us find such values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) in the subsystem B for which \( M_p(B) \) is minimal and denote this value as \( M_p(B_1) \), let us also find the values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) in the subsystem B taking into account the value of the operating parameter \( M_p(A_1) \) for which \( M_p(B) \) is minimal under these conditions and denote this value as \( M_p(B_2) \).

Let us find such values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) in the subsystem C for which \( M_p(C) \) is minimal and denote this value as \( M_p(C_1) \), let us also find:
- \( M_p(C_2) \), where the values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) correspond to \( M_p(A_1) \);
- \( M_p(C_3) \), where the values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) correspond to \( M_p(B_1) \) that only participate in the subsystem B;
- \( M_p(C_4) \), where the values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) correspond to \( M_p(B_1) \) taking into account the subsystem A;
- \( M_p(C_5) \), where the values of the operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) correspond to \( M_p(A_1) \).

Similarly let us find the values of \( Q_p(C_1-5) \) for the subsystem.

For each of the ten sets let us find the total losses of the system, from which we will choose the lowest ones, it is they that characterize the rational values of the operating parameters of the system as a whole.

**Fig. 2.** Block diagram of the algorithm for minimizing losses when implementing the technology.

For interval undefined characteristics, which may also be the indicators of losses when processing food raw materials (by waste size), in the absence of any additional conditions, the undefined entropy is a rectangle \( (B, p) \), \( B \in [A_{\text{min}}; A_{\text{max}}], p \in [0; (\ln 2)^{-1}] \).

The presented system, consisting of three subsystems (Fig. 1), is not indicative and is only given to illustrate the proposed algorithm, because such an approach is inexpedient with three consecutive operations. In this case, it is possible to quickly determine the weak link by simply comparing the losses. However, in complex technologies, where the number of operations is significant and at the same time there is waste recycling by returning it to the flow, and also heat recovery, simple comparison is clearly not enough. In addition, the ranges of mass and energy losses can enter one into another or be significantly shifted from one another for different operations.

In particular, an increase in mass or energy losses in the previous operations may lead to their decrease in the subsequent operations. For example, the fine grinding of the raw material is more energy-intensive compared with coarse grinding, leads to a decrease in the duration of thermal and mass-exchange processing of products and, as a consequence, a decrease in energy losses. In this case, it is necessary to optimize the operation of a technological flow on the basis of a system analysis and the probabilistic entropy approach, which, in fact, is based on determining the number of degrees of freedom of each subsystem and the system as a whole.

Virtually any technological operation can be characterized as a set of parameters that are at certain levels of development of machine-hardware design of technology. To estimate the level of stability and integrity using a fuzzy entropy component, it is possible to take the material and energy losses of each
subsystem, given that the expert is able to distinguish them among a set of parameters that significantly affect a technological operation.

As for the material characteristics $A_i$ of food products, there are always preferences based on their minimal losses when implementing technological operations, which indicates a more complex (non-rectangular) form of the preference function for the fuzzy value $A_i$.

Let $f_i(x)$ be the membership function of the characteristic $A_i$. In view of the conceptual meaning of the preference function, if $f(B_i) > f(B_j)$, then the value $B_i$ is more preferable (more desirable) than the value $B_j$ by a factor of $\lambda = f(B_i)/(B_j)$, and then with sufficiently high values of $n$ and the equal lengths of the intervals $A_i$ and $A_j$, the following proportion is valid: $n_i / n_j = f(B_i)/(B_j)$ or $n_i / f(B_i) = n_j / f(B_j) = \mu$.

Since the last relation is valid for any pairs of the indices $i$ and $j$, we arrive at the conclusion that for all values of $i$ the equalities $n_i = \mu f(B_i)$ are valid, from which it is not difficult to deduce that $p_i = \lim_{n \to \infty} n_i / n = kf(B_i)$ is valid for all $i$. Consequently, fuzzy entropy is a curvilinear trapezoid with a base of the argument $x$ of which vary from $A_{\text{min}}$ to $A_{\text{max}}$ (along the abscissa axis), and for each fixed value of $x$ along the ordinate axis is equal to $kf(x)\log_2 kf(x)$. To complete the analysis, it is necessary to estimate the value of the coefficient $k$. If the preference function is of rectangular type, then, as follows from the result obtained above, the maximum value of entropy is reached if the following condition has been fulfilled:

$$kf(x) = kf_{\text{max}} = 1 / e,$$

from where $k = (f_{\text{max}} e)^{-1}$ follows, where $f_{\text{max}}$ is the maximum value of the preference function.

Preference functions of a trapezoidal type with an additional selected range of more preferable values are of greatest interest. In the case of a trapezoidal type function without a selected range of preferred values, the entropy value at the maximum point will be lower, since two buffer zones of a triangular shape with a base width $A_{\text{min}}$ from the left and $A_{\text{max}}$ from the right are added to the rectangular section. We assume that the decrease is characterized by the ratio of the corresponding horizontal sections of the domain of variation of the argument $x$ corresponding to the rectangular zone and the entire domain of definition of the preference function, that is:

$$k = (f_{\text{max}} e)^{-1} \frac{A_{\text{max}} - A_{\text{min}}}{(A_{\text{min}} + A_{\text{max}}) - (A_{\text{min}} - A_{\text{min}})} \frac{f_{\text{max}}}{f_{\text{max}} + \delta_{\text{left}} + \delta_{\text{right}}}.$$

In the presence of the selected ranges, another coefficient is added for similar reasons:

However, in some cases, not only the interval of changes in its possible values, but also the most desirable value of the indicator or its interval can be indicated for the loss indicator. In this case, instead of the trapezoidal membership function, it is proposed to use its modification, taking into account the specified additional condition (Fig. 3).

![Fig. 3. Membership function with the selected range of preferences](image)

<table>
<thead>
<tr>
<th>Measure of availability of a component (x)</th>
<th>Preference level (g(x))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

For a triangular shape $B_i$ (Fig. 3), $f_{\text{max}} = 8$, $A_{\text{min}} = 1$, $A_{\text{max}} = 6.5$, $A_{\text{max}} = 8$, $A_{\text{min}} = 1$, $\alpha = 1$, $\gamma_i = 1$, $\delta = 0.6$, $\delta = 0.4$, $\alpha_{\text{min}} = 6$, $A_{\text{max}} = 6.5$. 

......
This modification of the membership function, shown in Figure 3, is defined as the ratio:

\[
f_i(x) = \begin{cases} 
0, & \text{if } x < A_{\text{min},i} \text{ or } x > A_{\text{max},i}, \\
(R_i - \delta_i)(\alpha_i)^{-1}(x - A_{\text{min},i}), & \text{if } x \in [A_{\text{min},i}; A_{\text{min},i} + \alpha_i], \\
(R_i - \delta_i + \delta_i \frac{x - A_{\text{min},i} - \alpha_i}{A_{\text{min},i} - A_{\text{min},i} - \alpha_i}, & \text{if } x \in [A_{\text{min},i} + \alpha_i; A_{\text{min},i}], \\
R_i, & \text{if } x \in [A_{\text{min},i}; A_{\text{max},i}], \\
R_i - \hat{\delta}_i \frac{x - A_{\text{max},i} - \gamma_i}{A_{\text{max},i} - \gamma_i}, & \text{if } x \in [A_{\text{max},i}; A_{\text{max},i} - \gamma_i], \\
R_i - \hat{\delta}_i - (R_i - \hat{\delta}_i)(\gamma_i)^{-1}(x - A_{\text{max},i} + \gamma_i), & \text{if } x \in [A_{\text{max},i} - \gamma_i; A_{\text{max},i}]. 
\end{cases}
\]

Fig. 4. Schematic structure of a technological flow with a semi-rigid connection.

Table 4. \( M_p \) and \( Q_p \) versus operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) functional characteristics and their adjustable range in subsystems A and B

<table>
<thead>
<tr>
<th>Subsystem</th>
<th>Function</th>
<th>( M_p ) and ( Q_p ) versus operating parameters ( \pi_1, \pi_2, \ldots, \pi_n ) functional characteristics</th>
<th>Adjustable range of operating parameters ( \pi_1, \pi_2, \ldots, \pi_n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>( M_p = (0.0053\pi_2 - 1.1)\pi_1 + (-0.05\pi_2 + 14)) ( Q_p = (0.0075\pi_2 + 1.2)\pi_1 + (-0.004\pi_2 - 0.04) )</td>
<td>( 3 \leq \pi_1 \leq 8 ) ( 40 \leq \pi_2 \leq 80 )</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>( M_p = -0.015\pi_1\pi_2\pi_3 + 0.1075\pi_2\pi_3 + 3.1\pi_1\pi_3 - 24.3\pi_3 - 0.035\pi_2\pi_2 + 0.23\pi_2 + 2\pi_1 + 9 ) ( Q_p = -0.0105\pi_1\pi_2\pi_3 + 0.024\pi_2\pi_3 + 0.34\pi_1\pi_3 + 8.88\pi_3 + 0.02\pi_2\pi_2 - 0.11\pi_2 + 0.4\pi_1 - 0.2 )</td>
<td>( 0.8 \leq \pi_3 \leq 1.2 )</td>
</tr>
</tbody>
</table>

Thus, for the calculated and empirical estimates of energy costs, material losses, ranges of variation of line parameters, technological limits and quality indicators of the finished and semi-finished products, as well as to eliminate the subjectivity of estimating the existing technological flows and predicting the stability of subsystems and the system as a whole at the design stage, it is reasonable to use the conditional entropy of a subsystem (the level of losses when implementing a technological operation) specified by fuzzy entropy. In this case, the process of a change in the initial entropy of a technical system can be described as the process of accumulation of the structural information calculated as the difference between the maximum and the real values of entropy, i.e. the growth of stability.

As an example, let us describe a technological system that consists of two subsystems A and B with a semi-rigid connection (Fig. 4).

In this case, we are interested in material and energy losses when the product goes through subsystems A and B. Let there be \( M_p \) and \( Q_p \) versus operating parameters \( \pi_1, \pi_2, \ldots, \pi_n \) functional characteristics and their adjustable range the data of which are presented in Table 4.

Let us find such values of the operating parameters \( \pi_1, \pi_2 \) in subsystem A for which the values \( M_p(A) \) and \( Q_p(A) \) are minimal and denote these values as \( M_p(A1) \) and \( Q_p(A1) \).

\[
M_p(A1) = 4.4 \text{ for } (\pi_1 = 8; \pi_2 = 80), \quad Q_p(A1) = 4.3 \text{ for } (\pi_1 = 3; \pi_2 = 40).
\]

Then let us find the values of the operating parameters and \( \pi_3 \) in subsystem B for which the values \( M_p(B) \) and \( Q_p(B) \) are minimal and denote these values as \( M_p(B1) \) and \( Q_p(B1) \).

\[
M_p(B1) = 5.01 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 1.2), \quad M_p(B2) = 20.4 \text{ for } (\pi_1 = 8; \pi_2 = 80; \pi_3 = 1.2),
\]

\[
Q_p(B1) = 14.1 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 1.2), \quad Q_p(B2) = 40.0 \text{ for } (\pi_1 = 8; \pi_2 = 80; \pi_3 = 1.2).
\]
\[ Q_p(B1) = 4.44 \text{ for } (\pi_1 = 3; \pi_2 = 80; \pi_3 = 0.8), \]
\[ Q_p(B2) = 6.68 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 0.8). \]

Given that total of material losses is:

\[ M_{P,\text{common}} = 1 - \prod_{i=1}^{n} (1 - M_i), \]

where \( n \) is the number of subsystems, \( M_i \) are losses for the \( i \)-th subsystem, referred to the mass of the initial material of the \( i \)-th operation, and also that the total energy losses are:

\[ Q_{P,\text{common}} = 1 - \prod_{i=1}^{n} (1 - Q_i), \]

where \( n \) is the number of subsystems, \( Q_i \) are the losses for the \( i \)-th subsystem, referred to the total losses, we obtain the values of total losses:

\[ M_{P,\text{common}} = 13.8 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 1.2), \]
\[ M_{P,\text{common}} = 23.9 \text{ for } (\pi_1 = 8; \pi_2 = 80; \pi_3 = 1.2), \]
\[ M_{P,\text{common}} = 23.6 \text{ for } (\pi_1 = 3; \pi_2 = 80; \pi_3 = 0.8), \]
\[ M_{P,\text{common}} = 18.4 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 0.8), \]
\[ Q_{P,\text{common}} = 14.4 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 1.2), \]
\[ Q_{P,\text{common}} = 27.1 \text{ for } (\pi_1 = 8; \pi_2 = 80; \pi_3 = 1.2), \]
\[ Q_{P,\text{common}} = 9.3 \text{ for } (\pi_1 = 3; \pi_2 = 80; \pi_3 = 0.8), \]
\[ Q_{P,\text{common}} = 10.7 \text{ for } (\pi_1 = 3; \pi_2 = 40; \pi_3 = 0.8). \]

It is important to determine which of the subsystems reduces the stability of the technological system. It becomes clear from the obtained values that subsystem \( A \) is stable, because with \( \pi_3 = 3; \pi_2 = 40 \) the losses are minimal, and subsystem \( B \) is unstable in view of the high spread of the parameter \( \pi_3 \). The determination of the stable variation range of the parameter \( \pi_3 \) of the problem subsystem depends on the fuzzy entropy component the calculation method of which is presented above.

The fuzzy entropy of the system is expressed through material and energy fuzzy entropies for each of the operations. Figures 5 and 6 present the obtained graphs of the entropy component.

It is known that entropy changes from zero to a certain maximum value, moreover, the value "0" will be when the a priori probability \( P = 0 \) and \( P = 1 \), i.e. when there is practically no distribution and there is no uncertainty in the system. Let us assume such values \( Q_{P,\text{common}} \) and \( M_{P,\text{common}} \) that the values of their entropies asymptotically approach zero, for example: \( Q_{P,\text{common}} = 11.73 \) and \( M_{P,\text{common}} = 17.89 \).

**CONCLUSIONS**

By predicting the stability of technical and technological subsystems and systems as a whole by making out material and energy balances, minimizing energy and material losses is possible, as well as determining the ranges of variation in the parameters of line operation, technological limits and quality indicators of the finished and semi-finished products. At the same time, the systemic complex analysis of technological flows allows us to study the processes of an entropy change in a technical system (the growth of system stability) by describing the processes of accumulation of structural information, calculated as the difference between the maximum and the real value of fuzzy entropy.

Thus, the use of the conditional fuzzy entropy of the system to identify the ranges of variation of adjustable operating parameters for the stable operation of lines and the elimination of subjectivity of estimates for the operating technological flows and the prediction of stability of operation of subsystems and the system as a whole at the design stage is original and competitive.
REFERENCES


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STUDY OF THE PROCESS OF CONCENTRATION AS A FACTOR OF PRODUCT QUALITY FORMATION

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Abstract: As a result of the analysis of the technological system for the production of dry granulated food concentrates, it has been revealed that the key contradictions in the formation of product quality arise in the subsystem of concentration of an extract and whey mixture. In the present paper on the improvement of the technology of instant granular products, including those on the basis of a chokeberry extract and milk whey, the process of concentration of their mixture has been studied, and the quality of the instant granular breakfast prepared using a concentrate has been estimated. To solve technical contradictions, studies were carried out using an example of the chokeberry extract, obtained both from the dried and frozen raw materials subjected to grinding in a mixture with curd whey. The combined concentration of whey and the extract made it possible to avoid the foaming characteristic of whey concentration, and also to exclude the process of mixing high viscosity masses from the technological flow, and the replacement of the processes of “drying and grinding” with “freezing and grinding” made it possible, first, to intensify the process and, second, to ensure a more complete extraction of BAS. A mathematical model of the process has been created, the regimes and parameters of concentration have been developed in a rotary-film evaporator operating under vacuum; the regulated indicators of nutritional value, the terms and modes of storage have been determined.

Keywords: Concentrating, extract, chokeberry, whey, concentration process, process model, quality indicators


INTRODUCTION

The indicators of the quality of a technological flow, such as reliability, controllability, efficiency, product quality, etc., are interrelated and interdependent and condition each other. The improvement of some indicators often causes the deterioration of others. The analysis of the bottlenecks in the technological system aims at finding the key contradictions. To find the key contradiction and reveal its essence is half the success when creating a new technology [4, 5]. It should be borne in mind that the knowledge of regularities, limited by a process in a machine or apparatus, without taking into account the regularities characteristic of the system as a whole, will not lead to an optimal solution of a problem. To resolve technical contradictions effectively, a theoretical and experimental study of all processes and their subsequent optimization are required to reduce the sensitivity of a particular process in the system to the fluctuations in the input parameters [4].

STUDY OBJECTS AND METHODS

In the present paper on the improvement of the technology of instant granular products [6, 7, 8], including those on the basis of a chokeberry [10, 11, 19, 21, 22] and whey [21, 23] extract, the process of concentration of their mixture has been studied and the quality of the instant granular breakfast prepared using a concentrate has been estimated. The combined concentration of whey and the extract made it possible to avoid the foaming characteristic of whey concentration, and also to exclude the process of mixing high viscosity masses from the technological flow, and the replacement of the processes of “drying and grinding” with “freezing and grinding” made it possible, first, to intensify the process and, second, to ensure a more complete extraction of BAS.

The process of concentrating a chokeberry extract together with curd whey (with a ratio of 3 : 1) to 55–60% of solids is carried out using a vacuum rotary-film evaporator at a temperature of 48–50°C, which allows us to preserve thermolabile substances, due to this, the derived semifinished product in the form of a concentrate has both chemical and microbiological stability.

Of particular importance, from the point of view of forming the quality of the finished products, is the process of concentrating an extract and whey mixture [13, 14, 15]. The mathematical model of an evaporation process in a rotary-film evaporator has...
been constructed and the modes of concentrating extracts have been studied.

To study the intensification of the process of concentrating the mixture of chokeberry and curd whey extracts, a vacuum rotor-film evaporator was designed and manufactured.

A feature that distinguishes this device from similar devices is the evolved evaporation surface regulated by a set of disk elements installed on the rotor shaft, which are film formers [16, 19] and an adjustable shaft speed.

The initial solution in the evaporator is a mixture of whey and extract, is fed through the inlet to the maximum filling of the apparatus, limited by the contact of the liquid with the lower edge of the disc shaft. This requirement is caused by the fact that the efficiency of the apparatus depends on the area of the evaporation surface, and the wetting of the discs, the area of the film and the uniform distribution depend on the area of contact between the disc and the liquid when the rotor moves. The heat transfer processes in vacuum provide the evaporation and concentration of a solution.

In the heat-exchange rotor-film apparatus of the developed design, there is no contact between the heat transfer medium and the process medium during heat exchange, the heat is transferred through a transfer wall.

The operation of a rotary-film evaporator depends on a variety of factors [12, 13, 14]: the conditions of the process; the geometric dimensions of plates; the thickness of the film layer of the solution; the rotor design; the properties of the process medium; the rotor speed. When designing any technical system, ours is no exception; the main task is to provide the maximum intensity of the process with the least (optimal) energy costs.

In our case, this is the calculation of the main characteristics of a rotary-film evaporator when concentrating a mixture of curd whey with a chokeberry extract. In this regard, it seems reasonable to consider the heat-mass transfer and hydrodynamic processes that occur inside a vacuum rotor-film evaporator.

**RESULTS AND DISCUSSION**

The liquid film formed by self-wetting on the surface of the plate will perform a rotational motion therewith. At the same time, internal and external forces act on each film particle, the main ones of which are: gravity; the centrifugal force; adhesion to the surface of the disc and the force of the surface tension of the liquid.

The centrifugal force depends on a rotor speed, disc radius and film weight:

\[ F = (2 \pi n)^2 \cdot R \cdot M, \]  

where \( M \) is the reduced mass of the film, kg; \( n \) is a rotor speed, \( s^{-1} \); \( R \) is the equivalent radius of the disk, m.

The given mass of the film is determined as the product of the thickness by the area occupied thereby on the surface of the disk by the density:

\[ M = \rho \cdot S \cdot b, \]  

where \( \rho \) is the density of the liquid, kg/m³; \( S \) is the area of the film on the disk, m²; \( b \) is the thickness of the liquid film, m.

The calculations show that the centrifugal force is low, with the surface area of the disc in our apparatus \( S = 10^{-2} \) m², the film thickness, according to an approximate calculation, \( b = 10^{-5} \) m, and the liquid density within \( \rho = (0.9–1.0)10^3 \) kg/m³, (the mass in this case is approximately equal to \( 10^{-4} \) kg), it can also be neglected.

The gravity of the film spread on the surface of the disk can also be neglected, due to its low thickness. Moreover, this thickness changes increasingly when the solution is concentrated and corresponding to an increase in viscosity. The liquid film is retained on the surface of the disk by the adhesion forces and does not have its own motion, and the possible displacement of part of it relative to the disk surface during one rotation under the action of the above forces will be insignificant and depends on the emerging internal shear forces that obey Newton's law. In this case, the force of the surface tension of the liquid caused by the intermolecular interaction inside the liquid is proportional to the dynamic viscosity of the solution and the shear rate gradient over the layer thickness, and the force of adhesion with the surface of the disk exerts resistance to the rupture of the film and its shear.

The heat-mass transfer, under such conditions when the hydrodynamic component is insignificant and close to zero in calculations, is simplified, since most of the similarity criteria describing this process are based on a velocity gradient.

Thus, based on the above calculated parameters characterizing the process, we can introduce a number of assumptions:

– the liquid film formed by adhesion on the surface of the rotor disk has a constant thickness and the relative shear of its layers is close to zero;

– the process temperature does not change with time and the heat exchange regime is steady inside the device;

– the heat flow is constant in the apparatus chamber;

– the losses of heat for concentration and convection during thermal calculations is included in the total value of the heat flow.

As can be seen from the graph of dependence of the evaporation area on the height of the liquid level presented in Fig. 1, with a decrease in the liquid level in the apparatus, it increases nonlinearly, as it was expected.

![Fig. 1. Dependence of the area of evaporation surface on the height of the liquid in the evaporator](image-url)
Assuming that the rotor speed is \( n \) (s\(^{-1}\)), we can calculate the initial mass flow rate using the following formula:

\[
G_H = n \cdot S \cdot b \cdot \rho, \quad (3)
\]

where \( S \) is the area of film formation (evaporation surface), m\(^2\); \( b \) is the thickness of the liquid film, m; \( \rho \) is the density of the processed liquid, kg/m\(^3\);

The efficiency of the evaporator for the secondary steam can be determined in this case using the formula:

\[
W = n \cdot S \cdot b \cdot (1 - \frac{X_H}{X_k}). \quad (4)
\]

The value of \( W \) for a device with a constant liquid level, and hence with the same evaporation area, when processing the same medium, will depend on the rotor speed, which theoretically can vary from 0 to the speed of the drive's engine (\( n_d \)).

The efficiency of the vacuum rotor-film evaporator for the secondary steam, depending on equation (4), depends on the final concentration of the evaporated liquid, which cannot exceed 100\% and must be known in advance, in accordance with the process conditions. In this case, the amount of heat supplied to the evaporated liquid is used to heat it from the initial temperature to the boiling point in a given vacuum and, in fact, for evaporation. In accordance with these assumptions, the heat flow in the apparatus can be determined using Fourier's law.

Thus, the most important parameter (along with others) was found – the rotor speed, which can vary within a certain range. Substituting \( G_H \) from formula (3) and \( W \) from equation (4) into the heat balance equation and expressing \( n \) from it, we obtain the following relationship:

\[
n = \frac{Q}{\rho \cdot S \cdot b \cdot (c \cdot (T_e - T_i) + (1 - X_H / X_k))}. \quad (5)
\]

The analysis of the obtained formulas shows:

1. The efficiency of the evaporator in accordance with equations (3) and (4) can continuously increase depending on an increase in the rotor speed and a decrease in the liquid level, hence, the area of the evaporation surface increases.
2. The amount of heat, and consequently the amount of energy consumed in accordance with the heat balance equation, increases.

If Item 1 fully corresponds to the solution of the problem of intensifying the concentration process in a vacuum rotary-film evaporator, the consideration of Item 2 showed that a continuous increase in the efficiency of the device due to an increase in the rotor speed while maintaining the overall dimensions leads to the fact that the amount of the heat required cannot be provided in full. Hence the need for the optimal use of thermal energy (heat flow), which provides a technological process. Thus, it was necessary to introduce a fourth assumption on the constancy of the heat flow in the evaporator. Therefore, in this case \( Q \) will not depend on an increase in the rotor speed \( n \).

In the proposed device of the rotary-film evaporator, the device is heated by the thermal envelope, and consequently, a wall of the body of a given thickness, the area of which can be determined on the basis of the geometric dimensions of the evaporator under study, will be used as the heat transfer surface:

\[
F = \pi \cdot L \cdot D_{an}, \quad (6)
\]

where \( L \) is the length of the device, m; \( D_{an} \) is the internal diameter of the body, m.

The main characteristics of the proposed apparatus can be calculated theoretically on the basis of the accepted mathematical relationships which are known or obtained on the basis of the known ones [16, 19].

Based on the above equations, the block diagram of the algorithm for calculating the main design parameters was developed (Fig. 2).

Fig. 2. Calculation algorithm.
Fig. 3. Change in efficiency for the secondary steam depending on the liquid level of the rotary-film evaporator (● – experiment; □ – calculation).

Table 1 presents the initial and calculated parameters.

The mathematical model of the developed vacuum rotor-film evaporator was constructed on the basis of which a method for calculating its basic characteristics was developed, taking into account the operating features of the apparatus, the conditions of the technological mode for processing solutions due to their specific properties.

Table 1. Initial and calculated parameters and their designation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Designation in a formula</th>
<th>Designation in a program</th>
<th>Dimension designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial temperature of the solution</td>
<td>$T_H$</td>
<td>T1</td>
<td>°C</td>
</tr>
<tr>
<td>Boiling point of the solution</td>
<td>$T_K$</td>
<td>T2</td>
<td>°C</td>
</tr>
<tr>
<td>Temperature of the heat-transfer medium</td>
<td>$T_m$</td>
<td>T3</td>
<td>°C</td>
</tr>
<tr>
<td>Initial concentration</td>
<td>$X_H$</td>
<td>X1</td>
<td>%</td>
</tr>
<tr>
<td>Final concentration</td>
<td>$X_K$</td>
<td>X2</td>
<td>%</td>
</tr>
<tr>
<td>Average specific thermal capacity of the solution</td>
<td>$C$</td>
<td>C1</td>
<td>J/kg·K</td>
</tr>
<tr>
<td>Specific heat of evaporation</td>
<td>$r$</td>
<td>R</td>
<td>J/kg</td>
</tr>
<tr>
<td>Thermal conductivity of the liquid</td>
<td>$\lambda$</td>
<td>LM2</td>
<td>W/m·K</td>
</tr>
<tr>
<td>Dynamic viscosity coefficient</td>
<td>$\mu$</td>
<td>M</td>
<td>Pa·s</td>
</tr>
<tr>
<td>Solution density</td>
<td>$\rho$</td>
<td>R0</td>
<td>kg/m³</td>
</tr>
<tr>
<td>Diameter of the disks</td>
<td>D</td>
<td>D1</td>
<td>m</td>
</tr>
<tr>
<td>Diameter of connecting elements</td>
<td>d</td>
<td>D2</td>
<td>m</td>
</tr>
<tr>
<td>Thickness of the disks</td>
<td>$\delta$</td>
<td>L1</td>
<td>m</td>
</tr>
<tr>
<td>Length of connecting elements</td>
<td>l</td>
<td>L2</td>
<td>m</td>
</tr>
<tr>
<td>Number of discs</td>
<td>$N$</td>
<td>N</td>
<td>pcs</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>$b_{st}$</td>
<td>B1</td>
<td>m</td>
</tr>
<tr>
<td>Thermal conductivity of the material</td>
<td>$\lambda_{cm}$</td>
<td>LM2</td>
<td>W/(m·K)</td>
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<td>Gravity acceleration</td>
<td>g</td>
<td>U</td>
<td>m/s²</td>
</tr>
<tr>
<td>Pi character</td>
<td>$\pi$</td>
<td>P1</td>
<td>–</td>
</tr>
<tr>
<td>Design parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of the liquid level</td>
<td>$H$</td>
<td>H</td>
<td>m</td>
</tr>
<tr>
<td>Area of the rotor evaporation surface</td>
<td>$S$</td>
<td>S</td>
<td>m²</td>
</tr>
<tr>
<td>Efficiency for the secondary steam</td>
<td>$W$</td>
<td>W</td>
<td>kg/s</td>
</tr>
<tr>
<td>Rotor speed $N1$</td>
<td>$n$</td>
<td>N1</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Thickness of the liquid film</td>
<td>$C$</td>
<td>B2</td>
<td>m</td>
</tr>
<tr>
<td>Heat flow (the amount of heat)</td>
<td>Q</td>
<td>Q</td>
<td>J/s</td>
</tr>
</tbody>
</table>
As can be seen from Fig. 4, with an increase in the rotor speed, the final concentration of the processed product (other things being equal to the process) drops sharply.

The solution concentration cannot be higher than 100% and lower than the initial value of $X_H$ [14, 19]. Substituting these values as boundary conditions in equation (5), we obtain the maximum and minimum rotational speed:

when $X_K = X_H$

$$n = \frac{Q}{(\rho \cdot b \cdot S \cdot c \cdot (T_K - T_H))},$$

(7)

when $X_K = 100\%$

$$n = \frac{Q}{\rho \cdot b \cdot S \cdot [(1 - 0,01 \cdot X_K) \cdot (1 - C_p \cdot T_K) + C \cdot (T_K - T_H)]}$$

(8)

Thus, the operating range of the rotor speed is determined.

The points obtained from formulas (7) and (8) are indicated on the X axis as the minimum and maximum values of the rotational speed $n$. In this case, the X axis was divided into several intervals.

Fig. 5 shows a change in the dominant characteristics of the vacuum rotor-film evaporator depending on the rotor speed.

Considering the "behavior" of the curves in each of the characteristic segments, it can be noted that

1. In the interval of a change in the rotation speed from 0 to $n_{\text{min}}$, the efficiency of the evaporator increases by the amount of evaporated liquid; and the final concentration of the solution reaches 100%. The amount of heat supplied will not be completely absorbed by an evaporation process, which will result in the additional heating of the final product, which is unacceptable for the processing of the liquids containing biologically active or thermolabile substances. The operation of the device in this range will result in the unreasonable consumption of energy and loss of quality of the final product. In this case, the speed value equal to $n_{\text{min}}$, which can be calculated using formula (8), will be optimal for obtaining a product with the maximum concentration under the given process conditions.

2. In the interval from $n_{\text{min}}$ to $n_{\text{max}}$, the efficiency for the secondary steam will increase for some time, and then, approaching the speed $n_{\text{max}}$, smoothly decrease, tending to a certain minimum value. In this interval, a point is fixed that corresponds to such a value of the rotor speed at which the efficiency of the evaporator for the secondary steam corresponds to the maximum value. The concentration of the resulting solution will not reach the maximum value.

3. With a further increase in the number of rotations of more than $n_{\text{max}}$, the efficiency for the secondary steam remains practically unchanged. Since the amount of heat is constant, this amount will not be enough to intensify the process of evaporation. In this case, the heat is only consumed to heat the liquid supplied to the evaporator. Then, in this interval, the device will operate as a heat exchanger and it will be inappropriate to use it to concentrate liquids.

Thus, one of the main optimization parameters for the design and operation of devices based on a vacuum rotary-film evaporator was set: is the rotational speed of the rotor $n$, which can be calculated from equation (5).

By transforming the obtained data, it is possible to calculate an optimal method for obtaining a mixture of curd whey extracts and a chokeberry extract using a special program for the given geometric dimensions and process conditions, determining in advance the efficiency of the rotary evaporator. Table 2 shows the initial data for this calculation and the optimal operating parameters of the rotary-film evaporator.

The discrepancy between the experimental data and the calculated ones for the reference liquid (water) did not exceed 10% with allowable deviations for mathematical models up to 30%. Hence it can be concluded that the calculated values of the main parameters of the rotary evaporator for extracts will correspond to the real ones with an accuracy of 10%.
Table 2. Summary table of parameters

<table>
<thead>
<tr>
<th>Name of the parameter</th>
<th>Designation in a formula</th>
<th>Designation in a program</th>
<th>Numeric value</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial temperature of the liquid</td>
<td>$T_{th}$</td>
<td>T1</td>
<td>20</td>
<td>°C</td>
</tr>
<tr>
<td>Boiling point of the liquid</td>
<td>$T_{b}$</td>
<td>T2</td>
<td>45</td>
<td>°C</td>
</tr>
<tr>
<td>Temperature of the heat-transfer medium</td>
<td>$T_m$</td>
<td>T3</td>
<td>80</td>
<td>°C</td>
</tr>
<tr>
<td>Initial concentration</td>
<td>$X_{th}$</td>
<td>X1</td>
<td>6</td>
<td>%</td>
</tr>
<tr>
<td>Final concentration</td>
<td>$X_f$</td>
<td>X2</td>
<td>78</td>
<td>%</td>
</tr>
<tr>
<td>Average specific thermal capacity of the liquid</td>
<td>$C$</td>
<td>C1</td>
<td>3425.1</td>
<td>J/kg·K</td>
</tr>
<tr>
<td>Specific heat of evaporation</td>
<td>$r$</td>
<td>R</td>
<td>$2.4 \times 10^6$</td>
<td>J/kg</td>
</tr>
<tr>
<td>Thermal conductivity of the liquid</td>
<td>$\lambda$</td>
<td>LM2</td>
<td>0.48</td>
<td>W/(m·K)</td>
</tr>
<tr>
<td>Dynamic viscosity coefficient</td>
<td>$\mu$</td>
<td>M</td>
<td>$0.6 \times 10^3$</td>
<td>Pa·s</td>
</tr>
<tr>
<td>Solution density</td>
<td>$\rho$</td>
<td>R0</td>
<td>1003.1</td>
<td>kg/m³</td>
</tr>
<tr>
<td>Diameter of the disks</td>
<td>$D$</td>
<td>D1</td>
<td>0.25</td>
<td>m</td>
</tr>
<tr>
<td>Diameter of connecting elements</td>
<td>d</td>
<td>D2</td>
<td>0.023</td>
<td>m</td>
</tr>
<tr>
<td>Thickness of the disks</td>
<td>$\delta$</td>
<td>L1</td>
<td>0.0008</td>
<td>m</td>
</tr>
<tr>
<td>Length of connecting elements</td>
<td>l</td>
<td>L2</td>
<td>0.01</td>
<td>m</td>
</tr>
<tr>
<td>Number of discs</td>
<td>$N$</td>
<td>N</td>
<td>58</td>
<td>pcs</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>$b_{st}$</td>
<td>B1</td>
<td>0.001</td>
<td>m</td>
</tr>
<tr>
<td>Thermal conductivity of the material</td>
<td>$\lambda_{cn}$</td>
<td>LM2</td>
<td>17.5</td>
<td>W/(m·K)</td>
</tr>
<tr>
<td>Gravity acceleration</td>
<td>g</td>
<td>U</td>
<td>9.8</td>
<td>m/s²</td>
</tr>
<tr>
<td>Pi character</td>
<td>$\pi$</td>
<td>P1</td>
<td>3.14</td>
<td>–</td>
</tr>
<tr>
<td>Optimal parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height of the liquid level</td>
<td>$H$</td>
<td>H</td>
<td>0.106</td>
<td>m</td>
</tr>
<tr>
<td>Area of the rotor evaporation surface</td>
<td>$S$</td>
<td>S</td>
<td>3.9</td>
<td>m²</td>
</tr>
<tr>
<td>Efficiency for the secondary steam</td>
<td>$W$</td>
<td>W</td>
<td>$0.93 \times 10^4$</td>
<td>kg/s</td>
</tr>
<tr>
<td>Rotor speed N1</td>
<td>$n$</td>
<td>N1</td>
<td>1.02</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>Thickness of the liquid film</td>
<td>$C$</td>
<td>B2</td>
<td>0.00168</td>
<td>m</td>
</tr>
<tr>
<td>Amount of heat (a heat flow)</td>
<td>Q</td>
<td>Q</td>
<td>87.237</td>
<td>J/s</td>
</tr>
</tbody>
</table>

At the same time, attention is drawn to the fact that, under equal experimental conditions, the fixed parameters of the process using an experimental sample of the apparatus are as follows: with an increase in the evaporation area by a factor of 10, the efficiency of the evaporator for the secondary steam increased by 6 times with the same thermal load of the device.

Such a significant increase in the coefficient of utilization of the thermal energy supplied to the evaporator is a sign of its high efficiency; while the energy costs in such devices are insignificant in comparison with the achieved effect of process intensification.

Taking into account the changes in the subsystem in obtaining a concentrated mixture of extract and whey [23–26], a new technology of instant granular breakfast was proposed.

Fig. 6 presents the technological scheme of production of the mixtures being developed.

After inspection, the fruit and berry raw materials are dried at a temperature of 50–55°C, which allows us to preserve thermolabile substances or frozen at a temperature of -22 degrees Celsius.

Then the fruits are ground to a size of 0.5–1.5 mm [25], which allows us to achieve the necessary phase contact of the surfaces between the extractant and the plant mass, which allows us to significantly reduce the hydrodynamic resistance, especially when using aqueous solvents, when the swelling of plant raw materials is possible.

The ground fruit and berry raw materials are extracted. At the same time, the raw materials / extractant ratio for the dried raw materials is 1/6–1/7; for the frozen raw materials – 1/8–1/10 at an extraction temperature of 40–50°C.

Water, ethanol or their solutions are used in different concentrations as solvents. Varying the extractants and the characteristics and conditions of the process allows us to change the spectrum of the extracted substances, as well as to fractionate them. Applying them in combinatorics or sequentially, it is possible to achieve the complete extraction of extractive substances from the raw materials. In this case, the extracts obtained have not only a different biological activity, but also a completely different type of action. The raw materials that have the same groups of high-molecular substances and the same structural and mechanical properties in their composition, can be extracted together [17, 19, 22].

After extraction, the resulting mixture is filtered and the extract is separated from the meal. The latter is dried to a humidity of 8–10% and ground to a particle size of 100–200 microns.
At the next step, the powdered sugar is premixed in a 3:1 ratio with dry cheese whey and is supplied into a mixer where the resulting mixture and the remaining dry and liquid ingredients that form part of the breakfast formulation are mixed. This order of feeding ingredients into the mixer allows them to be spread uniformly and, as a result, to obtain a more homogeneous breakfast mixture.

The prepared polydisperse multicomponent mixture moistened to 8–10% is fed into a pelletizer with a dispersant. The dispersant provides the homogeneity of granules, and also increases efficiency due to more intensive nucleation. In the process of granulation, the amount of the liquid phase is corrected to the optimum by spraying the concentrated mixture of the liquid constituent of the formulation through a nozzle.

The resulting breakfast granules are subjected to convective drying at a temperature of 50–55°C to a humidity of 8–9%.

The dried granulate is fed to a vibrating screen, where it is sorted by size: 1–3 mm ones are fed into the hopper; the ones that have a size of less than 1 mm and more than 3 mm are fed for further processing or grinding.

The finished product – "dry instant granular whey-based breakfast" is sent for packing and packaging.

From a physico-chemical point of view, the technology for the production of instant granular products suggests the creation of systems with a certain phase composition and structure (the presence of capillaries throughout the cross section of granules). Therefore, the granulation process requires the presence of an automatic process control system, and therefore, a permanent control over the concentrations of liquid, solid and gaseous phases at all stages of the formation of a porous system (mixing, coagulation, condensation, crystallization).

The analysis of the performed diagnostics of granulation processes made it possible to establish that the law of constancy of the volume phase composition of disperse systems gives the opportunity to conduct both the theoretical and practical analysis of various technologies, technological stages and operations with the aim of obtaining materials with specified...
properties. In addition, it makes it possible to improve
the effectiveness of technological control in the
production of materials on the basis of disperse
systems, to simplify the methods for their study, and
also allows the introduction of standardization
elements in scientific research.

The fractional composition of the product has been
studied. It has been revealed that most of the granules
of both samples are 1.5–2.0 mm in size, which agrees
well with the law of normal distribution, and confirms
our assumptions about the correct choice of the
technological parameters of the granulation process, as
well as the estimation of their effect on the quality of
granules. The shape of this curve characterizes the
monodispersity of the obtained granulate.

The capillary-porous structure of granular products
significantly affects the properties of the finished
product. To characterize the porosity, we used the
porosity index, which is determined by the ratio of the
volume of the water that penetrates into the granule of
the dry product and the volume of the granule:

\[
\Pi_{por} = \frac{V_2 - V_3}{V_1} \times 100\% \tag{9}
\]

where \(\Pi_{por}\) is a porosity index; \(V_1\) is the volume of a
granule, cm\(^3\); \(V_2 - V_3\) is the difference in water levels
before and after the complete disintegration of
granules, cm\(^3\).

Fig. 7 shows the phase diagrams that characterize
the changes in the ratio of the solid, gas and
liquid components in the technology of granulating
a chokeberry-based product with the addition
of whey.

Table 3 presents the formulation of the developed
product.

<table>
<thead>
<tr>
<th>Name</th>
<th>Content, kg/100 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatmeal flour</td>
<td>50.0</td>
</tr>
<tr>
<td>Chokeberry extract (the</td>
<td>10.0</td>
</tr>
<tr>
<td>substance content is 60%)</td>
<td></td>
</tr>
<tr>
<td>Powdered sugar</td>
<td>24.0</td>
</tr>
<tr>
<td>Dry cheese whey</td>
<td>8.0</td>
</tr>
<tr>
<td>Curd whey (the substance</td>
<td>3.1</td>
</tr>
<tr>
<td>content is 60%)</td>
<td></td>
</tr>
<tr>
<td>Chokeberry meal</td>
<td>4.7</td>
</tr>
<tr>
<td>Valetek-3 Art Life premix</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Based on the study of the dynamics of
organoleptic, microbiological and physico-chemical
properties in the process of production and storage, the
regulated indicators of the quality of the instant
breakfast based on the extract of chokeberry and curd
whey have been established. The obtained results have
become the basis for establishing a shelf life –
6 months from the date of manufacture.

The developed breakfast meets the requirements
for specialized products with directional functional
properties in composition, properties and a nutritional
value, and can be a reliable means of correcting the
deficiency of vitamins, minerals and the related
metabolic processes [20].

The ready-to-drink beverage is prepared by
dissolving 25 g of instant granular breakfast in 200 ml
of hot (85–95°C) water. This volume of the beverage
provides the necessary preventive amount of the adult's
need for essential nutrients, taking into account
their content in the traditional daily diet.

Technical documentation has been developed and
approved; the industrial production is carried out at
Scientific and Production Association “Healthy
Nutrition” [“Zdorovoe pitanie”] (Kemerovo, Russia).
### Table 4. Nutritional value of granular breakfast

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Content in 200 ml (25 g) of reconstituted breakfast, mg</th>
<th>Recommended level, day (MR 2.3.1.2432-08) in brackets – a service rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A, retinol equivalent</td>
<td>0.15 ± 0.006</td>
<td>0.9 (16.0)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.0014 ± 0.56·10⁻³</td>
<td>0.01 (14.0)</td>
</tr>
<tr>
<td>Vitamin E, tocopherol equivalent</td>
<td>1.25 ± 0.03</td>
<td>15.0 (8.3)</td>
</tr>
<tr>
<td>Thiamine (B₁)</td>
<td>0.24 ± 0.004</td>
<td>1.5 (16.0)</td>
</tr>
<tr>
<td>Riboflavin (B₂)</td>
<td>0.38 ± 0.11</td>
<td>1.8 (21.1)</td>
</tr>
<tr>
<td>Pyridoxine (B₆)</td>
<td>0.33 ± 0.10</td>
<td>2.0 (16.5)</td>
</tr>
<tr>
<td>Niacin, vitamin PP (B₃)</td>
<td>2.77 ± 0.08</td>
<td>20.0 (13.8)</td>
</tr>
<tr>
<td>Pantothenic acid (B₅)</td>
<td>1.1 ± 0.03</td>
<td>5.0 (22.0)</td>
</tr>
<tr>
<td>Cyanocobalamin (B₁₂)</td>
<td>0.0006 ± 0.18·10⁻⁴</td>
<td>0.003 (20.0)</td>
</tr>
<tr>
<td>Folic acid (B₉)</td>
<td>0.068 ± 0.002</td>
<td>0.4 (17.0)</td>
</tr>
<tr>
<td>Biotin (vitamin H)</td>
<td>0.029 ± 0.87·10⁻⁴</td>
<td>0.05 (58.0)</td>
</tr>
<tr>
<td>Ascorbic acid (C)</td>
<td>20.82 ± 0.62</td>
<td>90.0 (23.1)</td>
</tr>
<tr>
<td>Choline</td>
<td>15.8 ± 0.47</td>
<td>500 (3.2)</td>
</tr>
<tr>
<td>Bioflavonoids (vitamin P)</td>
<td>63.4 ± 1.9</td>
<td>250 (25.4)</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.98 ± 0.06</td>
<td>1300 (0.1)</td>
</tr>
<tr>
<td>Potassium</td>
<td>26.48 ± 0.8</td>
<td>2500 (1.1)</td>
</tr>
<tr>
<td>Calcium</td>
<td>11.22 ± 0.33</td>
<td>1000 (1.1)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>6.25 ± 0.18</td>
<td>400 (1.6)</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>43.02 ± 1.3</td>
<td>800 (5.4)</td>
</tr>
<tr>
<td>Iron:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>0.28 ± 0.01</td>
<td>10.0 (2.8)</td>
</tr>
<tr>
<td>female</td>
<td></td>
<td>18.0 (1.5)</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.10 ± 0.003</td>
<td>2.0 (5.0)</td>
</tr>
<tr>
<td>Copper</td>
<td>0.05 ± 0.002</td>
<td>1.0 (5.0)</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.14 ± 0.003</td>
<td>12.0 (1.2)</td>
</tr>
</tbody>
</table>

### REFERENCES

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elements of food machines on the basis of formation of models of technological blocks]. Kemerovo: Kuzbassvuzizdat Publ., 2010. 139 p.


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**INTRODUCTION**

In June 2016, the Government of the Russian Federation approved the Strategy for improving the quality of food products in the Russian Federation until 2030, one of the clauses of which states that "in order to ensure the quality of food products at all the stages of their life cycle, quality management systems should be introduced in food manufacture and processing organizations" [1]. Thus, the development and implementation of quality systems have become one of the top priorities of the heads of processing and food enterprises.

According to the international standard ISO 9001:2015 [2], the quality management system should be based on the application of the process approach and the Deming cycle: "Plan – Do – Check – Act" (PDCA), because it is this approach that allows an organization to plan its processes and their interaction.

At present, procedures for identifying and simulating the processes of various industrial enterprises have already been studied and developed [3, 4], which make it possible to make a complex analysis for all the stages of the product life cycle and form a visual structure of an organization, which is the first and most important stage in the construction of a quality management system. But a lot of developers face the problem of estimating the effectiveness of these processes since there is no single approach and methodological recommendations for accomplishing this task. Along with this, the organizations that already have certified QMS often face the problem of estimating the effectiveness of these processes, and an inherent condition for the correct functioning of a system. There is the same problem when integrating a quality management system into a security management system taking into account the requirements of 9000 and 22000 international standards, which is especially characteristic for food production [5].

The approaches to estimating the effectiveness of the quality management system of an enterprise that are available in the domestic [6, 7] and world practice [8–11] do not often take into account the specifics of food enterprises and do not affect all the stages of a product life cycle. At the same time, such factors as the
sanitary state of production, a wide range of products, multistage processing lines and short production terms significantly affect the structure and characteristics of a quality management system.

The study aimed at forming a mathematical model of the integrated estimation of the effectiveness of processes of the quality management system of a food enterprise.

**STUDY OBJECTS AND METHODS**

The object of the study was a procedure for estimating the effectiveness of processes of a food product life cycle using the example of the analysis and study of a low-capacity meat-processing plant in Moscow.

**Creating a treelike hierarchy.** When creating a treelike hierarchy we guided by a number of principles [12]. First, the overall indicator is considered as a certain hierarchical set of properties; secondly, different scales for measuring the single indicators of properties of an object should be unified in a scale with a uniform dimension, i.e. the transformation of scales was carried out; thirdly, any property at each of the levels should be characterized by two measurable parameters: a single property indicator and its weight coefficient, and, fourthly, the sum of weight coefficients of properties of one level of the hierarchy must be predetermined and constant:

\[ \sum_{j=1}^{n} M_{ij} = 1, \]

where \( n \) is the number of parameter properties at the \( i \)-th level (\( j = 1, 2, 3, 4... n \)).

**Fishburn’s weight coefficient system.** To determine the weight coefficients, Fishburn’s weight system was used, which only provides the knowledge of a degree of preference of some indicators to others. One indicator may express strong preference, a preference-indifference relation or indifference relative to another [13].

A set of scales decreasing by the arithmetic progression rule corresponds best to this system of decreasing alternative preferences:

\[ p_i = \frac{2(N-1)}{(N+1)N}, i = 1, ..., N, \]

where \( p_i \) is the weight coefficient of importance of the \( i \)-th factor; \( i \) is the number of the current factor; \( N \) is the total number of factors.

A set of equal weights best corresponds to the system of indifferent alternatives:

\[ p_i = \frac{1}{N}, i = 1, ..., N. \]

The choice of Fishburn’s weight coefficients is due to the fact that Fishburn’s weights are rational fractions, the numerator of which contains the units of a natural series decreasing by 1 from \( N \) to 1, for example, \( 4/9, 3/9, 2/9 \), forming one in sum, and the denominator contains the sum of the arithmetic progression of the first terms of a natural series at a pitch of 1. Thus, the preference is expressed in a decrease in the rational weight coefficient fraction numerator of the weakest of the alternatives by one.

Below are Fishburn’s weight fractions for all the mixed systems of preference relations for two, three and four single indicators (Table 1).

**Obtaining the rankings.** The series of preferences were created using the sequential comparison method. The preference of Object \( A \) before \( B \) is denoted as \( A>B \). The equality of objects from the point of view of the level of the quality estimated by the expert was reflected as “indifference” and it was designated as \( A\approx B \). A series is fully ordered in the case when there is no sign of “indifference” therein, and partially ordered if there is the given sign there.

The order of ranking was as follows: the experts compared two independent objects \( A \) and \( B \), while obtaining the result \( A<B \) or \( B>A \). Each successive object \( C \) was alternately compared to each of the elements of the already formed series, beginning with the first one. The process was repeated until a more preferable object was found to the left of the compared object, and a less preferable object – to the right. Then the compared object \( C \) is put in the ranking between the specified objects. After the comparison of all the objects \( A, B, C, D, E \) a series of preferences, say, \( A>B>C>D>E \), is obtained.

**Functional modeling methods.** The study used the methods of IDEF0 functional modeling [14]. The notation IDEF0 allowed to show processes as a composition of functional blocks that are graphically a set of rectangles and arrows (Fig. 1).

Table 1. Fishburn’s weight coefficient system

<table>
<thead>
<tr>
<th>No.</th>
<th>F</th>
<th>p₁</th>
<th>p₂</th>
<th>p₃</th>
<th>p₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>( R_i=R_j )</td>
<td>1/2</td>
<td>1/2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>( R_i\approx R_j )</td>
<td>2/3</td>
<td>1/3</td>
<td>1/3</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>( R_i=R_j \approx R_k )</td>
<td>1/3</td>
<td>2/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>( R_i\approx R_j\approx R_k )</td>
<td>2/5</td>
<td>2/5</td>
<td>1/5</td>
<td>2/5</td>
</tr>
<tr>
<td>4</td>
<td>( R_i=R_j \approx R_k \approx R_l )</td>
<td>3/6</td>
<td>2/6</td>
<td>1/6</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>( R_i\approx R_k\approx R_l \approx R_m )</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>( R_i\approx R_k\approx R_l \approx R_m )</td>
<td>4/10</td>
<td>3/10</td>
<td>2/10</td>
<td>1/10</td>
</tr>
</tbody>
</table>

Fig. 1. Main elements of process modeling methods.
Fig. 2. Level A0 diagram "Activity for the production of cooked sausages": BS – boiled sausages, NTD – normative and technical documentation, PMP – preventive maintenance plan.
The functional model is a set of blocks with "inputs" and "outputs", resources and control actions which are detailed to the required level. Decomposition allows us to study each process of the product life cycle without detaching from the higher processes, but with sufficient detail. A process or operation is represented as a quadrilateral, each interaction with other processes or the environment – in the form of an arrow. The arrows in the IDEF0 notation have several meanings: an administrative impact, resources or mechanisms, inputs and outputs are among them.

At the upper level, each process is represented as a "black box" that converts the inputs into outputs. This definition almost completely coincides with the definition of the process laid down in the standard ISO 9000 : 2015 [15], so that the IDEF0 notation is widely used in modeling production processes [16, 17].

RESULTS AND DISCUSSION

Processes identification. At the first stage of the study, the processes of a product life cycle were identified, aimed at defining and forming the organization structure, because the incorrect modeling and identification of processes lead to the creation of a heavy and unmanageable system. In addition, when developing QMS, it is important to determine the processes of exactly the level the management of which will be most rational and effective.

Process structures of three levels were modeled using the IDEF0 notation: A0 is the highest level without detail, A1 is the level with the details of processes of a product life cycle (Fig. 2), A2 is the level of details of each of the 7 processes of Diagram A1: marketing research, procurement and supply, production, engineering maintenance, packaging and storage, inspection, verification, testing and implementation. In addition, based on the identification principles [19], the processes not included in Diagram A1 were identified: Process A8 – launching products into manufacture and Process A9 – product conformity estimation.

Formation of a mathematical model. To estimate the effectiveness of QMS processes, the qualitative methods were used, which resulted in the generation of individual performance indicators, formulas for determining the values of these indicators were developed and scoring scales for their unification were determined. When forming the scoring scales, the range each criterion is within was revealed, while the maximum number of points was given to the best criterion value [20].

For the further formation of a complex performance indicator, the single indicators, which are a set of unordered factors, were to be systematized, and weight coefficients in order of the significance of each of the indicators were to be fixed. Fishburn's weight system was used to determine the weight coefficients of the single indicators.

A mathematical model was developed for estimating the effectiveness of the quality system called the $Ep$ model:

$$ Ep = \langle H, S, F \rangle, \quad (4) $$

where $H$ is the treelike hierarchy of performance indicators; $S$ is the score scale of single indicators in the hierarchy, scores; $F$ is the system of preferences relations of some indicators to others of the same level in the hierarchy. Whereby:

$$ S = \{1, 2, 3, 4, 5\}, \quad (5) $$

$$ F = \{R_i(\varphi) R_j | \varphi \in \{\approx, <\})\}, \quad (6) $$

where $\approx$ is a preference relation and $<$ is an indifference relation.

The proposed model described the tree hierarchy $H$ using a direct acyclic graph without horizontal edges and loops within one comparison series with a common root vertex:

$$ H = \langle R_i, A_{ij} \rangle, \quad (7) $$

where $\{R_i\}$ is a set of vertices of single indicators; $\{A_{ij}\}$ is a set of arcs, $R_0$ is the root vertex that characterizes the performance of processes in a complex manner.

![Fig. 3. Direct graph $H$ of performance indicators of the quality management system of a food enterprise.](image-url)
The arcs were arranged as follows in the treelike graph: the vertex of the lower rank corresponds to the beginning of the arc, and the vertex of the next level of the hierarchy, which is one less than the previous one, corresponds to the end of the arc. To estimate the effectiveness of the quality management system in accordance with the assigned weight relations at the same level of the hierarchy, were taken into account the values of the parameters of all levels of the hierarchy, expressed by aggregated at the next stage. At the same time, the weight coefficients correspond to the end of the arc. To estimate the performance indicator, respectively. The expert group estimated the importance of each group of processes – an engineering equipment maintenance process; – a procurement volume indicator; – an implementation process; – a packaging and storage process; – a conformity estimation process; – a marketing processes; – a sales growth indicator; – an assortment indicator; – a delivery terms indicator; – a product quality indicator; – an output indicator; – an index of absence of performers’ mistakes when testing samples; – a repair quality indicator; – a repair time indicator).

Based on the obtained system of relations $F$, the weight coefficients $a_{ijk}$ were determined for the single indicators within each level of processes. The complex estimation of the effectiveness of the quality management system was presented in the form of a four-level hierarchical set of the indicators $R_0$, $R_0$, $R_{ik}$ and the weight coefficients $a_c, a_p, a_{ijk}$ (Fig. 4), the sum of which is constant and equal to one at each level.

To derive a formula for the complex estimation of QMS effectiveness of a food enterprise, data were aggregated at the next stage. At the same time, the processes of all levels of the hierarchy, expressed by the values of the parameters $R_0$, $R_p$, $R_{ik}$, and their order relations at the same level of the hierarchy, were taken into account in accordance with the assigned weight coefficients $a_c, a_p, a_{ijk}$. The result of aggregation of the data is the formula:

$$ F = \{ R_i / R_j \approx R_{ij} \} , \quad (11) $$

Using the proposed mathematical model for the qualitative and quantitative estimation of the effectiveness of quality management systems of a food or processing enterprise allows us to comply with the requirements of international standards in full, using the current production information as the initial data. To ensure the quality management principle – constant improvement – and to analyze the performance dynamics, a formula was proposed for calculating the total deviation of the current value from the planned one:

$$ dR_0 = \sum_{l=1}^{n} dR_l , \quad (11) $$

where

$$ R_l = \sum_{i=1}^{m} (R_i \times a_i) , \quad (9) $$

and

$$ R_{ij} = \sum_{j=1}^{n} (R_{ij} \times a_{ijk}) , \quad (10) $$

The deviation indicator of the current value $dR$ makes it possible to control the degree of achievement of the planned level of effectiveness and, if necessary, to provide corrective actions.
Fig. 4. Treelike diagram of the performance indicators of quality management system processes.
Fig. 5. Algorithm for monitoring the effectiveness of processes in a quality management system.
Development of an algorithm for estimating the effectiveness of quality management systems.

The final stage of the study was the development of an algorithm for monitoring and estimating the effectiveness of QMS processes. The algorithm allows us to monitor systematically a change in the effectiveness of processes, to determine deviations from the planned values and identify the causes of these deviations (Fig. 5).

The algorithm includes three successively interconnected functional blocks: a monitoring block for the current effectiveness of processes of the quality management system, a process performance analysis block and an input control block. In the first block, data are collected and processed to estimate the state of processes, in the second – the calculation of the deviation of the current value \( dR \), and in the third – the formation of corrective actions or the correction of the planned values, as well as documenting the results of performance monitoring.

### DISCUSSION

Forming a process model is a complex task that requires special methods and tools to solve. The use of the IDEF0 notation is considered by a lot of authors to be an obsolete tool, however, in our opinion, this is not so [19, 20]. When identifying processes of various levels, the use of IDEF0 functional modeling remains a very convenient and effective tool for displaying processes of various levels and their connections. In addition, the ideology of this approach is almost completely identical to the requirements of ISO 9000 international standards, which sufficiently makes the work of QMS developers easy.

The approach proposed by the study was tested in practice when developing a quality management system for a low-capacity meat-processing plant (Table 2). In estimating the effectiveness of the quality management system, the actual value of the single indicators was first determined, then, using Formula 5, the complex effectiveness of QMS processes was determined, the deviation from the achievement of the planned value was calculated using Formula 8. The obtained data contributed to the estimation of the success of a process approach for an enterprise and the correction of further improvement actions. The methods described in the study have been included in the organization standard STO "Methods for estimating the effectiveness of the life cycle processes of boiled sausages".

A further activity area is the adaptation of the proposed methods to other enterprises taking into account branch specificity, as well as the development of a complex indicator of the estimation of the integrated quality and safety systems.

### CONCLUSION

The study identifies the processes of the quality management system of a food enterprise. All the processes are divided into 3 levels, each of which is subordinate to the superior. There are 9 processes at the lower level: marketing research; procurement and supply; production; engineering maintenance; packing and storage; inspection, verification, testing; implementation; launching new products into manufacture; product conformity estimation. A mathematical model for estimating the effectiveness of processes has been developed, including a treelike hierarchy, a scale for estimating single indicators and a system of relations of preference of some indicators to others for the same level of the hierarchy. Formulae for calculating the QMS performance indicator \( F_i \) and the total deviation from the planned value \( dF \) have been developed. The data obtained have been summarized as an algorithm for estimating the effectiveness of the quality management system of a food enterprise.

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**Table 2. Effectiveness of the process "Activities for the production of boiled sausages"**

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Basic processes</th>
<th>Control processes</th>
<th>Secondary processes</th>
<th>Total process effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planned value</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Present value for the 1st quarter</td>
<td>3.08</td>
<td>3.20</td>
<td>3.10</td>
<td>3.12</td>
</tr>
<tr>
<td>Deviation value for the 1st quarter d, %</td>
<td>0.92</td>
<td>0.80</td>
<td>0.90</td>
<td>0.88</td>
</tr>
<tr>
<td>Present value for the 2nd quarter</td>
<td>3.44</td>
<td>3.25</td>
<td>3.45</td>
<td>3.40</td>
</tr>
<tr>
<td>Deviation value for the 2nd quarter d, %</td>
<td>0.56</td>
<td>0.75</td>
<td>0.15</td>
<td>0.60</td>
</tr>
<tr>
<td>Present value for the 3rd quarter</td>
<td>3.90</td>
<td>3.50</td>
<td>4.05</td>
<td>3.84</td>
</tr>
<tr>
<td>Deviation value for the 3rd quarter d, %</td>
<td>0.10</td>
<td>0.50</td>
<td>-0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>New planned value</td>
<td>4.00</td>
<td>4.00</td>
<td>4.20</td>
<td>4.05</td>
</tr>
</tbody>
</table>


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PROFILE OF SUGARS IN A GRAPE-WINE SYSTEM AS THE IDENTIFYING INDICATOR OF THE AUTHENTICITY OF WINE PRODUCTS

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Abstract: The current problem of winemaking is the confirmation of a method for producing table and liqueur wines to protect the economic interests of producers and protect the health of consumers. It is possible to determine the nature of the sugars contained in wine on the basis of regularities in the dynamics of the glucose-fructose index (GFI) and the proportion of disaccharides in the total sugar content in the process chain "raw materials – finished products". The study objects included: the grapes grown on the territory of the Crimean Peninsula, European, autochthonous technical varieties, as well as the varieties of a new selection; domestic and foreign wine materials and wines; model samples and wine falsifications. The content of disaccharides in terms of sucrose, glycerol, glucose and fructose was determined by high-performance liquid chromatography. As a result of the studies of the wines obtained by arrested fermentation, there are some trends in the reduction of GFI with a decrease in the level of endogenous sugars: for the wines with a sugar content of 230–270 g/l, the range of GFI is 0.75–0.94, with a mass concentration of sugars of 10–20 g/l – 0.05–0.14. In the case of a sugar concentration in wine of more than 120 g/l, it is necessary to study a sample for the glycerol content as a marker of fermentation depth in order to increase the reliability of conclusion. The values of the indicators characteristic of high-sugar grapes are typical for the wines obtained by sweetening with a grape must concentrate: GFI – not more than 1.0, the proportion of disaccharides is not more than 1.2%; falsifications are characterized by the profile of sugars atypical for grape products: GFI is higher than 1.02, the share of disaccharides is more than 2%.

Keywords: Glucose, fructose, sucrose, glycerol, high-performance liquid chromatography, concentrated grape must, falsification, banned supplements

INTRODUCTION

The winemaking plants of the Crimea are able to produce the high-quality wines, both liquor and table, obtained by arrested fermentation. The production of wines in this way is possible due to the significant accumulation of sugars in a grape berry, which is a consequence of favorable agroclimatic conditions for growing a grape plant, especially on the southern coast of Crimea. The content of sugars in a grape berry and the features of their qualitative composition depend on a number of factors: the species and variety of grapes, the climatic features of the region and the year of harvest, the stage of maturity at the time of harvesting [1–4]. In the future, the sugar content of grapes determines the trend of use of raw materials (the production of a must concentrate, the production of table or liqueur wines) and the features of technology for its processing. The predominance of fructose can cause slow fermentation and / or arrested fermentation [5], which is unacceptable in the production of dry wines. The difficulties of fermentation, in this case, are explained by the different degree of yeast glucophily. It is known that different yeast races, related to the species Saccharomyces cerevisiae, traditional for winemaking are characterized by a tendency to actively assimilate glucose, non-saccharomycetes (Candida, Zygosaccharomyces and others) are distinguished by high fructosophility [6–8]. Despite the fact that the activity of sugar consumption by yeast cells is a genetically determined sign, the peculiarities of fermentation also affect this process. Under the unfavorable conditions of fermentation (the non-correspondence of temperature conditions to the physiological optimum, the deficiency of nitrogenous substances and the presence of fermentation inhibitors), the fermenting capacity may not be fully realized [9, 10].

To correct the sugar content in wines, a must concentrate is used, including the rectified must produced from grapes (Federal Law No. 171-FZ of November 22, 1995 "On State Regulation of Production and Circulation of Ethyl Alcohol and Alcohol Products and Restriction on Alcohol Consumption", http://www.garant.ru/products/ipo/prime/doc/71335844/#ixzz4XP5WJ7Jo). However, semi-dry, semisweet and sweet wines of high quality categories, such as wines with protected geographical indication (PGI) and wines with protected appellation of origin (PAO), can be produced only by incomplete alcoholic fermentation. The wines with the preserved
natural (endogenous) sugars have more pronounced organoleptic characteristics and are appreciated much higher than those produced with the application of sugars (exogenous), but they are difficult to produce and require a high level of technological discipline.

The use of sugar-containing products with a non-grape basis is prohibited in the production of wines. Such sugar-containing substances include the glucose-fructose syrup (GFS) obtained by the enzymatic hydrolysis of starch-containing raw materials. A distinctive feature of GFS is the glucose content of more than 50%, as well as the presence of a disaccharide of maltose, which is a by-product of starch hydrolysis [11].

It is also unacceptable to sweeten wines with fruit juice concentrates, which are characterized by an individual profile of sugars in accordance with the characteristics of raw materials of various botanical species [12], in particular, a high content of sucrose is noted in apples [13].

There are various approaches to identifying and determining the authenticity of juice and wine products, including a variety of analytical methods. The method of gas chromatography is used to determine the presence of syrup supplements in juices (GOST 32800-2014 Juice products. Detection of glucose and fructose syrups addition by capillary gas chromatography, http://standartgost.ru/g/%D0%93%D0%9E%D0%A1%D0%9A%D0%ae2_32800-2014). A modern informative method for controlling the authenticity of wines is the mass spectrometry of stable oxygen and carbon isotopes [14–16], but the wide spread of this analytical study is limited to the significant cost of high-tech equipment.

To identify and determine the authenticity / identify the falsification of wines, the Magarach Institute developed a system of indicators that includes: the profile of organic acids, the content of glycerol, phenolic and aroma-forming substances and some other components. We proposed some criteria for verifying the authenticity of the grape origin of various products – physical and chemical characteristics, a phenolic content, an organic acid composition and the composition and content of sugars [17, 18]. The use of high-performance liquid chromatography and capillary electrophoresis allows to obtain the exact information about the state of soluble carbohydrates in any liquid product within a short period of time [19–21].

At the moment, the information on changes in these indicators, in particular, sugars, in literature during the process cycle in the "grapes-wine material-wine" chain, taking into account the sugar content of the raw materials and various categories of wine quality is not provided, which can reduce the reliability of conclusions in the identification of wine products.

The study aims at the profile of sugars at all stages of the process cycle in order to determine a method for producing different types of wines.

**STUDY OBJECTS AND METHODS**

The study objects included:
- the grapes of white (Aligote, Verdelho, Kok Pandas, Kokour White, Muscat White, Rkatsiteli, Sary Pandas, Sersial, Sauvignon Green, Tokay, Sabbath, Chardonnay) and red (Bastardo Magarachsky, Cabernet Sauvignon, Kefesia, Pinot Franc, Ekim Kara) European and autochthonous technical varieties of the species *Vitis vinifera*, as well as 2 varieties of a new selection (Golubok, the selection of Institute of Viticulture and Winemaking named after V.E. Tairov (the Ukraine)), a complex hybrid (Severyn x the pollen mixture of the varieties Sorok Let Oktayabrya, Odesskiy Ranniy and No.1-17-54 (Alicante Bouschet x Cabernet Sauvignon)), Bukovinka and the selection of the Magarach Institute, the hybrid of the varieties Pukhlyakovsky x Zeybel 13-666);
- the sweetening grape (a must concentrate of various manufacturers) and non-grape (glucose-fructose syrup) components;
- the table and liqueur wine materials and wines produced in the conditions of microvinification and at the plants of the Crimea;
- foreign-made wines (Azerbaijan, Germany, Italy, Portugal, France, Chile);
- the model samples that imitate table semi-dry, semi-sweet and sweet wines, as well as the liqueur wines obtained by adding various sugar-containing components to dry table and liqueur wine materials;
- the samples of the finished products provided by controlling authorities the falsification of which was established by us in accordance with a method for identifying the authenticity of grape wine materials and wines [17].

The samples were divided into groups in accordance with the mass concentration of sugars according to the normative documentation of the Russian Federation (http://standard.gost.ru/wps/portal):
- the table wines produced by arrested fermentation: No. 1 – semi-dry, No. 2 – semi-sweet, No. 3 – sweet (Table 1);
- the liqueur wines produced by arrested fermentation: № 1–7 – in accordance with the conditions encountered in various finished products (Table 2);
- the sweetened wines (the application of a grape must concentrate in dry wine material (Table 1, 4–6 and Table 2, No. 8–10) and the falsifications obtained by adding a non-grape component (Table 1, 7–9 and Table 2, No. 11–13) were combined according to the content of sugars into larger clusters due to lack of a reliable difference.

The informativeness of the glucose-fructose index was verified using the samples of elite liqueur wines (the Crimea), obtained by arrested fermentation that we referred to different groups in the content of sugars according to Table 2: 10 g/l – Madeira Dry (Group No. 1); 30–40 g/l – Madeira Massandra, Madeira Crymskaya (Group No. 2); 60 g/l – Port White and Magarach Red (Group No. 3); 95–110 g/l – Sevastopol Port White, Port White and Crymsky Port Red, Port White and South Coast Port Red (Group No. 4); 160–180 g/l – Surozh Dessert Kokour, Bastardo Magarachsky, South Coast Cahors (Group No. 6); 220–270 g/l – Magarach Muscat White, Red Stone Muscat White, Livadia Muscat White (Group No. 7). Group 5 includes the wines of a lower quality category that are not elite wine products.
The table and liqueur wine materials were produced according to classical schemes. The wine materials produced under microvinification conditions were fermented using pure yeast cultures from the "Collection of microorganisms of winemaking of "Magarach". In the experimental samples of wine materials, the content of sugars was varied within the range of 0–230 g/l, in accordance with the normative documentation for table and liqueur wines [http://standard.gost.ru/wps/portal].

The flow sheet of table wines involved the following procedures [22]:

I – from white grapes: destemming → grape crushing → sulfitation → pressing the crushed grapes → clarification of must → the crushed grapes fermentation culture must → racking → clarifying the wine material.

II – from red grapes: destemming → grape crushing → sulfitation → the fermentation of pure yeast culture crushed grapes to the fermentation of 2/3 of sugars → pressing the crushed grapes → afterfermentation → racking → clarifying the wine material.

The flowsheet of liqueur wines suggested:

– destemming → grape crushing → sulphation → the crushed grapes fermentation culture crushed grapes → pressing the crushed grapes → afterfermentation to obtain the required sugar content → fortification → racking → clarifying the wine material.

The moment of fermentation arrest depended in all cases on the type of wine and the maintenance of the required standards for the content of sugars in the finished products.

The sweetening ingredients were: the permitted supplement – a grape must concentrate, the banned supplements – glucose-fructose syrup.

Varying the sweetening methods was provided by adding glucose-fructose syrup and a grape must concentrate to dry wine material (table wines) or dry wine material with the addition of alcohol (liqueur wines).

The mass concentration of organic acids, sugars and glycerol was determined when separating one sample by high-performance liquid chromatography (a Shimadzu LC Prominance chromatograph, Japan). The determination was made according to the preliminary calibration of the device for the standard solutions of pure substances using a refractometric (glucose, fructose, disaccharide, glycerol) and spectrophotometric (citric, tartaric, malic acid) system detector, taking into account the time of each individual substance efflux. The operating wavelength for the determination of organic acids was 210 nm. The sample was separated using a Supelcogel C610H column filled with a sulfonated polystyrene/divinylbenzene sorbent (the column size is 300 × 7.8, the sorbent particle size 9.0 μm, Supelco®, Sigma-Aldrich), in an isocratic mode (an 0.1% aqueous solution of orthophosphoric acid, the rate is 0.5 ml/min). Before the analysis of samples or to obtain calibration dependences, the refractometric detector of the system was additionally calibrated for the standard solutions of substances, and the obtained analytical characteristics were noted. The final calculation of the mass concentration of glucose and

### Table 1. Mass concentration of sugars in table wines and their falsifications

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Number of samples</th>
<th>Mass concentration of sugars, g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n = 17</td>
<td>5–17</td>
</tr>
<tr>
<td>2</td>
<td>n = 34</td>
<td>18–40</td>
</tr>
<tr>
<td>3</td>
<td>n = 41</td>
<td>more than 45</td>
</tr>
<tr>
<td>Wines with the addition of a grape must concentrate to dry wine material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>n = 27</td>
<td>5–17</td>
</tr>
<tr>
<td>5</td>
<td>n = 15</td>
<td>18–40</td>
</tr>
<tr>
<td>6</td>
<td>n = 5</td>
<td>more than 45</td>
</tr>
<tr>
<td>Falsification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>n = 7</td>
<td>5–17</td>
</tr>
<tr>
<td>8</td>
<td>n = 8</td>
<td>18–40</td>
</tr>
<tr>
<td>9</td>
<td>n = 5</td>
<td>more than 45</td>
</tr>
</tbody>
</table>

### Table 2. Mass concentration of sugars in liqueur wines and their falsifications

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Number of samples</th>
<th>Mass concentration of sugars, g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n = 13</td>
<td>10–20</td>
</tr>
<tr>
<td>2</td>
<td>n = 36</td>
<td>30–40</td>
</tr>
<tr>
<td>3</td>
<td>n = 22</td>
<td>50–60</td>
</tr>
<tr>
<td>4</td>
<td>n = 31</td>
<td>70–120</td>
</tr>
<tr>
<td>5</td>
<td>n = 8</td>
<td>130–140</td>
</tr>
<tr>
<td>6</td>
<td>n = 31</td>
<td>160–180</td>
</tr>
<tr>
<td>7</td>
<td>n = 16</td>
<td>230–270</td>
</tr>
<tr>
<td>Wines with the addition of a grape must concentrate to dry fortified wine material</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>n = 10</td>
<td>10–60</td>
</tr>
<tr>
<td>9</td>
<td>n = 15</td>
<td>70–120</td>
</tr>
<tr>
<td>10</td>
<td>n = 7</td>
<td>160–270</td>
</tr>
<tr>
<td>Falsification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>n = 11</td>
<td>10–60</td>
</tr>
<tr>
<td>12</td>
<td>n = 8</td>
<td>70–120</td>
</tr>
<tr>
<td>13</td>
<td>n = 6</td>
<td>160–270</td>
</tr>
</tbody>
</table>

The total sample size was 74 batches of the grapes grown on the territory of the Crimean Peninsula, 94 samples of sweetening grape (a must concentrate of various manufacturers) and non-grape (glucose-fructose syrup) components, 400 samples of wine materials and wines (including model and falsified samples). The studies were carried out within the period of 2010–2016 based at the department of wine chemistry and biochemistry of All-Union Scientific Research Institute of Winemaking and Viticulture Magarach.

When studying grapes, the average sample of the berries previously separated from the stems, weighing 20–50 grams was selected and ground in a homogenizer. The obtained homogeneous mass was filtered through a glass filter to separate gross impurities. The indicators were determined in must after centrifugation (the speed of the centrifuge rotor is 7000 rpm, the separation time is 10 minutes).
fructose was performed taking into account the refraction data for the respective groups of substances (tartaric and malic acids) that have the same efflux time as the listed carbohydrates, by the mathematical recalculation of the data previously obtained using a UV detector. In the case of the presence of suspensions or insoluble particles in the visual estimation of the sample, they were preliminarily separated using a centrifuge (the speed of the rotor is at least 6–7 thousand rpm, the duration is 5–7 minutes). The relative error of the method (δ) did not exceed 10% with the confidence probability $P = 0.95$.

The content of sugars was calculated as the sum of the mass concentration of disaccharides (sucrose and maltose in terms of sucrose), glucose and fructose.

The glucose-fructose index (GFI) is a quotient of the glucose and fructose content in the sample, herewith, the error of the final result did not exceed 0.01.

The mass concentration of phenolics was determined using the colorimetric method with the Folin-Ciocalteu reagent [23].

RESULTS AND DISCUSSION

The profile of sugars was studied at various stages of processing grapes and producing wine products: in fresh and concentrated must, in wine materials and the finished products.

The content of sugars (the sum of glucose, fructose and disaccharides) in grapes characterizes technical maturity and determines the way of its processing: the production of table or liqueur wines. The ratio of glucose and fructose (the glucose-fructose index) is one of the criteria for identifying the grape origin of sweeteners, and also determines the choice of yeast and fermentation conditions for obtaining wine products with the specified conditions.

To form a unified system for establishing the authenticity of wines, we carried out a study of the variation of GFI in grape must. 74 batches of the grapes grown on the Crimean Peninsula and collected at a sugar content of 160–330 g/l were analyzed.

As can be seen from the variety of the actual data presented in Fig. 1, with the equal sugar content in grape must, the values of GFI can be different, while the same GFI values can correspond to different levels of sugar accumulation. The variability of the index in the must of various batches of grapes is explained by a multitude of simultaneously and multidirectionally affecting factors, including the stage of physiological maturity [1–4]. The statistical processing of the results of the study (mode – 0.93, median – 0.97, a standard deviation – 0.03, dispersion – 0.001) shows that the GFI values are within the range of 0.89–1.04, which corresponds to the world data obtained for grapes growing in other wine-growing regions of the world [1–3].

We compared the index in the grapes of some varieties at a sugar content range of 200–230 g/l, which provides obtaining conditioned table and liqueur wines. The obtained results demonstrate that for each variety, as sugar is accumulated in grapes, there is a tendency to a decrease in the GFI values by 0.015–0.06 (within the studied sample), Fig. 2 presents the examples of actual values. According to the values of this index, we conditionally divided the studied grape varieties into three groups: the first group – the lowest GFI (0.94) – Aligote, Sauvignon Green; the second group – the intermediate GFI (0.97–0.99) – Muscat White, Rkatsiteli, Kokour White, Cabernet Sauvignon, Bastardo Magarachsky; the third group – the highest GFI (1.02–1.04) – the varieties of grapes of a new selection Golubok and Bukovinka.

Different values of GFI at the same level of sugars can be explained by the fact that in the Crimea conditions the grape varieties prone to high sugar accumulation (the second group) have a long vegetative period and the prolonged synthesis of carbohydrates, while glucose is not expended on the process of cell respiration. The grapes of the varieties with a shorter maturation period (the first group) stop the anabolic process earlier, the high sugar accumulation in this case is due to the evaporation of moisture through the skin of a berry; the stopped carbohydrate anabolism causes an active glucose intake, which reduces the glucose/fructose ratio (GFR) [24].

Fig. 1 Glucose-fructose index (GFI) in the must of grapes of white and red varieties (the list of varieties is provided in the methodical part).
Fig. 2. Glucose-fructose index (GFI) in the must of various grape varieties depending on sugar accumulation (in the studied sample). Grape variety: (1) Aligote, (2) Sauvignon Green, (3) Rkatsiteli, (4) Kokour White, (5) Muscat White, (6) Cabernet Sauvignon, (7) Bastardo Magarachski, (8) Golubok, (9) Bukovinka.

A characteristic feature of a grape berry is the insignificant accumulation of the disaccharides presented by sucrose, in comparison with glucose and fructose [12]. The study of the profile of sugars showed that the content of disaccharides in the must of grapes of the first and second group does not exceed 1.7 g/l, making up no more than 0.7% of the total sugar content; in the third group, the content of these carbohydrates is significantly higher and can reach 14 g/l (4%). This is due to the fact that the varieties of a new selection with the genes of the species *Vitis labrusca* have a distinctive feature of this species of grapes, prone to the higher accumulation of sucrose and glucose than the European varieties that belong to the species *Vitis vinifera*. The results are consistent with the data presented in the world literature on the biochemical features of grapes [cit. by 1].

The level of GFI in the grapes used for processing must be taken into account when choosing a yeast race for fermentation. It has been shown that yeasts differ significantly in the activity of fructose consumption [25] and in the case of a high proportion of fructose in grapes, as can be seen in the case of the grape varieties Sauvignon Green and Aligote, difficulties may arise due to the complete fermentation of sugars. This problem is typical for wine-making regions with a hot climate, where GFI in some cases is reduced to 0.77 at the time of harvesting [26].

When processing grapes for the production of a must concentrate, it is economically justified to use raw materials with the maximum content of sugars. In addition, technological costs are reduced due to the exclusion or reduction of the acid loss of fresh must, which is due to a low content of titratable acids in high-sugar grapes. However, a must concentrate is more expensive than the sugar-containing products widely represented in the market and used in the food industry, which prompts unfair wine producers to use them in the production of the wines that are falsified wine products.

One of the distinguishing features of the origin of concentrated juices, as already noted, may be the profile of sugars. In our previous studies, it was shown that when concentrating must, the values of GFI characteristic of this batch of grapes remain unchanged [27]. This allows us to use this indicator as a reliable criterion that confirms the grape origin of must before and after concentration.

The study of grape and non-grape products showed (Table 3) that in a grape must concentrate that belongs to the species *Vitis vinifera*, the glucose-fructose index does not exceed 1.0, the proportion of disaccharides is not more than 0.6%, which is close to the values typical for mature grapes with a high sugar content, which is used to obtain this product. Glucose-fructose syrups and the falsifications of a grape must concentrate differ from an authentic grape product in GFI that exceeds 1.04 and a higher proportion of disaccharides – more than 2%. In the case of the production of a must concentrate from grapes of the species *Vitis labrusca* or its hybrids, there is an increase in the limits established for *Vitis vinifera*.

The next stage of our studies was the study of the peculiarities of the profile of sugars in wine materials and the finished products obtained by arrested fermentation or sweetening to provide the specified standards for the content of sugars. The obtained results demonstrate that the values of GFI in the table and liqueur wines obtained by arrested fermentation, by mixing dry wine materials with a grape must concentrate or sugar-containing non-grape products differ significantly (Fig. 3 and 4).

For the table and liqueur wines obtained by arrested fermentation, there is a direct positive relationship between the sugar content and the index value. The correlation coefficient for the whole sample of wines (n = 249) is $r = 0.87$ (at $p = 0.95$).

In sweet table wines (Group No. 3), the GFI values are on average 0.47 within the range of 0.16–0.73 (Fig. 3). For semi-sweet wines (Group No. 2), the average value of the indicator decreases to 0.18, the range is 0.08–0.31. Semi-dry wines (Group No. 1) differ by the minimum value of the index – 0.08, with the range of values of 0.02–0.21.
Table 3. Profile of sugars in various sweetening components

<table>
<thead>
<tr>
<th>Study object</th>
<th>number of samples, pcs.</th>
<th>GFI</th>
<th>Percentage of disaccharides, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>average</td>
<td>range</td>
</tr>
<tr>
<td>Grape must concentrate</td>
<td>72</td>
<td>0.95</td>
<td>0.88–0.99</td>
</tr>
<tr>
<td>Sugar-containing non-grape components</td>
<td>22</td>
<td>1.12</td>
<td>1.04–1.19</td>
</tr>
</tbody>
</table>

Fig. 3. Glucose-fructose index value in table wines and falsifications (the list of sample groups is provided in the methodological part, Table 1).

Fig. 4. Value of the glucose-fructose index (GFI) in liqueur wines and falsifications (the list of samples groups is provided in the methodological part, Table 2).

The allowed technological method for sweetening wine materials by adding a grape must concentrate (Group No. 4–6) gives the expected result – regardless of the sugar content, the value of the indicator was 0.95, within the range of 0.89–0.99, characteristic for GFI of grape must (Table 3). Using the example of the authentic samples obtained under microvinification conditions, it has been found that GFI of wine corresponded to GFI of a must concentrate, the difference did not exceed the error limit of the method. The models of table wines, obtained with the use of the forbidden sugar-containing components (Group No. 7–9), were characterized by a sugar profile unusual for grapes. Regardless of the mass concentration of sugars, the average index value was 1.12 with a range of 1.04–1.18. The minimum value significantly differs from the maximum, characteristic for the wine produced with the application of a grape product -1.0 (F = 319, at F_{crit} = 4.3).

The study of liqueur wines (Fig. 4) confirms the regularities established for table wines. In the case of the wines obtained by arrested fermentation (Group No. 1–7), GFI decreases as the fermentation of sugars increases by 85–90%: with a sugar content of 230–270 g/l, the range is 0.75–0.94 (on average 0.87), with a residual sugar content of 10–20 g/l, the range is 0.05–0.14 (on average up to 0.11). The highest variations of the index – from 0.23 to 0.83 – have been noted for the wines with a sugar content of 50–120 g/l. The glucose-fructose index in the samples produced with the application of grape (Group No. 8–10) and of non-grape products (Group No. 11–13) is 0.94–0.95 and 1.11–1.13, respectively.

The noticeable fluctuations of GFI in both the table and liqueur wines obtained by arrested fermentation can be explained by the different sugar content of the raw materials used for processing, by the considerable variability of industrial yeast races in the ability to assimilate fructose and by various fermentation conditions [10, 25, 26].

The established relationships between GFI and the amount of the fermented carbohydrates are confirmed when studying the high-quality liqueur wines produced at the leading wine-making plants of the Crimea. By reducing GFI and the content of sugars, they can be arranged as follows: Magarach Muscat White, Red Stone Muscat White, Livadia Muscat White → Surozh Dessert Kokour, Bastardo Magarachsky, South Coast Cahors → Sevastopol Port White, Crymsky Port White (Red), South Coast Port White (Red) → Magarach Port White → Madeira Massandra, Madeira Crymskaya → Madeira Dry.
Table 4. Physico-chemical indicators of authentic and falsified table and liqueur wines

<table>
<thead>
<tr>
<th>Variant*</th>
<th>Content, % vol.</th>
<th>Mass concentration, g/l</th>
<th>Estimate indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>Sugars</td>
<td>Citric acid</td>
</tr>
<tr>
<td>Dry table wine material (the sugar content is less than 4 g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete fermentation</td>
<td>14.2</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Semi-sweet table wine (the sugar content is 35 g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12.4</td>
<td>32.3</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>14.0</td>
<td>34.4</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>14.1</td>
<td>36.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Liqueur wine (the sugar content is 60 g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.2</td>
<td>60.5</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>17.8</td>
<td>60.4</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>17.9</td>
<td>63.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Liqueur wine (the sugar content is 160 g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15.9</td>
<td>161.0</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>16.5</td>
<td>157.9</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>16.3</td>
<td>159.0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Note. * 1 – fermentation arrest; 2 – the dry wine material sweetened with a grape must concentrate; 3 – the dry wine material sweetened with GFS.

When determining the authenticity of wines with a sugar content of 120–270 g/l, in some cases the range of GFI of wine overlaps with that of a must concentrate, which does not allow us to identify with a high degree of confidence a method for obtaining a particular sample.

We carried out the studies of wines of this range of sugars according to the additional physicochemical parameters (Table 4), proposed earlier for establishing the authenticity of wines: organic acids, phenolics and glycerol [17, 28–30]. In each group, the samples developed with the preservation of endogenous sugars (Variant 1) and their application in the form of a grape must concentrate (Variant 2) and GFS (Variant 3) were considered.

The results allow us to conclude that the application of a grape must concentrate leads to a logical increase in the content of tartaric acid as a natural component of a grape berry by 0.1–1.0 g, and GFS – to a decrease in its mass concentration by 0.1–0.2 g/l due to the dilution of a sample with a sweetening component. The content of malic and citric acids changes to a lesser degree.

Similar trends have been noted with regard to the mass concentration of phenolics – sweetening with a grape must concentrate increases the content of this component from 0.26 g/l to 0.3–0.56 g/l, depending on the applied volume that provides the required standards; sweetening with GFS does not significantly affect the content of the specified substances. The change in the concentration of organic acids and phenolic compounds is noted with respect to the control wine material before sweetening, however, the content of these components is within the limits established for grape wines [17].

It was noted during the experiment that the mass concentration of glycerol is within the range of 5.2–6.8 g/l for all the variants, with the exception of the liqueur wine with a sugar content of 160 g/l obtained by arrested fermentation. In the latter case, the concentration of this substance is much lower – 3.1 g/l, which corresponds to the literature data on the accumulation of fermentation products in wines [28].

The high value of this indicator, typical for the wines obtained by complete fermentation, is not compatible with an index of more than 0.85, characteristic of the wines with a small amount of fermented sugars, and indicates sweetening. This approach allows us to confirm the authenticity of high quality wine technology (PGI, PAO), in the production of which the applied sugar-containing components should not be used.

In all the experimental variants with sweetening, the values of GFI correspond to the ranges established for sugar-containing grape and non-grape components (Table 3), and are 0.91–0.94 and 1.04–1.18, respectively.

The significant indicator that allows us to prove the falsification of wine products is also the share of disaccharides in the total content of sugars. In variants 3, the value of this indicator is 2.5–2.8%, which exceeds the range characteristic of the authentic wines that contain grape sugars – 0.5–0.9% (Variant 1 – fermentation arrest) and 0.8–1.2% (Variant 2 – sweetening with a grape must concentrate). This allows us to include the profile of sugars in the system of indicators to authenticate wines.

The analysis of the imported wine products showed (Table 5) that the values of GFI in the studied sample do
not always coincide with the ranges established by us for domestic wines. In samples of semisweet wines No. 2 and 3, the value of GFI (0.83 and 0.84) is higher than that for similar domestic wines and approaches to the wines that contain exogenous grape sugars. Sample No. 6 differs from the general trend in a high value of GFI, but at the same time it is characterized by a high sugar content, which is not typical for domestic table wines. The deviation from the range has also been noted for liqueur samples No. 7 (0.88) and No. 16 (0.59), which may also be due to the peculiarities of the biochemical characteristics of grapes at the time of harvesting and wine technology specific for a particular region. This can be explained by the fact that when obtaining wines there is a variety of technological and biotechnological techniques that can be legislated for separate wine regions, which does not apply to other countries, including Russia. In all cases, the GFI value did not exceed 1.0, which indicates the absence of falsifications in this sample. This is consistent with the foreign literature data [31], as well as the results obtained by LA. Valgina, who showed that the range of the glucose/fructose ratio, characteristic for the imported table wines is 0.7–0.8 [http://mgutm.ru/files/graduates-and-doctors/avtoreferat_valgina_la.pdf]. The proportion of disaccharides in all the analyzed samples did not exceed 0.5%, which is also characteristic of authentic wines. A further study of the wine products of different regions of the world is planned to compare their physicochemical parameters with the ranges established for domestic wines, with the presence of endo- or exogenous sugars.

**CONCLUSIONS**

The dynamics of the profile of sugars in the "grape-wine" system has been studied for the first time:

– the ratio of different sugars in grapes is determined by the variety-specificity and level of sugar accumulation;

– when concentrating grape must, the glucose-fructose index and the proportion of disaccharides remain unchanged;

– during fermentation, GFI and the content of disaccharides decrease, their values depend on the amount of the fermented sugars;

– GFI and the proportion of disaccharides in the wines that contain exogenous sugars correspond to the values of the parameters of the applied sugar-containing component;

– sweetening the wine material with a sugar-containing non-grape product determines the profile of sugars uncharacteristic for grape wines.

Identifying indicators and the ranges of their variation for authentic wines with various contents of sugars – GFI and the share of disaccharides have been established; to increase the reliability it is necessary to determine the content of glycerol as a marker of fermentation depth.

The revealed regularities make it possible to identify the table and liqueur wines obtained by arrested fermentation, mixing with a grape must concentrate, as well as the inadmissible modification of their composition by introducing sugar-containing non-grape components.

**Table 5. Value of the glucose-fructose index (GFI) in imported wines**

<table>
<thead>
<tr>
<th>№</th>
<th>Sample</th>
<th>Country of origin</th>
<th>Sugar content, g/l</th>
<th>GFI Range set for domestic wines</th>
<th>Value in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Brackenheim Riesling Kabinett</td>
<td>Germany</td>
<td>23.7</td>
<td>0.08–0.31</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>Casa Verde Cabernet Sauvignon/Merlot Semi-Sweet</td>
<td>Chile</td>
<td>36</td>
<td>0.08–0.31</td>
<td>0.83</td>
</tr>
<tr>
<td>3</td>
<td>Winemaker Sauvignon-Blanc Chardonnay Semi-Sweet White</td>
<td>Chile</td>
<td>37</td>
<td>0.08–0.31</td>
<td>0.84</td>
</tr>
<tr>
<td>4</td>
<td>Chateau d'Yquem Sauternes AOC 1-er Grand Cru Superieur</td>
<td>France</td>
<td>52</td>
<td>0.16–0.73</td>
<td>0.15</td>
</tr>
<tr>
<td>5</td>
<td>Eiswein Riesling Blue Nun</td>
<td>Germany</td>
<td>169</td>
<td>0.23–0.86</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>Vin Santo del Chianti Classico</td>
<td>Italy</td>
<td>210</td>
<td>0.23–0.86</td>
<td>0.72</td>
</tr>
<tr>
<td>7</td>
<td>Blandys Alvada Rich</td>
<td>Portugal</td>
<td>58</td>
<td>0.34–0.85</td>
<td>0.68</td>
</tr>
<tr>
<td>8</td>
<td>Calem Old Friends White Porto</td>
<td>Portugal</td>
<td>63</td>
<td>0.34–0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>9</td>
<td>Calem White and Dry Porto</td>
<td>Portugal</td>
<td>65</td>
<td>0.34–0.85</td>
<td>0.45</td>
</tr>
<tr>
<td>10</td>
<td>Graham’s 10 Year Old Tawny Porto</td>
<td>Portugal</td>
<td>113</td>
<td>0.34–0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>11</td>
<td>Calem Special Reserve Porto</td>
<td>Portugal</td>
<td>113</td>
<td>0.34–0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>12</td>
<td>Calem Old Friends Ruby Porto</td>
<td>Portugal</td>
<td>115</td>
<td>0.34–0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>13</td>
<td>Alabashli</td>
<td>Azerbaijan</td>
<td>120</td>
<td>0.34–0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>14</td>
<td>Calem Friends White Porto</td>
<td>Portugal</td>
<td>126</td>
<td>0.34–0.85</td>
<td>0.88</td>
</tr>
<tr>
<td>15</td>
<td>Agstafa</td>
<td>Azerbaijan</td>
<td>129</td>
<td>0.34–0.85</td>
<td>0.90</td>
</tr>
<tr>
<td>16</td>
<td>Calem Old Friends Ruby Porto</td>
<td>Portugal</td>
<td>148</td>
<td>0.55–0.91</td>
<td>0.59</td>
</tr>
<tr>
<td>17</td>
<td>Calem Lagrima Porto</td>
<td>Portugal</td>
<td>164</td>
<td>0.55–0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>18</td>
<td>Macvin du Jura Blanc</td>
<td>France</td>
<td>178</td>
<td>0.74–0.98</td>
<td>1.00</td>
</tr>
</tbody>
</table>
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IDENTIFICATION OF TISSUE-SPECIFIC PROTEINS AND PEPTIDES FORMING INNOVATIVE MEAT PRODUCTS CORRECTIVE PROPERTIES TO CONFIRM AUTHENTICITY OF MEAT RAW MATERIALS

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Abstract: Proteomic methods and approaches to the detection of tissue-specific and tissue-generating proteins and peptides – which form corrective properties – in studied meat samples and specially developed meat products were successfully tried out in 2016–2017. The methods allow one to confirm protein and peptide authenticity and also detect bio-markers of proteolytic changes in meat after slaughter. The following proteomic techniques were used in the present research: two-dimensional O’Farrell electrophoresis with isoelectrofocusing in ampholin and immobilin pH gradients, the detection of proteins on two-dimensional electrophoregrams by staining with Coomassie R-250 and silver nitrate, and mass spectrometric identification of proteins by means of MALDI-TOF and MS/MS methods. Contractile actomyosin complex proteins, such as myosin light chains and troponymosins, were the most informative among proteins of species specificity. It is also necessary to mention that earlier experiments allowed us to choose enzymes which play a part in carbohydrate metabolism (glyceraldehyde 3-phosphate dehydrogenase and β-enzolase) as markers. In addition to the listed proteins, myoglobins, actins, and several other proteins in horse meat have showed high species specificity and have been detected well. A system of species specificity (authenticity) of meat raw materials was suggested. The system allows the presence of pork, beef, horse, and camel meat to be detected in both raw and heat-treated products if the content is 5% and more. The data has been obtained by means of bioinformatics, a highly useful tool for formulating an algorithm to identify the protein markers for the Atlas “Proteomic profiles of farm animals meat proteins”. “Proteomic profiles of farm animals muscle proteins”.

Keywords: Beef, pork, horse meat, camel meat, proteomics, muscle proteins, peptide fingerprint, 2D-electrophoresis, MALDI-TOF mass spectrometry, authenticity

INTRODUCTION

During last 10 years, scientists throughout the world have been researching protein and peptide substances in raw and processed meat products, which are formed as a result of technological treatment and bring about the quality, functionality, and safety of final food products. Peptides containing 2–30 amino acids have been defined. The peptides have hypotensive, opioid, antioxidant, antimicrobial, and other biological effects on a number of the most common mechanisms underlying various pathological processes. The investigation of meat proteins as potential sources of biopeptides includes the study of both proteome and metabolites formed due to the fermentation process as well hydrolysis of raw materials by gastrointestinal tract natural enzymes by means of proteomics and bioinformatics [1, 3]. The presence of hypotethical functional sequences and the formation of active sequences due to the action of own proteolytic enzymes, proteases and peptidases of bacterial origin were studied.

Numerous researches in the world practice are concerned with the study of the biological role of peptides in vivo, their absorption and resistance to gastrointestinal tract enzymes. Mechanisms of protein and peptide formation which bring about bio corrective and qualitative characteristics, as well as biosynthesis, folding, and catabolism processes are of great interest.

Bioinformatics as the tool of studying proteome in relation the hypothetical presence of various bioactive peptides and protein makers in it is becoming more and more popular. According to available data, muscle proteins of food-producing animals contain amino acid sequences with various biological properties such as beef collagen α1, myosin light chain (LC), and connectin [2]. Beef collagen α1 has hypotensive, opioid, anti-amnesic, and anti-thrombotic properties and stimulates ubiquitin-regulated proteolysis which inhibits dipeptidyl peptidase IV and adjusts gastric mucosa activity; myosin LC is rich with antimicrobial sequences; connectin contains peptides of antithrombotic, anti-amnesic, opioid, neuroprotective, immunomodulatory, antioxidant, and hypotensive activity, dipeptidyl peptidase IV inhibitors, and regulators of gastric mucosa activity. Beef, chicken, and pork acts contain inhibiting dipeptidyl peptidase IV sequences. Collagen and elastin include sequences with corrective properties due to high glycine and proline content. It should be noted that over 220
conditions.

“proteome” as a reaction to changed biological modifications, cell processes, and changes in proteins, their biological activity, post-translational functional proteomics includes extraction and separation of proteins/peptides, their identification, and data analysis. The most common method used to determine proteins or peptides in proteomics is mass spectrometry. The strategy is fairly applicable in many spheres; however, it is limited by the great biochemical heterogeneity of proteins and impossibility of accurate determination of less common proteins [7].

During recent decades, proteomic methods with high output are developing and improving rapidly, which has considerably changed experimental approaches for food science. The interest in using genomics, proteomics, and metabolomics in the science of meat to obtain useful information about meat characteristics is growing. Proteomics takes an important part in life sciences, including agriculture, food and animal sciences to make safe food products of high quality and improve ecological rationality of livestock farming [3]. Such important parameters as meat composition, sensory quality, and nutritional value are responsible for meat quality and its acceptability for customers. Meat quality is closely related to animal biological characteristics. Meat quality parameters such as delicacy, water-binding capacity, fractional content, autolytic changes, and others are complicated multi-component indicators; hence they would be characterized in detail on the basis of experimental approaches and techniques aimed at parallel studies of genes and proteins simultaneously.

Proteomics is a promising approach to study mechanisms underlying the meat quality and the effect of meat on human health. Proteomics is aimed to identify molecular markers, usually named bio-markers, which allow earlier and more accurate diagnostics of diseases in medicine, for instance. Currently, bio-marker search is of importance since bio-markers may be used to improve the great number of characteristics in meat production and processing.

The aim of the research was the integration of existing knowledge and its practical application to identify the protein/peptide markers by means of proteomic and bioinformatic methods on the basis of their proteomic profiles which confirm or refute claimed properties of the meat product.

STUDY OBJECTS AND METHODS

Experimental studies were conducted at “Scientific and Methodological Works, Biological and Analytical Researches Laboratory” of “Gorbatov Research Center for Food Systems” in collaboration with the “Protein Research Laboratory” of the Federal Research Center of Biotechnology of the Russian Academy of Sciences.

Subjects of the study in identifying specific peptide markers were myofibrillar and sarcoplasmic proteins, as they are presented in meat in large amounts; thus, the method can be expected to be highly sensitive. Moreover, these proteins are completely soluble in buffer. For the experiment, average samples of longissimus dorsi from chilled pork and pork after 5 days of autolysis, as well as cooled beef and horse meat were used. Among the specific peptide markers for each animal species, the most sensitive peptides were selected from a protein database to develop the high performance liquid chromatography method (HPLC) in combination with tandem mass spectrometry.

For protein extraction, 300 g of each kind of meat – beef, pork, horse, and camel – finely chopped were mixed with 4 ml of the extraction buffer (0.3 M of KCl, 0.15 M of KH2PO4, and 0.15 M of KH2PO4 with pH 6.5) and kept for two hours at room temperature with constant stirring by shaking. Then the samples were subjected to centrifugation for 60 minutes at 12,000 g and 40°C. 100 µM of the supernatant was evaporated in a stream of nitrogen at 39°C and placed into 100 µM of 6 molar urea. After reduction with dithiothreitol (DTT) and alkylation with iodoacetamide (IA), cleavage process was running on exposure to trypsin for overnight at 37°C with slow stirring by shaking. The samples were then diluted with deionized water in a ratio of 1 : 2 and desalted by using Strata-X (30 mg). For this, 1 ml of a mixture consisting of 5% methanol and 1% formic acid was washed with water. For the elution of the peptide mixture, 1 ml of methyl cyanide/water (90 : 10; 0.1% formic acid) was used. The eluate was placed into Eppendorf tubes with 5 µm of dimethylsulphoxide (DMSO). Two-dimensional O’Farrell electrophoresis with isoelectrofocusing in ampholin (IEF-PAGE) and immobiline (IPG-PAGE) pH gradients and detection of proteins on two-dimensional electrophoregrams by staining with Coomassie R-250 and silver nitrate were used as main proteomic techniques [1, 13].

Protein fractions selected for the identification were cut out of gel plates obtained by means of two-dimensional electrophoresis. Gel sections were cut out, the protein was hydrolyzed with trypsin, and then tryptic peptides were extracted using time-of-flight mass spectrometry on the matrix (MALDI-TOF) in accordance with earlier published techniques [8, 9] with some modification [10]. The degree of extraction was determined by the identification of model proteins with known weight (PageRuler Protein Ladder, a mixture of 14 recombinant proteins with molecular weight from 10 to 200 kDa). The protein yield in the experiment was from 88 to 94%.

The study sample (0.5 µm) was mixed with the same volume of 20% methyl cyanide containing 0.1% of triflouracetic acid and 20 mg/ml of 2,5-dihydroxybenzoic acid (“Sigma”, USA) and subjected to air drying.

The mass spectrometric identification of the peptides was carried out after the separation of the mixture by HPLC method on the RP18 column by using MALDI-TOF MS and MS/MS spectrographic methods; for this, a MALDI-TOF time-of-flight mass spectrometer Ultrafllex (“Bruker”, Germany) with UV laser (336 nm) in the
positive ion mode within the weight range from 500 to 8,000 Da was used. The peptides were calibrated with known peaks of trypsin autolysis.

At least three MRM transitions (transitions in mode of multiple reaction monitoring) were selected for more accurate identification. Mass spectrometric parameters optimization was carried out from the study of peptide extracts or synthetic peptides. Various matrices were investigated to verify the specificity of the peptide markers found in the database and to eliminate false results; this is of importance because of the proteomic data Imperfection.

Proteins were identified by using peptide maps from the protein sequences database of NCBI (the National Center of Biotechnological Information) and the Mascot Software (http://www.matrixscience.com). Initial parameters of the search included one missed cleavage site in tryptic peptides, carbamidomethylation of cysteine, partial oxidation of methionine, and a mass-to-charge ratio discrepancy (m/z) about 25 ppm.

For benchmarking the proteomic profiles, modules of UniProtKB/the Swiss Institute of Bioinformatics and the database “Skeletal muscle proteomics” (http://mp.inbi.ras.ru) were used [11].

RESULTS AND DISCUSSION

The fractionation of the protein extractions from beef, pork, and horse longissimus samples by means of 2D-electrophoresis with isoelectrofocusing in ampholin pH gradient (IEF-PAGE) ensured a large number of protein fractions when staining with Coomassie R-250 [12]. The number of these fractions was determined automatically on digital images of two-dimensional electrophoregrams (2-DE) using ImageMaster 2D Platinum, version 7 (“GE Healthcare”, Switzerland). It should be noted that the total distribution of protein fractions detected was similar to that of muscle proteins in meat of earlier studied animals.

Fig. 1 demonstrates the similarity when comparing major fractions for tropomyosins and myosin light chains (MLC).

Proteins of contractile actomyosin complex, such as myosin light chains and tropomyosins, were selected as the most informative among species-specific proteins. Also, according to earlier experiments, enzymes which take a part in carbohydrate metabolism (glyceraldehydes 3-phosphate dehydrogenase, β-enolase) have been selected as markers. In addition to the listed proteins, myoglobins, actins, and several other proteins in horse meat have had high species specificity and have been detected well.

On the basis of data obtained in both present and previous experiments [13, 14], the system of species specificity (authenticity) of meat raw materials was suggested (described below). The system allows the presence of pork, beef, horse, and camel meat to be detected in both raw and heat-treated products if the content is 5% and more.

More detailed ways of identification are presented below. Thus, Fig. 2 shows 2-DE of average samples of pork longissimus and coupling.

![Fig. 1. Zones of tropomyosins and myosin light chains of M. Longissimus dorsi: (a) horse (Equus caballus); (b) pork (Sus scrofa); (c) beef (Bos taurus). The sections were cut out of gels whose decryption is presented in earlier published works [13, 14].](image)

![Fig. 2. 2-DE of average samples of pork longissimus and coupling.](image)
Multi-year proteomic researches of different species of meat raw materials (pork, beef, horse, and camel) as well as cooked meat products (sausages) demonstrate fractions which were identified as known tissue-specific proteins to present as major proteins: α- and β-tropomyosins (1), myosin light chains 1 MLC and MLC 2 (2 and 3), and myoglobin (4).

The number of muscle proteins was determined automatically on digital images of two-dimensional electrophoregrams (2-DE) using ImageMaster 2D Platinum, version 7 (“GE Healthcare”, Switzerland). The software finds stained spots and draws a contour over the stained area. The intermediate result of this analysis is represented in Figure 3.

Further, mathematical algorithms were used to determine a difference between light and dark pixels. Since the spots have different degree of staining and can blend into the background, the program sets three special parameters to identify protein fractions (spots). One of them (“Smooth”) enables the most marked spots identification and sharpness of the spot edge detection. The second parameter (“Saliency”) is used to identify weakly stained spots from the background and noise. “Min Area”, the third parameter, sets the minimum area of the spot; thus, all the spots with less area are not analyzed.

The next stage of the image processing includes the collection of information about spots and 3D models construction. The analysis involves such parameters as intensity, area, and volume of spots.

The intensity shows a degree of staining compared to the background; for this, the values of the most strongly stained pixels of a spot and the lightest area of the nearest background surrounding the spot are taken.

The spot area (in mm$^2$) is calculated from a mean value of the staining intensity. The spot edge determination in automatic mode includes some difficulties, therefore slightly larger area is assigned to the spots, and the index value is taken as 75% from the spot intensity. The area is expressed in mm$^2$.

The spot volume reflects the integrated optical density of the study fraction and is computed from the spot area magnitude which in turn is calculated strictly along the spot outline.

The final models represent sets of peaks; the higher intensity, the higher the peak and the larger the protein concentration in the fraction. The peak height is taken as 75% from the spot intensity [15].

Basic steps in the 3D model constructing are demonstrated in Fig. 4. The final result is shown in Fig. 5.

![Fig. 3. Intermediate result of images 2-DE analysis which detects stained fractions (spots) automatically.](image1)

![Fig. 4. 3D model construction algorithm of study image area. Arrows indicate a difference in calculation of intensity, area, and volume parameters (100*75).](image2)
Fig. 5. Zones of certain pork protein markers by computer densitometry techniques (a) α- and β-tropomyosins; (b) myosin light chains 1 MLC; (c) myosin light chains MLC 2; (d) myoglobin.

Thus, computer densitometry techniques are successfully applicable to use the species-specific protein markers in estimating a quantity and a type of protein in meat raw materials and meat products; correction factors can be used to take into consideration different protein content in raw materials.

On the basis of the data about the real proportion of meat raw materials in meat products produced according to standard formulations in study pork/beef samples (Fig. 6) and the confirmed species-specific bio-markers (through the example of myoglobin), the proper correction factor \( K_C \) was calculated. Its value turned out to equal 0.67 OD/C units to determine the major protein in pork (here, OD is the value of optical density of pork myoglobin fraction measured by means of computer densitometry, and C is the protein content in units). For beef myoglobin, \( K_C \) was determined and had the value of 1.5 OD/C units.

After correction of the results by using \( K_C \), a ratio of pork/beef was 56.3/43.7 for sample No. 1, 57.4/42.6 for sample No. 2, and 57.6/42.4 for sample No. 3 (in %).

Summarily, a ratio for the three samples was 57.1/42.9 that almost coincided with the results of the three other species-specific markers (Fig. 5).

The technique developed for the detection of species specificity of meat raw materials is highly useful to determine meat and beef in a volume ratio of 60 : 40.

Myoglobins of horse/beef/pork, for example, show discrete spots differing in Mm and pI that allows their presence on obtained elecrophoregrams to be determined visually but it is not easy to differentiate horse myoglobins from that of beef due to their high homology and similar electrophoretic characteristics. Therefore, we decided to use isoforms of β-enolase and muscle actin alpha instead of myosins as bio-markers in meat raw materials because of their higher species specificity, which means that they are detectable and identified well.

The data obtained in the present and previous experiments [16] were organized using bioinformatics, which has been highly useful for formulating an algorithm to identify the protein markers (Fig. 7) and expand the Atlas “Proteomic profiles of farm animal meat proteins”. Fig. 7 demonstrates all the detected species specificity protein markers in meat raw materials.
Fig. 7. Identification algorithm of species-specific proteins in raw materials of slaughter farm animals and poultry.
This investigation is also useful for meat-packing factories which use a variety of raw materials in single shift. The development of criteria for classifying product components as technologically inseparable impurities is a challenge with large-scale monitoring. First, undeclared components being contained in production must be shown on labels. Statements on the label “may contain insignificant amount…”, “contains trace amounts…”, and others do not give accurate criteria whether the declared component is technologically inseparable impurity or intentionally introduced into the product. This fact can lead to deliberate substitution of a small part of ingredients in the formulation (for example, the substitution of meat raw materials by vegetable protein, in particular, soya) without declaring the ingredient in the composition, but with the statement “may contain insignificant amount…”, “contains trace amounts…”, and others. Since the value of “insignificant amount” is not regulated by standards, dishonest manufacturers do not bear administrative liability.

The practical application of the developed algorithm in the future will involve work on comparative interpretation of the detected proteins which will be aim at finding deviations from technological formulas. Some aspects and primary results will be given in this paper.

To understand the autolysis process, it is necessary to know an effect of changes in muscle fiber and the cells of muscle fiber on muscle proteins. At the same time, it should be noted that these changes have been little investigated nowadays. Obviously, it is autolysis that leads to the breakdown of proteins – in particular, specific myofibrillar proteins and proteins of cytoskeleton, titin, and nebulin – and the formation of peptides. The protein breakdown begins in 6 hours after slaughter [17].

Nevertheless, it is not completely clear what factors cause the breakdown – own meat enzymes, or fragmentation as a result of amino acid modification chemically influenced [18], or direct hydrolysis caused by weak acid and acidic medium in muscles tissues.

The results of the identification (Table 1) showed that the most striking feature of autolysis is the formation of fast skeletal muscle troponin T fragments. The presence of ordinary troponin fraction caused the appearance and growth of three additional fragments with different Mm and pI. Increased amount of such protein fragments as piruvate kinase, α-enolase, creatine kinase M-type, glyceraldehyde-3-phosphate dehydrogenase, troponin I, adenylate kinase, alpha-cristallin, myosin light chains, and cofilin were observed.

Knowledge of protein changes during processing and the variability of the results will be of value in the improvement of treatment process. Numerous studies showed that variations in the rate of post-mortem glycolysis allow the meat of different delicacy to be obtained [19].

However, future studies are necessary for clearer understanding of complicated mechanisms of post-mortem changes.

**CONCLUSIONS**

In conclusion, the obtained results of proteomic studies of farm animals and poultry muscles is today a valuable contribution in developing highly sensitive techniques for the quality control of meat products based on analyses of muscle proteins species-specific isoforms.

**Table 1.** Results of mass spectrometric identification of pork M. Longissimus dorsi protein fractions on the fifth day of autolysis

<table>
<thead>
<tr>
<th>Protein name; some synonyms, (gene symbol)</th>
<th>Numbers Protein NCBI</th>
<th>Mm/pI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. pyruvate kinase PKM isoform X6 (PKM2)</td>
<td>545841009</td>
<td>58.0/6.80</td>
</tr>
<tr>
<td>2. alpha-enolase (ATP5A1)</td>
<td>927145216</td>
<td>52.0/5.80</td>
</tr>
<tr>
<td>3. creatine kinase M-type (CKM)</td>
<td>194018722</td>
<td>41.0/6.60</td>
</tr>
<tr>
<td>4. glyceraldehyde-3-phosphate dehydrogenase (GPDH)</td>
<td>329744642</td>
<td>34.0/7.30</td>
</tr>
<tr>
<td>5. glyceraldehyde-3-phosphate dehydrogenase (GPDH)</td>
<td>329744642</td>
<td>21.0/7.60</td>
</tr>
<tr>
<td>6. troponin T fast skeletal muscle type (TNTF)</td>
<td>46389777</td>
<td>28.0/7.90</td>
</tr>
<tr>
<td>7. troponin T, fast skeletal muscle (TNTF)</td>
<td>55741811</td>
<td>27.0/8.00</td>
</tr>
<tr>
<td>8. troponin T fast skeletal muscle type (TNTT3)</td>
<td>46389785</td>
<td>26.5/7.95</td>
</tr>
<tr>
<td>9. troponin I (TNI-F4)</td>
<td>190610684</td>
<td>20.0/7.60</td>
</tr>
<tr>
<td>10. troponin I (TNI-F4) +Acetyl(Protein N-term)</td>
<td>190610684</td>
<td>18.0/7.65</td>
</tr>
<tr>
<td>11. adenyylate kinase isoenzyme 1 isoform X2 (AK1)</td>
<td>350579686</td>
<td>21.0/6.70</td>
</tr>
<tr>
<td>12. alpha-cristallin B chain (CRYAB) +Acetyl (Protein N-term)</td>
<td>54584505</td>
<td>19.0/6.65</td>
</tr>
<tr>
<td>13. alpha-cristallin B chain (CRYAB) +Acetyl (Protein N-term)</td>
<td>54584505</td>
<td>17.0/6.40</td>
</tr>
<tr>
<td>14. MLC1f (MYL1)</td>
<td>117660874</td>
<td>20.0/4.80</td>
</tr>
<tr>
<td>15. myosin regulatory light chain 2, skeletal muscle isoform (HUMMLC2B)</td>
<td>54607195</td>
<td>15.0/4.65</td>
</tr>
<tr>
<td>16. cofilin-2 isoform 1 (CFL2)+Acetyl (Protein N-term)</td>
<td>NP 668733.1</td>
<td>16.0/6.35</td>
</tr>
</tbody>
</table>
In the beginning of the 21st century, proteomic and bioinformatic techniques had taken a significant part in protein biochemistry [6]. Accumulated results of numerous researches represent information arrays on the basis of which various common and specialized databases are formed and placed in the internet. Among them, the informational resource in the database UniProt called “Completeproteomes of Homosapiens” should be particularly noted. It included more than 70,000 papers by middle of 2012; however, only 25,000 (35.2%) of them represented the results of direct studies of the corresponding protein. Thus, it is evident that the majority of the papers (64.8%) require additional experimental data. Proteomic techniques and the development of bioinformatics resources can play a significant role here, which indicates high topicality of such researches.

Along with studying different aspects of protein polymorphism and other fundamental problems of muscle protein biochemistry, proteomic technologies have the tendency to be widely used in solving biomedical problems directed at a number of applied tasks – from detecting potential diagnostic protein polymers, targets for pharmacological interventions, to developing methods of quality control of various food products produced from animal muscle tissue [19].

The final stage in studying proteomic protein profiles for many scientists is the obtaining of two-dimensional electrophoregrams, as they do not have any considerations how to use modern instrumental and bioinformatic resources to confirm/refute their hypotheses or even just to identify.

The data obtained in the present and previous experiments have been organized, proteomic and bioinformatic methods of studying protein markers have been applied practically, and the identification algorithm of species-specific proteins in slaughter farm animals and poultry meat – which makes it possible to confirm the authenticity of raw materials – has been developed.

In future research, we are planning to carry out work on comparative interpretation of the identified proteins in order to apply in practice the developed foundations and the identification algorithm aimed at detecting deviations from technological formulas and predicting functional and technological properties of meat products. At present, these tasks are of importance for autolytic processes studying of raw materials under the conditions of changing animal genotype and forage base.

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DETERMINATION OF ENZYME INHIBITION AND ANTIOXIDANT ACTIVITY IN SOME CHESTNUT HONEYS

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Abstract: Honey has always been seen as the main source of healthy natural food and folk medicines. It has been prized due to bioactive components that are responsible for different therapeutic effects. Phenolic compounds are one of the parts of these components. It is claimed that these have been antioxidant agents. But it also has to be evaluated by different perspectives in biomechanics except antioxidative effects. A variety of diseases may be treated by the inhibition of some individual enzymes. A pharmaceutical drug and synthetic agents are used to treat and avert illness even though there is a potential risk named drug resistance. Nowadays, the most effective treatment seems to be the combined administration of natural foods. The study aims at investigating hyaluronidase (HYA), xanthine oxidase (XOD) and the urease enzyme inhibition of some chestnut honeys from different locations of Giresun and Ordu in Turkey. Moreover, the antioxidant activities of the prepared chestnut honey extracts were investigated by using different methods. The total phenolic (TP), total flavonoid (TF), FRAP, CUPRAC assays and DPPH, and ABTS inhibition potential were carried out using in vitro models. The enzyme IC_{50} values in the samples ranged from 0.793 to 12.639 mg/ml for HYA; from 0.029 to 0.106 g/ml for XO; from 0.002 to 0.054 g/ml for urease, respectively. In conclusion, honey extracts exhibited good potentials towards the inhibition of activities of the studied enzymes, and the samples also suggest a practical value for surveying natural inhibitors for specific clinical purposes. Moreover, all results can provide a basis of future studies on the alternative medicinal application related to honey.

Keywords: Chestnut honey; hyaluronidase; xanthine oxidase; urease; inhibition; antioxidant


INTRODUCTION

Honey is valuable functional food that provides an important part of the energy needed by a body cell and also known as a traditional medicine source [1]. Its content is changeable and depends on a lot of factors, such as the botanical (floral or vegetable) and geographical (regional or territorial) origins and species of bee [2], although it consists of approximately 75–80% of carbohydrates among which there are such principal constituents as fructose and glucose, 17–20% of water and 1–2% of other substances [3].

Honey is commercially available and varies greatly in quality all over the world. It is largely assessed on the basis of some physico- and bio-chemical parameters such as color, moisture, HMF, diastase activity, proline, acidity, electrical conductivity, phenolic contents, and some enzyme inhibition sources are a strong indicator for the prediction of honey quality [4]. Although it is known that these quality parameters can vary only due to the botanical and geographical origins of honey, other factors are also important, including the climate, environmental conditions and processing that honey has undergone [5].

Turkey, due to its geographical location, has a wide scale relative to monofloral honeys. Chestnut honey, having high bioactivity, is part of monofloral honey. This honey with a light bitter taste is offered in the market and is often produced especially by the eastern Black Sea region beekeepers and it is seen as a source of alternative medicine for local people. It is claimed that the regular consumption of these bee products might contribute to a reduction in several forms of ROS-mediated pathological injury, notwithstanding the geographical origin of the honey [6]. Antioxidant studies can create the basis of these claims.

Enzyme inhibitors are mainly bioactive secondary metabolites that bind to an enzyme and decrease its bioactivity and catalytic activity. Moreover, blocking enzyme activity can kill a pathogen or correct a metabolic imbalance [7]. Although a lot of synthetic and chemical products are used as useful inhibitors, natural products become popular for enzyme inhibition. Since the resistance of synthetic and chemical drugs has become a major clinical and public health problem. Natural enzyme inhibitors are often mediated by its specificity and its effectiveness that designated the absorption desirable to inhibit the enzyme [7]. Honey acts as natural enzyme inhibitors. Hyaluronidase (HYA), xanthine oxidase (XOD) and urease inhibitors are especially one of them.

Hyaluronic acid (HA) is a natural biopolymer that is responsible for some biological progress (synovial fluid, eye vitreous fluid etc.) in bacteria and higher animal metabolism. β-D-glucuronidase, β-N-acetyl-
hexosaminidase and especially hyaluronidases play an important part during the degradation of this natural polymer [8, 9]. Moreover, the HA substrate has to keep on metabolic control with HYAs which could change tissue permeability by accelerating their dispersion and delivery [3].

Xanthine oxidase (XOD, EC 1.17.3.2) catalyses the oxidation of xanthine or hypoxanthine to uric acid, and also creates a superoxide radical formed during the metabolic process [10]. This radical has to be neutralized by antioxidant systems and inhibitors which slow down or stop the effect of the level of the enzyme. There are some known inhibitor chemicals to inhibit XOD. Allopurinol is one of these inhibitor chemicals that can cause some negative effect, such as hepatitis, nephropathy, hypersensitivity etc. [10].

Urease (urea amidohydrolase, E.C.3.5.1.5) is a nickel containing metallo-enzyme found in plants, bacteria, fungi and soil. This enzyme is used by plants to break down urea into carbon dioxide and ammonia. The indophenols method was used to determine urease activity by measuring ammonia production [11]. Besides these given inhibitors, some natural products can act as broad-based urease inhibitors due to their bioactive contents [6].

Finally, the quality of chestnut honey is related to its bioactive levels. Some geological, geochemical, seasonal, floral source and climatic parameters are responsible for the bioactive levels such as antioxidant values and enzyme inhibition concentrations. There have recently been a lot of considerable studies on the biological activity of chestnut honey. But the work on enzymatic inhibition is also extremely limited. For this reason, the aim of this study was to investigate hyaluronidase, xanthine oxidase and urease enzyme inhibition of ten chestnut honeys from Giresun and Ordu and also to determine their antioxidant properties. Hence, the research approaches and findings presented in this paper would be useful for investigating new alternative sources, such as bee products.

**STUDY OBJECTS AND METHODS**

**Chemicals and instruments.** All chemicals were of an analytical grade and were used as received without further purification. All chemicals were also purchased from Sigma Chemical Co. (St. Louis, MO, USA). *In vitro* spectrophotometric measurements were performed by using a Mapada UV-6100 PCS spectrophotometer (Shanghai Mapada Instruments Co., China).

**Melissopalynological characteristics of floral honey.** Botanical origin denomination with melissopalynological analysis is necessary for getting information about discriminatory abilities of honeys. For this reason, the samples were collected by experienced beekeepers during the harvest season of 2015 and were handled by melissopalynological analysis for sample tagging. The method was described by Louveaux et al. (1978) and the procedure progress was the same with Nair et al. (2013). For analysis, approximately 10 g of honey were dissolved in 20 ml of distilled water. This mixture was divided into two centrifuge tubes of 15 ml and centrifuged for about 5 min at 3000 rpm. Distilled water was added to the sediment again repeating the previous operation. Approximately 5 ml of glycerine-water 1 : 1 were added to the sediment and it was left for 30 min. After this time, the sample was centrifuged. The sediment was removed with the aid of a stylet embedded in glycerine jelly and deposited on a microscopic slide sealed with paraffin wax [13]. For pollen identification, sample materials were microscopically observed and compared with the reference slide. The frequency classes of pollen grains were attributed as predominant pollen (more than 45%). The percentage of chestnut (*Castanea sativa* Mill.) pollen from the samples ranged from 78% to 90%. Table 1 presents pollen analyses and honey properties.

**Sample preparation.** Approximately 10 g of the honey sample was extracted with 50 ml of distilled water in a flask attached to a condenser at 60°C for more than 6 h. The extract was subsequently filtered to remove particles, and the final volume was adjusted with distilled water.

**Enzyme inhibition assays.** All the enzyme inhibition assays were done in triplicate and given as IC_{50} values, were determined as the concentration of a compound that provides 50% of inhibition of maximal activity. IC_{50} was determined graphically from the inhibition curves by plotting enzyme activity against sample extract concentrations that were known as a natural inhibitor.

**Hyaluronidase (HYA) inhibition.** The slightly modified Sigma protocol was fixed for determining HYA inhibition activity [14]. Briefly, the reaction mixture consisted of 100 ml of hyaluronidase (1.67 U/mg), 100 ml of a phosphate buffer (200 mM, pH 7, 37°C) with 77 mM sodium chloride and 0.01% BSA mixed with 25 ml of a sample extract solution. After pre-incubation at 37°C for 10 min, the reaction was initiated by the addition 100 ml of a substrate solution in the form of hyaluronic acid (0.03% in 300 mM sodium phosphate, pH 5.35). The assay mixture was incubated at 37°C for 45 min. The undigested hyaluronic acid was precipitated with 1 ml of an acid albumin solution made up of 0.1% bovine serum albumin in 24 mM sodium acetate and 79 mM acetic acid, pH is 3.75. After leaving the mixture at room temperature for 10 min, the absorbance was measured at 600 nm using a Mapada UV-6100 PCS spectrophotometer (Shanghai Mapada Instruments Co., China).

Table 1. Sample codes, origin and microscopic pollen analysis results

<table>
<thead>
<tr>
<th>Codes</th>
<th>Collection Region</th>
<th>Dominant Pollen Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Giresun/ Center</td>
<td>Chestnut 83%</td>
</tr>
<tr>
<td>H2</td>
<td>Giresun/ Görele</td>
<td>Chestnut 85%</td>
</tr>
<tr>
<td>H3</td>
<td>Giresun/ Keşap</td>
<td>Chestnut 90%</td>
</tr>
<tr>
<td>H4</td>
<td>Giresun/ Tirebolu</td>
<td>Chestnut 84%</td>
</tr>
<tr>
<td>H5</td>
<td>Giresun/ Center</td>
<td>Chestnut 81%</td>
</tr>
<tr>
<td>H6</td>
<td>Ordu/ Gülüyağı</td>
<td>Chestnut 80%</td>
</tr>
<tr>
<td>H7</td>
<td>Ordu/ Kabadüz</td>
<td>Chestnut 78%</td>
</tr>
<tr>
<td>H8</td>
<td>Ordu/ Orençik</td>
<td>Chestnut 83%</td>
</tr>
<tr>
<td>H9</td>
<td>Ordu/ Çambaşı</td>
<td>Chestnut 82%</td>
</tr>
<tr>
<td>H10</td>
<td>Ordu/ Center</td>
<td>Chestnut 84%</td>
</tr>
</tbody>
</table>
Xanthine oxidase (XOD) inhibition. The xanthine oxidase inhibitory activity was measured using the method by Ryu et al. (2012) with slight modifications. The assay mixture of XO inhibitory activity consisted of 0.5 ml of the test compound, 0.77 ml of a phosphate buffer (pH is 7.8) and 0.07 ml of bovine milk XO (Sigma–Aldrich, St. Louis, USA), which was prepared immediately before use. After pre-incubation at 25°C for 15 min, the reaction was initiated by the addition of 0.66 ml of a substrate solution into the mixture. The assay mixture was incubated at 25°C for 30 min. The reaction was stopped by the addition of 0.2 ml of 0.5 N HCl and the absorbance was measured at 295 nm.

Urease inhibition. The urease assay was explained step by step. The reaction mixtures including 100 μl of Jack Bean Urease, 400 μl of a buffer (100 mM urea, 0.01 M K2HPO4, 1 mM EDTA and 0.01 M LiCl, pH is 8.2) and 500 μl of the honey extract were incubated at room temperature for 15 min. 500 μl of a phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 500 μl of an alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each tube. The increasing absorbance at 625 nm was measured after 50 min using a UV/vis spectrophotometer [6].

**Determination of antioxidant capacity.**

Total phenolic content (TPC) assay. The total phenolic content (TPC) of honey extracts was analyzed using the Folin–Ciocalteau assay [15]. To this end, 680 ml of distilled water, 20 ml of aqueous extracts and 400 ml of 0.2 N Folin–Ciocalteau were mixed and then vortexed. After 2 min, 400 ml of Na2CO3 (7.5%) was added and the mixture was incubated for 2 h at room temperature. After incubation in the dark, the absorbance at 760 nm was measured before distilled water. The concentration of TPCs was calculated as mg of gallic acid equivalents (GAE) per 100 g of a sample, using a calibration curve determined using gallic acid standard solutions.

Total flavonoid content (TF) assay. The flavonoid compounds in honey samples were determined using the spectrophotometric method [16] and expressed as mg QE (Quercetin Equivalents)/100 g honey. Regarding this method, the extracted solutions were prepared in a different concentration (0.5 ml) and mixed with 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 mol/l potassium acetate, and 4.3 ml of 80% ethyl alcohol. The samples were kept at room temperature for 40 min and the absorbance was measured at 415 nm.

Ferric reducing antioxidant power (FRAP) assay. The ferric reducing antioxidant power is based on the reduction of the Fe3+-TPTZ complex under acidic conditions. Regarding this method, the increase in absorbance of a blue-colored ferrous form (Fe2+-TPTZ complex) is measured at 593 nm [17]. The working FRAP reagent was prepared as required by mixing 25 ml of 0.3 M acetate buffer at pH equal to 3.6 with 2.5 ml of a 10 mM FeCl3 × 6H2O solution. An amount of 100 μl of the sample was mixed with 3 ml of a freshly prepared FRAP reagent. Then, the reaction mixture was incubated at 37°C for 4 min. A calibration curve was applied using an aqueous solution of ferrous sulfate FeSO4 × 7H2O.

Cupric reducing antioxidant capacity (CUPRAC) assay. The cupric reducing antioxidant capacity (CUPRAC) of the honey extracts was determined using the method of Apak et al. (2004). Trolox was also tested under the same conditions for a standard calibration curve. 1 ml of a CuCl2 solution (0.01 M), 1 ml of a neocuproine ethanolic solution (0.0075 M) and 1 ml of an NH4-acetate buffer solution were added to a test tube and mixed; (x) ml of the sample extract followed by (1.1 – x) ml of water were added (the total volume = 4.1 ml) and shaken well. The absorbance against a reagent blank was measured at 450 nm after 30 min. CUPRAC values were expressed as a mmol Trolox equivalent per 100 g of the sample.

DPPH– and ABTS•+ radical scavenging activity assays. The DPPH• and ABTS•+ assay was based on the method of Brand-Williams et al. (1995) and van den Berg et al. (1999), respectively. For the DPPH method, various concentrations (0.75 ml) of compound extracts were mixed with 0.75 ml of 0.1 mM DPPH in methanol.

In addition, for the ABTS method, the stock solutions included 7 mM ABTS and 2.4 mM potassium persulfate. This stock was diluted by mixing 1 ml of an ABTS (7 mM) solution with methanol to obtain the absorbance of 0.700 ± 0.001 units at 734 nm using a spectrophotometer. 20 ml of the honey extract and 2 ml of the diluting ABTS solution volume were mixed and incubated to react for 12 h at room temperature under dark conditions.

All the results of these assays were compared to each other and were expressed as SC50, the concentration of the samples causes 50% of the scavenging of a relevant radical.

Statistical analysis. All the tests were repeated in triplicate and the data were expressed as the mean ± standard deviation. All the calculations were performed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA). P < 0.01 was considered as indicative of significance as compared to each group.

**RESULTS AND DISCUSSION**

Results. In this study, in vitro inhibition results of chestnut honey based on hyaluronidase, xanthine oxidase and urease enzymes were primarily clarified. According to its content and enzyme mechanisms, honeys have been shown to have different inhibitory effects. All chestnut type honey extracts were shown to inhibit three enzymes according to IC50 values and varying concentrations (Table 2). The inhibition values determined in chestnut honeys were hyaluronidase inhibition values from 0.793 to 12.639 mg/ml, xanthine oxidase from 0.029 to 0.106 g/ml and urease from 0.002 to 0.054 g/ml. The highest inhibitory activity of urease, hyaluronidase and xanthine oxidase was determined in H2 (0.793 mg/ml), H3 (0.029 g/ml) and H9 (0.002 g/ml) tagged chestnut honeys, respectively. While the lowest urease inhibitory activity was found H5, the lowest hyaluronidase and xanthine oxidase inhibitory activity was found in H9. While a positive correlation (R2 = 0.886) between hyaluronidase and xanthine oxidase enzyme inhibition was detected, negative weak correlation (R2 = 0.473) was seen between hyaluronidase and urease enzyme inhibition (Table 3).
### Table 2. IC<sub>50</sub> values of each enzyme and antioxidant properties of honey samples*

<table>
<thead>
<tr>
<th>Code</th>
<th>HYA</th>
<th>XO</th>
<th>Urease</th>
<th>TP</th>
<th>TF</th>
<th>FRAP</th>
<th>CUPRAC</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
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<td></td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/ml)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (g/ml)</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (g/ml)</td>
<td>mgGAE/100g</td>
<td>mgQE/100g</td>
<td>µmol FeSO&lt;sub&gt;4&lt;/sub&gt;·7H&lt;sub&gt;2&lt;/sub&gt;O/100g</td>
<td>mmol Trolox/100g</td>
<td>SC&lt;sub&gt;50&lt;/sub&gt; (mg/ml)</td>
<td>SC&lt;sub&gt;50&lt;/sub&gt; (g/ml)</td>
</tr>
<tr>
<td>H1</td>
<td>0.978 ± 0.036</td>
<td>0.047 ± 0.003</td>
<td>0.030 ± 0.001</td>
<td>111.746 ± 1.211</td>
<td>3.363 ± 0.122</td>
<td>82.223 ± 3.000</td>
<td>90.333 ± 0.493</td>
<td>25.422 ± 0.047</td>
<td>0.153 ± 0.003</td>
</tr>
<tr>
<td>H2</td>
<td>0.793 ± 0.019</td>
<td>0.032 ± 0.005</td>
<td>0.026 ± 0.001</td>
<td>78.703 ± 1.724</td>
<td>3.324 ± 0.029</td>
<td>61.895 ± 0.995</td>
<td>71.200 ± 0.700</td>
<td>22.026 ± 0.650</td>
<td>0.131 ± 0.006</td>
</tr>
<tr>
<td>H3</td>
<td>1.347 ± 0.043</td>
<td>0.029 ± 0.002</td>
<td>0.031 ± 0.001</td>
<td>146.804 ± 2.935</td>
<td>4.391 ± 0.122</td>
<td>89.256 ± 2.631</td>
<td>97.067 ± 0.603</td>
<td>19.344 ± 0.082</td>
<td>0.100 ± 0.002</td>
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<tr>
<td>H4</td>
<td>1.022 ± 0.104</td>
<td>0.042 ± 0.002</td>
<td>0.032 ± 0.004</td>
<td>109.740 ± 1.952</td>
<td>3.578 ± 0.140</td>
<td>63.877 ± 2.935</td>
<td>78.667 ± 0.666</td>
<td>25.052 ± 0.046</td>
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<tr>
<td>H5</td>
<td>8.398 ± 0.952</td>
<td>0.050 ± 0.003</td>
<td>0.054 ± 0.000</td>
<td>90.528 ± 0.946</td>
<td>3.049 ± 0.169</td>
<td>67.840 ± 3.812</td>
<td>80.300 ± 0.436</td>
<td>25.950 ± 0.123</td>
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<td>0.005 ± 0.000</td>
<td>65.591 ± 5.302</td>
<td>1.786 ± 0.055</td>
<td>58.667 ± 2.065</td>
<td>65.000 ± 0.656</td>
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<td>0.261 ± 0.006</td>
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<td>H7</td>
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<td>0.091 ± 0.001</td>
<td>0.008 ± 0.000</td>
<td>19.280 ± 1.181</td>
<td>1.495 ± 0.058</td>
<td>9.767 ± 0.907</td>
<td>11.000 ± 0.755</td>
<td>79.054 ± 0.903</td>
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<td>H8</td>
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<td>1.820 ± 0.135</td>
<td>9.933 ± 0.666</td>
<td>11.667 ± 0.416</td>
<td>88.221 ± 2.207</td>
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<td>H9</td>
<td>9.035 ± 0.193</td>
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<td>59.333 ± 1.955</td>
<td>67.167 ± 0.814</td>
<td>30.095 ± 0.086</td>
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<td>H10</td>
<td>11.340 ± 0.118</td>
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<td>0.018 ± 0.001</td>
<td>26.724 ± 1.214</td>
<td>1.887 ± 0.088</td>
<td>17.767 ± 1.060</td>
<td>30.533 ± 0.666</td>
<td>83.358 ± 1.309</td>
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*Note.* In all the results given, the analyses were performed in triplicate and given as ± standard deviation.

### Table 3. Correlation coefficients of each analysis

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<th>Urease</th>
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<th>TF</th>
<th>FRAP</th>
<th>CUPRAC</th>
<th>DPPH</th>
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<td>-.859(**)</td>
<td>-.896(**)</td>
<td>-.806(**)</td>
<td>-.815(**)</td>
<td>.838(**)</td>
<td>.855(**)</td>
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<td>-.916(**)</td>
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<td>.900(**)</td>
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<td>.971(**)</td>
<td>-.510(**)</td>
<td>-.892(**)</td>
<td>-.816(**)</td>
<td>-.931(**)</td>
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<td>ABTS</td>
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<td>.984(**)</td>
<td>-.487(**)</td>
<td>-.907(**)</td>
<td>-.827(**)</td>
<td>-.934(**)</td>
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**Note.** **The correlation is significant at a 0.01 level (2-tailed).**
The polyphenolic contents of the samples were evaluated in two different ways: total phenolic contents (TPC) and total flavonoids (TF). Total phenolic contents of the honeys ranged from 19.222 to 146.804 mg GAE/100 g (Table 2). The phenolic contents were the highest in H3 honey, while H1 honey and H4 honey exhibited the highest levels according to the others. The lowest phenolic content was found in H7 and H8 samples (19.222 and 19.304 mg GAE/100 g). The total flavonoids contents of the ten honeys ranged from 1.565 to 4.431 mg QE/100 g. The highest and the lowest values were found in H3 and H7 honey, respectively. The results were shown in Table 2.

In electron transfer reaction-based methods, when the oxidant is reduced, it changes color, and the degree of a color change is related to antioxidant capacity. Cu (II) ion reducing antioxidant capacity (CUPRAC) and iron (III) Reduction / Antioxidant Power (FRAP) of mainly ET-based methods were applied for our samples. When the results ranged from 9.8 to 89.3 µmol of a FeSO4 × 7H2O/100 g sample in a FRAP assay, the results for the CUPRAC method were 11.0–97.1 mmol of a Trolox/100 g sample within the range (Table 2). While the highest antioxidant activities of honey samples in both methods were found in H3 and H1 samples, the lowest activities were in H7 and H8 honey samples.

ABTS and DPPH radical scavenging methods are commonly used to measure a free radical scavenging ability in various natural products. The SC50 value was determined as the concentration of a compound that gives 50% of inhibition of the maximal activity and the low SC50 value indicates that the sample showed high radical scavenging activity. In the results of the DPPH and ABTS radical scavenging method, the SC50 values of ten chestnut honeys ranged from 19.344 to 88.221 and from 0.10 to 0.43 mg/ml (Table 2). According to both methods, while the highest radical scavenging activity was observed in H3, lower results were in H8. In all the antioxidant tests, the high linear correlation coefficient was determined using the same methodological methods: ABTS-DPPH (R² = 0.984), FRAP-CUPRAC (R² = 0.990), and TP-TF (R² = 0.919) (Table 3).

When comparing enzyme inhibition and antioxidant activity, a negative correlation was found between the inhibition of xanthine oxidase and total phenolic (R² = -0.899), total flavonoids (R² = -0.860), FRAP (R² = -0.913) and CUPRAC (R² = -0.916), while using DPPH (R² = 0.971) and ABTS (R² = 0.984) a positive correlation was determined (Table 3). A negative correlation was between the inhibition of hyaluronidase and total phenolics (R² = -0.859) and total flavonoids (R² = -0.919) and FRAP (R² = -0.806) and CUPRAC (R² = -0.815). A positive correlation was also detected between DPPH (R² = 0.838) and ABTS (R² = 0.855) (Table 3). No correlation was found between the inhibition of the urease enzyme and the antioxidant tests.

Discussion. Besides the nutritional value of honey owned, it has significantly stood out in recent years to have a biological potential. Honey is a powerful antioxidant source with a rich phenolic content. It has antimicrobial, antibacterial, anti-inflammatory, anticancer and antiviral effects as well. These effects are affected by several factors, such as the floral source involved and seasonal, geographical and environmental conditions. Based on this reality, when compared with the studies in the literature it is sometimes seen as the same type of honey with a higher or lower activity. There are some available studies in the literature on chestnut, forest rose, heather, oak and flower honey. Chestnut honey used in the study is believed to be good ethno-medicine for asthma, respiratory disease and cancer [21]. In vitro and in vivo studies of health effects of chestnut honey showed that it is not only asthma, respiratory disease and cancer, but also chestnut honey that can be consulted for different health problems. De Vasconcelos et al. (2010) reported that it was used to dress chronic wounds, burns or skin ulcers due to its antibacterial activity. Alvarez-Suarez et al. (2012) studied chestnut honey for proving its cellular effect and the results showed that chestnut honey had a strong antioxidant activity and it might provide defense and promote cell functions in erythrocytes. Choi et al.’s (2012) study concerned accelerating wound healing and promoting early HO-1 protein expression in mice with chestnut honey. And these findings also indicate that chestnut honey can promote wound healing in diabetics with early HO-1 protein expression. Although it could be seen in different studies on chestnut honey that the investigation of some enzyme inhibition degrees could be evaluated as limited.

Although enzyme inhibitor activities in natural products are quite extensive when compared to other enzyme studies, their biological values have not been completely clarified. Nowadays, pharmaceutical searches focus on enzyme inhibition studies because these studies have led to the discoveries of drugs useful in a variety of physiological conditions. Enzyme inhibitors are molecules that interact in some way with an enzyme to block their activity towards natural substrates. Urease is a metalloenzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia, its inhibitors have recently attracted great attention as potentially new anti-ulcer drugs [25]. Urease, providing an opportunity for bacteria to live at low pH in the stomach that can result in cancer plays a role in gastritis and peptic ulcer pathogenesis [6]. Urease inhibitor studies are performed for the therapy of the diseases caused by bacteria. Its inhibition is very important for the treatment of Helicobacter pylori related diseases. If that would inhibit urease, an inhibitor has been shown to inhibit an antiulcerative effect. As Helicobacter pylori cannot live in an acidic medium resulting from urease inhibition, this fact is the evidence that urease inhibition is antibacterial. In this sense, hydroxamic acids are known as the best inhibitors of the urease enzyme. The discovery of urease natural inhibitors will be important for alternative medicine for the treatment of diseases such as gastric ulcer. All ten chestnut honeys in the study inhibited urease in a manner dependent on varying IC50 values and concentrations (Table 1). Sahin (2016) found the IC50 of urease inhibition of chestnuts of a different origin between 0.010 and 0.034 g/ml.

Another important enzyme is hyaluronidase in the metabolic system. It is associated with a lot of pathological diseases and its inhibitors show an anti-inflammatory, anti-allergic, anti-tumor, anti-aging, anti-
rheumatoid, anti-toxin and antimicrobial effect [26, 27]. Hyaluronidase fragments are reported to be associated with cancer [26]. In addition, HA inhibitors have led to new therapeutic concepts for the treatment of throat and breast cancer associated with the hyaluronan-hyaluronidase system in pathophysiological conditions [26, 28]. There was an aspect of the study by Isoyama et al. (2005) that inhibitors of hyaluronidase (HAase) might be useful as contraceptives, because they inhibit the acrosomal reaction initiated by testicular HAase. Therefore, the synthetic and natural inhibitors of hyaluronidase have recently attracted the attention of researchers [27]. In the literature, there are few studies about the effect of honeys on hyaluronidase inhibition. Kolayli et al. (2016) found that oak, heather and chestnut honeys have the highest anti-hyaluronidase activity. The chestnuts used in the present study showed a low IC50 value (inhibitory high).

In this study, the third enzyme used to examine inhibition effects of chestnut honey extracts was xanthine oxidase that is responsible for oxidative damage that causes a lot of pathological diseases such as gout, hyperuricemia, hepatitis, carcinogenesis and aging [30]. Xanthine oxidase regulation is an important means in the prevention of a lot of diseases. In the previous studies, Boumerfeg et al. (2012) investigated the antioxidant and radical scavenging effects of the Teucrium polium and its active fractions by applying various established in vitro xanthine oxidase inhibition assays. The study of natural compounds clearly indicated that Teucrium polium was a potent scavenger of O2•. So, it could prevent the formation of ROS. Another study from Sowndhararajan et al. (2012) was carried out to evaluate the xanthine oxidase inhibitory potential of a methanol extract of Erythrina indica Lam. leaves and stem bark. The obtained results of that study showed that Erythrina indica stem bark exhibited a good XO inhibitory activity and therefore may contain bioactive constituents useful in the treatment of XO induced diseases. Besides these natural products, there were some details about bee products for xanthine oxidase inhibition in literature, too. The enzyme inhibitor potential of the respective bee products was estimated by Sahin (2016), it was designed for a comparative study on the enzyme inhibitors of some bee products. Sahin (2016) viewed the effects on the inhibition of xanthine oxidase of honey and the highest inhibitory effect was detected in chestnut honey. In this study, all the samples depending on a different floral effect showed the significant inhibition of xanthine oxidase. As a result, honey is an important inhibitor depending on a floral source with the degree of inhibition against urease, hyaluronidase and xanthine oxidase enzymes. Regular honey consumption contributes to a reduction in inflammatory injuries and strengthening the human immune system.

The molecular diversity of antioxidant substances in a sample can always interfere with the formation of a linear relationship between the results obtained with the applied methods. It is also apparent from the results of the literature: it is not appropriate to provide information about the antioxidant capacity of a sample with a single antioxidant method. In other words, there is not an antioxidant method that detects all antioxidants as a radical source, the antioxidant capacity was examined using different methods. It has also been found that there is a correlation among the methods used. Thus, each method used bridges the gap of other method’s methodologies.

It is worth mentioning that honey contains a lot of biologically active components such as polyphenols, vitamin C, organic acids, catalase, glucose oxidase, amino acids and proteins that may react with reactive oxygen species (ROS). Some studies have also proposed that these compounds may help to slow down aging due to antioxidant abilities [6, 33, 34]. Especially, the phenolic contents of honeys are directly responsible for their antioxidant degree, so they can be evaluated as natural and therapeutically products. There were a great many related studies in this area to support this reality. The phenolic content of monofloral honey of the flora of Turkey is within the range of 16–78 mg GAE/100 g [33], the total phenolic content of chestnut honey Turkey ranges from 19.05 to 108.21 mg GAE/100 g [34] and the phenolic content of chestnut honey in Black Sea was 38.90–65.30 mg GAE/100 g in a sample [6]. The average polyphenol content was 14.67 mg GAE/100 g in a sample of a different botanical origin in Southern Italy [35], while in this content from Croatia chestnuts was within the range of 18–29.2 mg GAE/100 g in a sample [36]. The total amount of phenolic chestnuts in the present study (19.2–146.8 mg GAE/100 g in a sample) is more than literature. H3 chestnut honey has the highest phenolic content in all honey samples. When compared to other studies, they reported that there was a correlation between the amount of total phenolic and biological activity of honey [33, 37].

Antioxidant, antimutagenic and free radical scavenging activities are generally found to increase with flavonoids that are parts of phenolic compounds [38]. The total flavonoid assay was used for the determination of a cumulative flavonoid ingredient in honey samples. In addition, the total flavonoid contents of the analyzed honeys were comparable to the previously reported values. Kolayli et al. (2016) reported that the total flavonoid contents in chestnut honeys in Turkey were within the range of 6.0–7.6 mg QE/100 g in a sample. In another study a high average of the flavonoid content of chestnut honeys was provided as 10.8 mg QE/100 g in a sample [33]. Perna et al. (2012) found the total amount of flavonoids in a chestnut of a different botanical origin in Italy as 7.92 mg QE/100 g in honey. In our study, the total flavonoids ranging from 1.5 to 4.4 mg QE/100 g in the sample obtained from Giresun and Ordu seems to be between the values given in the previous studies.

On the basis of the chemical reactions involved, the total antioxidant capacity assays can also be grouped into two categories: hydrogen atom transfer (HAT) based methods and single electron transfer (SET) based methods. FRAP and CUPRAC are part of SET-based methods and detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals. The FRAP method measures the ability of reduction to Fe3+ from Fe2+ in antioxidants [39]. All the studied chestnut honey samples were found to have a reduced ability to
ferric iron (9.8 ± 1.3 – 89.3 ± 1.4 μmol Fe (II)/100g in a sample) (Table 1). Sahin (2016) also revealed the antioxidant capacity of chestnut honey using the FRAP method that it was within the range from 3.111 ± 0.078 to 4.690 ± 0.094 μmol Fe (II)/g in a sample. In addition, Can et al. (2015) found 4.30 ± 0.13 μmol Fe (II)/g in a sample. According to the obtained results, the antioxidant capacity of honey largely depends on the botanical origin of honey and phenolic compounds. In this study, the total phenolic and flavonoid contents showed as a result of a FRAP test a positive correlation as 0.948 and 0.805, respectively. Thereby, a higher polyphenolic content indicates a higher reduction in ferric iron. One of the methods in the total antioxidant capacity is a CUPRAC test based on an electron transfer which is a method for measuring the ability of reduction to Cu^+ from Cu^{2+} in antioxidants. High CUPRAC value indicates a high antioxidant capacity. All the honey samples in the present study had a high CUPRAC value (11.0–97.1 mmol Trolox/100 g). There were a few studies on the determination of antioxidant activity using the CUPRAC method. Kaygusuz et al. (2016) found a CUPRAC value of 23.8 ± 1.5 to 17.18 ± 1.52 μmol Trolox/g in a sample from chestnut honey with the origin of Trabzon. A high positive correlation value (R^2 = 0.990) was identified between FRAP and CUPRAC antioxidant activities. That was normal because it was known that these methods were based on nearly the same redox hypothesis. The DPPH and ABTS radical scavenging methods are commonly used to measure the free radical scavenging ability of a lot of natural products [41]. When comparing these radical scavenging activity assays, it was found that they have some different advantages and disadvantages. According to Sahah and Modi (2015), DPPH is known as stable and commercially available organic nitrogen radicals, is widely reported methods for the determination of antioxidant activity. It can also be preferred for its operational simplicity in the preparation of chemicals and a short incubation time. In addition, Sahah and Modi (2015) mentioned about the ABTS radical scavenging activity method widely reported for the measurement of antioxidant activity. Despite its frequent use, the assay has some drawbacks such as forming an unstable radical and calculating non-reproducible results. In each method, the SC_{50} value was defined as the concentration of a compound that gives 50% of inhibition of the maximal activity and the low SC_{50} value indicates that the sample showed a high radical scavenging activity. DPPH (19.344–88.221 mg/ml) and ABTS (0.10–0.43 mg/ml) results in the present study demonstrated that all honeys had an antioxidant activity. Since the reaction conditions used by other authors in the literature were different, it was difficult to make direct comparisons between the previous study data and the ABTS and DPPH values in the current study. However, we could say that our study results were compatible with the results presented by other authors [1, 21, 25]. Between the DPPH and ABTS radical scavenging methods, a positive correlation with the value R^2 = 0.984 was detected. In addition, the negative correlation between the total phenolic contents as a result of DPPH and ABTS tests, respectively (R^2 = –0.892 and R^2 = –0.907), indicates that a high phenolic content indicates a high antioxidant capacity. There was a correlation between all the antioxidant tests used in this study.

In conclusion, a correlation between the anti-oxidative effects and the degree of some enzyme inhibitions based on the biochemical and nutritional substantiality of chestnut honeys can be evaluated as the evidence. As is known, the factors that affect the chestnut honey composition lead to the correlation of bioactive properties as some enzyme inhibitions and anti-oxidation degrees. The current study results claimed that all the honey samples that had the anti-oxidative levels and different inhibition concentrations of hyaluronidase, xanthine oxidase and urease, are beneficial for human consumption. Their richness of bioactive properties was found to be relatively moderate among the honey samples collected in different regions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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In the production crystallization of glucose, there are special problems at the nucleation stage, which requires seed crystals. The need for them reaches 10–15% of the weight of the solution, reducing the productivity of equipment. In this paper, the results of the studies on the identification and creation of effective seed crystals for the nucleation of anhydrous glucose in the presence of surface active agents (SAA) have been described. The nucleation process was controlled according to a change in the transparency of solutions and microscopy. The following have been tested as seeds: small (< 60 μm) commercial anhydrous glucose crystals and the same crystals wet with propanol; large (> 200 μm) and small (< 60 μm) hydrate glucose crystals wet with propanol. The large and small hydrate glucose crystals preliminarily wet with propanol or another aliphatic alcohol is recognized as the best of the tested seeds. When these crystals were mixed with a supersaturated glucose solution at a temperature of 60°C, they rapidly (within 15–30 min) disintegrated into a lot of uniform tiny particles with a size of 1–5 μm, became crystallization centers and began to grow rapidly in the form of alcohol suspensions of ground anhydrous glucose crystals and crystals of any sizes of hydrate glucose have been proposed.

Keywords: Glucose, nucleation, seed crystals, surface active agents, alcohol suspensions of crystals


INTRODUCTION

Crystalline glucose is a valuable food product and medicine. Glucose is used in healthcare, the food and pharmaceutical industries and veterinary medicine. It refers to strategic products, ensuring the health of the nation and the national security of the country. It is vital to use it as solutions for high blood losses, heart failure, shock and other severe conditions of the body. At present, due to lack of domestic production, Russian health care is supplied with crystalline glucose and injection solutions from imports. Improving the crystalline glucose production technology to organize its production in Russia in the order of import substitution is an urgent task.

On an industrial scale, glucose is obtained in a hydrate α-form with crystallization at a temperature below 50°C, in the form of anhydrous α-glucose – within the temperature range of 50–108°C and an anhydrous β-form at a temperature above 108°C. This article is devoted to the study of anhydrous α-glucose crystallization.

In the production of crystalline glucose, the process of its crystallization is the most complex and responsible, since the technological mode of crystallization with high yield and quality of crystals depends on a dozen different physical and technological parameters [1].

In theory, the process of crystallization is conditionally divided into two stages: nucleation and crystal growth. The emergence of a small volume consisting of 10 or 100 molecules of a new phase in a supersaturated mother liquor, from which a viable crystalline embryo of a critical size capable of further growth is formed, is understood by nucleation. The number of molecules or ions forming a nucleus of a critical size is not the same for different substances, and the conditions for existence and growth are also different. The general regularities of the existence of a critical nucleus for various substances were formulated by J. Gibbs, M. Volmer and other researchers [2, 3, 4].

Herewith, there are two types of nucleation: homogeneous – with the self-adherent formation of nuclei and heterogeneous – with the use of seed crystals.

In the process of glucose crystallization, the stage of nucleation of crystals is the most complex, since it is less studied, more difficult to control, the regime disturbance is practically not amenable to correction, and the final result thereof is usually determined only at the end of crystallization.
In the production of glucose in anhydrous form, crystals are seeded using a "shock" method—a conventionally homogeneous one and a method for applying the total amount of seed crystals—a heterogeneous one [1]. The "shock" method is used in the crystallization of anhydrous glucose under isothermal conditions (with boiling masscuit in a vacuum apparatus). The nucleation of crystals occurs rapidly in the labile zone of supersaturations of the solution and requires a low expenditure of seed crystals (15–20 g per ton of syrup). This nucleation process can be referred to conditionally homogeneous ones, since the violent nucleation over the whole volume of the solution is caused by a relatively small amount of seed crystals relative to the volume of the solution. The problem of the method consists in the visual, approximate determination of the amount of the new crystallization centers that are formed, which disturbs the optimum crystallization regime, the production of masscuit crystals with inhomogeneous crystals, and the presence of conglomerates [5, 6]. The heterogeneous nucleation method is used in the crystallization of anhydrous glucose under polythermal conditions (with cooling masscuit in a crystallizer), at which the formation of nuclei is much slower and leads not only to the inhomogeneity of the crystals, but also to an increase in the duration of the crystallization process [7, 8]. Due to this, the need for seed crystals significantly increases (up to 5–10% by weight of syrup) in the conditions of industrial crystallization.

The problem of production nucleation is urgent in the preparation of a lot of crystalline substances, since the crystallization process has general basic regularities. In this regard, scientific achievements in the development of new methods for accelerating nucleation in the adjacent production of fructose, lactose, pharmaceutical preparations, aerosols and other chemicals are of interest [9–16].

In the experiments of fructose nucleation [9], the effect of the initial supersaturation of the solution on nucleation has been shown. With the supersaturation coefficients characteristic for the metastable zone, the obtained crystals were comparatively large, but inhomogeneous in size. With higher supersaturations, the crystals were smaller, but more homogeneous. The yield of crystals increased with an increase in supersaturation, reached its maximum, and then decreased because of the problems with the separation of small crystals from the mother liquor. In the experiments with the crystallization of ibuprofen [10], the boundaries of the metastable crystallization zone were determined from the control of the onset of nucleation. At the same time, the boundary concentration of the solution was determined at the time of the appearance of the nuclei fixed visually. In the experiments with a seed, the nucleation was detected immediately near the saturation boundary. In the experiments without a seed, the induction period was long.

In the production of lactose, the acceleration of nucleation and the growth of crystals was achieved by mixing due to the vibrations of the solution [11], bubbling the solution with cold air with short cyclic temperature fluctuations and a total decrease therein in each cycle [12], passing the supersaturated solution flow through a hole controlled by Re = 1000 [13].

In [14] the original methods of homogeneous nucleation of paracetamol under continuous conditions without a seed and traditional solution cooling through the heat exchange surface have been considered. The main means of nucleation activation are the mixing of jets of counterflows of hot (66°C) and cold (22°C) solutions in a ratio of 8:1 and the use of radial and coaxial mixing of the combined flows in a mixer. The nucleation activation in this case is due to fluctuations in temperature, concentration and supersaturation in solutions [15]. An important condition for the successful formation of nuclei is the degree of supersaturation of the hot solution and the velocity of jets.

To activate the nucleation of a lot of substances, the ultrasonic treatment of solutions is successfully applied [16–18]. The installation of an ultrasonic generator on a pipe with a continuous flow of the solution accelerated the nucleation of paracetamol and provided the formation of homogeneous nuclei, excluding the secondary nucleation [16]. The authors [17, 18] studied the ultrasonic effect on the sedimentation of particles of glycine aerosols. The coagulation of aerosol particles and the acceleration of their sedimentation upon the application of an ultrasonic field have been shown. Such an effect of the ultrasonic field is also observed during the coagulation and precipitation of aerosols of dust and smoke particles. The acceleration of coagulation and sedimentation of particles is promoted by the introduction of the additional coagulation centers. In the experimental hydrate glucose crystallization [19], the ultrasound treatment of solutions also accelerated the process of homogeneous nucleation, but did not affect the growth of the nuclei obtained. The small nuclei did not grow to technical sizes with the subsequent propagation, which would allow them to separate from the viscous mother liquor during centrifugation. Therefore, the method was never applied in practice.

Of scientific and practical interest is the activation of nucleation with the help of surface active agents (SAA) due to the fact that according to the theory of crystallization the work of nucleation (Az) and, correspondingly, the radius of the critical nucleus (rk) depend on the surface tension of solutions, which follows from the known equations for spherical-shaped nuclei [2, 3, 20]:

\[ A_z = \frac{16\pi \sigma M^2}{3kT \ln \left( \frac{C_p}{C_s} \right)^2}, \]  
\[ r_k = 2 \frac{\sigma}{kT \ln \left( \frac{C_p}{C_s} \right)}, \]  

where \( R \) is the gas constant; \( T \) is the absolute temperature; \( C_p \) and \( C_s \) are the concentrations of supersaturated and saturated solutions; \( \sigma \) is the surface tension of the solution; \( M \) is the molecular mass of the crystallizing substance.

It follows from the equations that the work of formation and the radius of the critical nucleus are directly dependent on the surface tension of the
solution, namely, the lower the surface tension of the solution, the sooner the nucleus is formed. Numerous studies confirm the effect of SAA [21–28] on the rate of nucleation, the size and shape of crystals of various substances during condensation or dispersion. The use of a nonionic SAA (KDPG-100) during phosphogypsum crystallization promoted the enlargement of its crystals, an increase in their homogeneity and the improvement of filtration conditions [21]. The experiments [22] show an increase in the number of particles of n-butyl methacrylate during its polymerization, depending on the concentration of SAA of sodium dodecyl sulfate. With the critical SAA concentration that corresponds to colloidal stability, the number of particles increased in proportion to the third degree of SAA concentration. In the next paper [23], as a result of the numerical simulation of the process of nucleation and the growth of nanoparticles in the presence of SAA, it has been revealed that the adsorption of SAA molecules on the surface of nanoparticles slows down their growth in supersaturated solutions. With an increase in SAA concentration, the average size of nanoparticle decreases; at the critical concentration of SAA, when their molecules completely cover the surface of nanoparticles, the growth of the latter completely ceases. In the crystallization of goethite [24], the effect of SAA was expressed not only in an increase in the fraction of fine (2–5 μm) particles, but also in the formation of SAA and goethite compounds.

In our experiments [25], under the effect of SAA (distilled acetylated monoglycerides), there was a decrease in the rate of nucleation and the growth of glucose crystals, and the effect of SAA in the form of aliphatic alcohols on glucose nucleation was positive: the surface tension of glucose solutions decreased, the nucleation and crystal growth accelerated. Such a positive effect of the use of aliphatic alcohols is promising for improving the seeding method in glucose production in order to reduce the need for seed crystals and increase the efficiency of equipment.

This article is devoted to the search for effective seeding methods for accelerating the nucleation of glucose using SAA, the creation of new types of seed crystals with a long period of use to simplify the nucleation of crystals.

The study purposes included:
– the tests of various types of seeds with SAA and the selection of the most effective ones for the acceleration of nucleation;
– the determination of the technological mode of preparation, shelf life and use of seed crystals of anhydrous and hydrate glucose with the use of SAA;
– the determination of the excessive concentration (ΔC) and temperature of syrup, most favorable for seeding and nucleation.

**STUDY OBJECTS AND METHODS**

The studies of the nucleation of anhydrous glucose with the use of seeds with SAA were carried out within the temperature range of 55–75°C typical for the mode of glucose production under production conditions [6, 7] of most favorable for seeding and nucleation.

A rotary evaporator with a flask capped to prevent the evaporation of water and alcohol and experimental horizontal crystallizers (0.6 l) equipped with a ribbon-type mixer and a water "jacket" was used as experimental units.

The formation of crystal nuclei was determined from the measurement of the transparency of the solution using a FEK-56 photocolorimeter [26]. The transparency of solutions was measured in a 10 mm cuvette with a length of a light filter wave of 580 nm in comparison with the initial solution. The size and shape of the crystals were monitored microscopically using Leica DMLM and MBI-4 microscopes.

The crystallization process was monitored according to a change in the solids of the intercrystalline solution and massicuite [27].

The difference between the amounts of dissolved glucose in the supersaturated and saturated solution (g/100 g of water) was taken as the excess concentration (ΔC):

$$\Delta C = C_p - C_n,$$

where $C_p$ and $C_n$ are the amount of the dissolved glucose in a supersaturated and saturated solution. The difference between the amounts of dissolved glucose in the supersaturated and saturated solution (g/100 g of water) was taken as the excess concentration (ΔC):

$$\Delta C = C_p - C_n,$$

where $C_p$ and $C_n$ are the amount of the dissolved glucose in a supersaturated and saturated solution.

**RESULTS AND DISCUSSION**

**Effect of SAA on the kinetics of glucose nucleation.** The basis for carrying out the studies presented in the article was the found accelerating effect of SAA in the form of aliphatic alcohols on glucose nucleation. This set of experiments was the most instructive at 40°C in the form of hydrate glucose. It slowly crystallizes, which allowed the most reliable detection of the effect of SAA on glucose nucleation. Fig. 1 shows the nucleation of glucose nuclei according to a change in the transparency of solutions over time, depending on SAA supplements and seed crystals. First of all, attention is drawn to the difference in the duration of the induction periods, characterized by the unchanged transparency of the solution, equal to about 210 minutes for a pure solution, and the reduction thereof by half for the solution with propanol and practically the complete absence thereof for the solution with a seed and propanol. A crystalline seed has the largest accelerating effect on nucleation, and the presence of propanol significantly enhances it. Such activation is explained by a decrease in the work costs for the formation of a new phase as a result of the introduction of the finished solid phase and propanol, which reduces the surface tension of the solution, into the solution.
The nucleation process was controlled according to a change in the transparency of solutions during the nucleation period at 40°C and ΔC = 40 g/100 g of water: — — — — a pure glucose solution; with the addition by weight of the solution: — — — — propanol 1%; — — the crystals of hydrate glucose 0.01%; — — propanol 1% + the crystals of glucose 0.01%.

Selection of effective seeds for glucose crystallization. The large (> 200 μm) and small (< 60 μm) crystals of commercial hydrate and anhydrous glucose and the same crystals wet in alcohol were tested as seeds. The dosage of a seed was 0.15% by weight of syrup. The nucleation process was controlled according to a change in the transparency of solutions over time.

Fig. 2 presents the curves of changes in the transparency of solutions with different seeds.

The upper curve with the highest transparency of solutions characterizes the nucleation of glucose with a large (> 200 μm) anhydrous glucose crystals, which has a heat of crystallization two times higher – 4.72 kcal/mol. Dispersing various crystalline substances using SAA is also known in the derivation of other substances [29, 30].

The mechanism of transformation of alcohol wet crystals of hydrate glucose to the centers of crystallization of anhydrous glucose can be explained by several concomitant processes. One of the main such processes is the influence of high temperature of the solution, not peculiar to the conditions of existence of hydrate glucose crystals, which causes the weakening of the connection of crystallization water with glucose molecules in crystals, their decay and dissolution; the second equally active process is the interaction of the alcohol film of the crystal with the solution, namely, when the alcohol is dissolved in the water of the glucose solution in the film surrounding the crystal, the interphase tension decreases, the temperature rises, the volume of the film is compressed, contributing to the rapid destruction of the crystal to the smallest homogeneous particles [28]. Then, as the dispersion and partial dissolution of the crystal are concerned, the temperature of the solution near the surface of the crystals decreases and the supersaturation coefficient increases, which leads to the violent nucleation of anhydrous glucose. The low nucleation heat of anhydrous glucose nuclei is also promoted by the low heat of its crystallization – 2.36 kcal/mol compared to hydrate glucose, which has a heat of crystallization two times higher – 4.72 kcal/mol.
For clarity, Table 1 shows the effect of quantitative conversion during the nucleation of the hydrate glucose crystals wet with propanol into anhydrous glucose nuclei by the example of large crystals (220 μm). According to Table 1, their number in 1 g of hydrate glucose with a crystal size of 220 μm is 149, 256. When these crystals are dispersed in a solution to 1–5 microns in size, the number of the last particles increases to $9.1 \times 10^9 - 1 \times 10^{12}$, becoming the centers of crystallization of anhydrous glucose. Theoretically, due to this, the seed mass that corresponds to the total demand in crystallization centers – $8 \times 10^{10}$ per 100 kg of massecuite [1], can be reduced by 1000 times.

**Preparation of alcohol seed suspensions.** The preserved intactness of glucose crystals in the medium of absolute alcohols [25] served as a basis for the creation of the alcohol suspensions of seed crystals for a long-term use. The method is particularly relevant for the finely ground crystals subject to caking in a conventional way of bulk storage, which makes them unsuitable for seeding. When mixed with a viscous solution, they cannot be divided into separate particles, which leads to the formation of conglomerates. The alcohol suspensions of seed crystals, in contrast to them, are well distributed as separate particles when mixed with a glucose solution.

For glucose production, alcohol seeding suspensions from both anhydrous and hydrate glucose are of interest depending on a method for seeding crystals.

Due to the fact that anhydrous glucose crystals when mixed with a solution are not subjected to dispersion, they should be ground to obtain alcohol suspensions. We obtained the alcohol suspensions of seed crystals with a size of 1–10 microns from anhydrous glucose by mechanically grinding crystalline anhydrous glucose with the crystals of any size by means of a mill, for example PDI-70, with a filter to classify particles by size. The obtained crystals were poured into a container and filled with absolute alcohol in a ratio of 3 : 2. The container with the suspension was sealed and stored at room temperature for the subsequent use as a seed (at any time during a year of storage). One of the following was used as alcohols: propanol, butanol, isopropanol and isobutanol.

The seed crystals of hydrate glucose when mixed with syrup at a temperature above 50°C are dispersed, so there is no need for their mechanical grinding and long-term storage in alcohol. Commercial hydrate glucose crystals, in the absence of signs of caking, and large crystals, for example, grits after glucose sifting, should only be wet with absolute alcohol immediately before the use of them as a seed. Small seed crystals (< 30 microns) require alcohol storage due to their high caking. Before feeding the alcohol suspensions of crystals to the glucose syrup, they were previously separated from alcohol, filled with syrup, mixed and fed into a vacuum apparatus or crystallizer as a seed.

**Determination of the effect of excess concentration and solution temperature on glucose nucleation.** The nucleation of crystals and their subsequent growth largely depends on the temperature and degree of supersaturation of syrup at the time of seeding nuclei.

In the experiments, anhydrous glucose crystals with a size of 60 μm wet with propanol with a dosage of 0.15% by weight were used as seeds.

**Effect of excess concentration on glucose nucleation.** Fig. 4 shows the dependence of the transparency of the solution on the excess concentration of solutions. It follows from the figure that as the excess concentration increases, the transparency of solutions decreases as a result of the formation of nuclei and their growth simultaneously with the growth of seed crystals. With the excess concentration of 15 g/100 g of water, the transparency of the solution slowly decreases with time, which is due to the growth of the introduced seed crystals. At the same time, there is no formation of new nuclei. With the excess concentration of 44 g/100 g of water, the crystal growth accelerates, and with the excess concentration of 59 g/100 g of water, the pattern of the curve at the initial moment, that sharply decreases, along with the growth of seed crystals, demonstrates the formation of new nuclei and indicates a transition from the metastable concentration zone to the labile zone. With the excess concentration of 88 g/100 g of water, the transparency of solution drops sharply within 80 min, which indicates the predominance of nucleation over the growth of nuclei, characteristic of the labile zone of supersaturations.
Crystallization depending on the excess concentration of the solution ($\Delta C$), g/100 g of water: — 15; — 44; — — 59; — — — 88.

Fig. 5. Change in the transparency of solutions during crystallization depending on temperature, °C: — — — 70, — — — 55; at $\Delta C = 60$ g/100 g of water and a temperature, °C: — — 70, — — — 55.

Studies at a temperature above 50°C. Temperature plays a special role in glucose crystallization. In the studies on the effect of temperature on the rate of growth of crystals, a sharp increase in the rate of growth with an increase in temperature, which follows from the general theses of the theory of crystallization, has been proved [15]. In case of the crystallization of substances under the conditions limited by the diffusion mode, with an increase in temperature the diffusion coefficient increases and the thickness of the film decreases because of a decrease in the viscosity of the solution with an increase in temperature [31].

When the kinetic crystal-chemical stage is limiting, with an increase in temperature the size of a two-dimensional crystalline nucleus decreases, and, consequently, so does the work of its formation, which also increases the crystal growth rate [15]. In addition, a certain role is played by a decrease in the hydration of ions or molecules in the solution [32], which also accelerates the transition of a substance from a partially arranged layer to the crystal face. This is confirmed by the fact that in the area of low temperatures, the kinetics of crystal growth is often determined precisely by the rate of the crystallochemical stage, at the same time the diffusion stage is limiting for the same salts at high temperatures.

Determining the effect of temperature on glucose crystallization is not an easy task. It develops as an accelerating or delaying effect on a whole set of the reactions and processes involved in crystallization: tautomerism, diffusion, the surface reaction of the integration of molecules into the crystal lattice, the type of crystals, the solubility of glucose, the viscosity of solutions, the hydration of crystals. Consequently, the effect of temperature on the rate of crystallization of glucose develops as the combined effect of these factors. The rate of crystallization of anhydrous glucose is known to be much higher than that of hydrate glucose.

Fig. 5 shows a change in the transparency of the solution depending on temperature for two values of excess concentration. It follows from the figure that when the temperature decreases from 70 to 55°C, the rate of nucleation and crystal growth increases. This is confirmed by a decrease in the transparency of solutions with a decrease in temperature. With the excess concentration of 40 g/100 g of water, the flat pattern of the transparency curves indicates the predominant growth of the introduced seed crystals over time.

With the excess concentration of 60 g/100 g of water, the introduction of a seed intensified the nucleation to a higher degree. A sharp decrease in the transparency of solutions demonstrates the predominance of nucleation over crystal growth and leads to rapid saturation in the solution. A decrease in the rate of crystallization of anhydrous glucose with an increase in temperature was also determined in another paper [33]. Thus, with an increase in temperature from 55 to 75°C, the crystallization rate decreased from 4.01 to 3.52 mg/g min, or by 12%, which is explained by an increase in the viscosity of saturated glucose solutions with an increase in temperature.

Thus, the nucleation of glucose crystals in the presence of SAA accelerates with an increase in the excess concentration of solutions and with a decrease in temperature. At the same time, the concentration of the upper boundary of the metastable zone decreases from 82 to 80%.

On the basis of the obtained results, the technologial mode of crystallization of anhydrous glucose under isothermal conditions with a seed from the large hydrate crystals wet with propanol has been tested in the amount of 0.01–0.015% by weight of syrup. This amount was sufficient to meet the total demand of the process in crystallization centers. A feature of the nucleation process in the presence of SAA (propanol) was the introduction of a seed into the syrup in the metastable supersaturated zone (at a temperature of 70°C and the concentration of solids of 79–80% in the syrup). The process of nucleation during the crystallization of anhydrous glucose without SAA is carried out at the concentration of solids of syrup of 81–82% [1]. The tests showed that in order to meet the total demand for crystallization centers, the dosed mass of seed crystals should be increased by a factor of 1.5–2 compared to the calculated one (Table 1).
Table 1. Characteristics of seed crystals

<table>
<thead>
<tr>
<th>Size of the crystal, μm</th>
<th>Mass of the crystal, d</th>
<th>Number of crystals per 1 g</th>
<th>Seed weight, g (with the number of crystals of 8 × 10^{10} per 100 kg of massecuite)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hydrate glucose</td>
</tr>
<tr>
<td>220</td>
<td>6.7 × 10^{-6}</td>
<td>149256</td>
<td>–</td>
</tr>
<tr>
<td>165</td>
<td>2.8 × 10^{-6}</td>
<td>357143</td>
<td>–</td>
</tr>
<tr>
<td>Anhydrous glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.92 × 10^{-9}</td>
<td>1.1 × 10^{7}</td>
<td>68</td>
</tr>
<tr>
<td>5</td>
<td>0.11 × 10^{-9}</td>
<td>9.1 × 10^{6}</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>0.92 × 10^{-12}</td>
<td>1.1 × 10^{12}</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Fig. 6. Kinetics of nucleation and growth of the crystalline mass of anhydrous glucose under isothermal conditions with a seed from the hydrate crystals wet with propanol, when boiling massecuite in a vacuum apparatus at a temperature of 70–68°C.

Fig. 7. Change in the transparency of the solution during the nucleation of anhydrous glucose with a seed from the large (200 μm) hydrate glucose crystals wet with propanol at a temperature of 48°C and ΔC, g/100 g of water: – – – 80, – 90.

It can be assumed that some of the hydrate particles dissolved when mixed with the syrup. The crystalline mass continued to grow for 6 hours (Fig. 6). The massecuite was of high quality, it contained about 50% of homogeneous crystals with a size of 0.15–0.20 mm and was easily divided into crystals and intercrystal molasses, which is the evidence in favor of the proposed seed types.

Studies at a temperature below 50°C. Of theoretical and practical interest are the studies on the detection of conditions of the nucleation and growth of anhydrous glucose crystals using SAA at temperatures below 50°C, especially when hydrate glucose crystals are used for seeding.

The temperature intervals of the formation of crystals of hydrate and anhydrous glucose were established by V.B. Newkirk [34–36]. Hydrate crystals (C_{6}H_{12}O_{6} × Н_{2}O), as a stable solid phase, are formed at a temperature from –5.3 to +50°C. At temperatures above 50°C, a stable solid phase is anhydrous glucose (C_{6}H_{12}O_{6}). Later on, J. Vaschatko and A. Smelik [37] specified the temperature of the transition of hydrate glucose to anhydrous glucose and the boundaries of the metastable zone for hydrate and anhydrous glucose. According to their data, the crystals of anhydrous glucose are spontaneously formed at a temperature of 52.2°C and higher; the crystals present in the solution can also grow with a decrease in temperature to 34.55°C.

Below are the results of the studies of nucleation of anhydrous glucose in the temperature zone of crystallization of hydrate glucose (at a temperature of 45–48°C) with the help of the seed crystals of hydrate glucose with a size of 200 microns wet with propanol.

Fig. 7 presents the results of the experiments at a temperature of 48°C. It follows from the figure that after introducing the crystals of hydrate glucose wet with propanol into the supersaturated solution, the transparency of solutions drops. This indicates the beginning of nucleation. The microscopic analysis showed that 30 minutes after the beginning of the experiment, the large hydrate crystals completely disintegrated to the smallest particles (as shown in the micrographs of Fig. 3) and began to grow rapidly in the form of anhydrous glucose crystals, although the form of a seed from hydrate crystals and the temperature zone should stimulate the growth of hydrate glucose crystals. The activation of nucleation of anhydrous glucose under the studied crystallization conditions is in the same way as in the experiments with a temperature above 50°C. The growth of nuclei with a high rate characteristic of anhydrous glucose continues with a decrease in temperature to about 36°C. And then, at a temperature close to 35°C, the crystals dissolve almost instantaneously until a pure solution is obtained. This is consistent with the data of J. Vaschatko and A. Smelik, who determined the lower temperature boundary of 34.55°C for the existence of anhydrous glucose crystals. According to some tests, nucleation occurs within the temperature range from 50 to 38°C.

The obtained data on the nucleation of anhydrous glucose at a temperature below 50°C with a seed from the hydrate glucose crystals wet with alcohol, were a prerequisite for the further improvement of the technological mode of crystallization of anhydrous glucose under polythermal conditions.
The deterioration of the quality of glucose and the accumulation of glucose decay products. The proposed nucleation method is more effective, requires less (by weight) seed crystals of hydrate glucose of any size to provide the complete demand in crystallization centers, and the resulting massecuite is characterized by high homogeneity crystals and the absence of conglomerates. The viscosity of the initial syrup and the intercrystalline solution is lower. The advantage of the tested technological mode is also saving technical water for the cooling of massecuite and drinking water for washing crystals in a centrifuge.

Tests of the technological mode of crystallization of anhydrous glucose at a low temperature. As already mentioned above, anhydrous glucose crystallizes under industrial conditions within the temperature range of 75–55°C [6, 7].

In laboratory conditions, the technological modes for crystallization of anhydrous glucose by reducing the temperature of massecuite from 60–65 to 37–38°C have been tested.

Fig. 8 shows the graph of crystallization of anhydrous glucose with a decrease in temperature from 60 to 38°C. The nucleation of crystallization centers was carried out with the help of the seed crystals of hydrate glucose with a size of 200 μm, wet with propanol in the amount of 0.015% by weight of syrup. During the nucleation, the concentration of solids in the syrup was 78% at a temperature of 60°C. The growth of the crystalline mass lasted for 13 hours.

The large crystals (200 microns) wet with alcohol, introduced into the syrup, disintegrated into small particles, became nuclei and grew in the form of anhydrous glucose. With the further mixing and gradual cooling, the nuclei grew to technical sizes of 0.10–0.15 mm by the end of crystallization and were homogeneous. The content of crystals in massecuite reached 43–45% after 12–14 hours; the crystals were easily separated from the mother liquor during centrifugation. The mother liquor was depleted from the initial concentration of solids of 78% to 62% in the centrifugation. The mother liquor is lower. The advantage of the tested technological mode is also saving technical water for the cooling of massecuite and drinking water for washing crystals in a centrifuge.

As is known, the driving force of crystallization is the supersaturated state of the solution, due to which the nuclei of a critical size are formed in the solution and their further growth takes place. To realize the crystal growth, the diffusion inlet of the molecules or ions of the crystallized substance to the crystal surface and the kinetic reaction of integrating them into the crystal lattice are needed. The rate of crystallization is limited by the slowest of these two stages. We established [38] that the kinetic surface reaction is limiting in the crystallization of hydrate glucose, while the diffusion component is, on the contrary, limiting in the crystallization of anhydrous glucose. Analyzing the conditions of the nucleation process of anhydrous glucose (Table 2) at a temperature > and < 50°C, we find that: the diffusion coefficient at a temperature of 40°C is 1.5 times higher (3.8·10–10 m²/s) than at a temperature of 65°C (2.36·10–10 m²/s). This is due to a decrease in the viscosity of saturated glucose solutions with a decrease in temperature, the value of which at a temperature of 40°C is 2.6 times lower than the viscosity of saturated solutions at a temperature of 65°C. For supersaturated solutions in the metastable zone, the difference between the values of viscosity at the analyzed temperatures is even higher. In addition, the reduction in the thickness of the film surrounding the crystal and the acceleration of the diffusion of molecules through it to the surface of the crystal contributes to the improvement of crystallization conditions at a temperature of 40°C.

An important role in the crystallization of glucose is played by the tautomeric reaction of the interconversion of α- and β-glucose [39, 40]. The faster the conversion of β-glucose into the α-form, the higher the crystallization rate of α-glucose. According to Table 2, the duration of tautomeric conversions at a temperature of 40°C is 172 minutes, at a temperature of 65°C – 7–10 minutes, which should accelerate the crystallization process at a temperature of 65°C.
Table 2. Comparative values of the parameters of anhydrous glucose crystallization at a temperature > and < 50°C

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>Diffusion coefficient, m²/s</th>
<th>Viscosity of saturated solutions, Pa·s</th>
<th>Duration of mutarotation, min</th>
<th>Excess concentration, g/100 g of water</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>3.81×10⁻⁵</td>
<td>0.0230</td>
<td>172</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0.0520</td>
<td></td>
<td></td>
<td>41</td>
</tr>
<tr>
<td>40</td>
<td>0.0370</td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>65</td>
<td>2.36×10⁻⁵</td>
<td>0.0650</td>
<td>7–10</td>
<td>0</td>
</tr>
<tr>
<td>65</td>
<td>0.214</td>
<td></td>
<td></td>
<td>106</td>
</tr>
<tr>
<td>65</td>
<td>0.30</td>
<td></td>
<td></td>
<td>139</td>
</tr>
</tbody>
</table>

Based on the analysis of the data of Table 2, the high rate of crystallization of anhydrous glucose at a temperature of < 50°C in comparison with hydrate glucose becomes clear. An increase in the diffusion coefficient and a decrease in the thickness of the film the crystal is enclosed in, create favorable conditions for the crystallization of anhydrous glucose in the diffusion mode. The delayed tautomerism is apparently compensated by the acceleration of diffusion. Further on, the analysis of the conditions of the crystallization of hydrate glucose is provided. As already mentioned above, the main stage that inhibits the process of crystallization of hydrate glucose is the kinetic reaction of the integration of molecules into the crystal lattice. At temperatures below 50°C, to crystallize hydrate glucose solutions with a concentration of 70–72%, in which glucose molecules have a high degree of hydration, are used [32]. Hydration makes it difficult for a molecule to jump from the solution to the crystal lattice. To do this, the glucose molecule must first get rid of water molecules, namely to break chemical bonds with water molecules, get rid of the intercrystalline solution and spend additional energy to include one water molecule in the crystal lattice. The use of SAA accelerates the nucleation of hydrate glucose, as shown in Fig. 1, but this effect is less distinct at the low rate of crystallization of hydrate glucose compared with anhydrous glucose. O.D. Linnikov [41] established that in the case of the kinetic mode of crystallization (NaCl and KCl), the basic energy of nucleation and growth of crystals is spent precisely on dehydration, since the diffusion component is comparatively less significant. The large energy costs for the nucleation and growth of hydrate glucose crystals are also confirmed by the heat of crystallization of hydrate glucose which is twice as high in comparison with anhydrous glucose.

CONCLUSION

As a result of the studies, new data have been obtained for the theory and practice of glucose production. The activating effect of SAA – aliphatic alcohols on the nucleation of glucose has been established. The tests of seed crystals of anhydrous and hydrate glucose, dry and wet with alcohol for nucleation, showed the advantages of crystals wet with alcohol. The seed crystals (of any size) of hydrate glucose wet with alcohol that make it possible to improve the homogeneity of crystals and to reduce significantly the required mass of seed crystals, have been recognized as the most effective. A method for producing seed crystals of anhydrous and hydrate glucose with a long shelf life has been proposed. The activating effect of the hydrate crystals wet with alcohol on the nucleation of anhydrous glucose at a temperature below 50°C has been established. In the experimental crystallization at a temperature of about 35°C, the crystals dissolved almost instantaneously. With positive results, the technological mode of crystallization of anhydrous glucose under polythermal conditions has been tested with a decrease in temperature from 60 to 38°C. The data obtained are recommended for further testing and use in the development of technological regulations for the production of crystalline glucose.

REFERENCES


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INTRODUCTION

Mass consumption products, including beverages, are of great importance among food products. Currently, nectars that are more affordable than juices and non-alcoholic carbonated beverages are becoming more popular with a large part of the population of Russia among non-alcoholic beverages.

In non-alcoholic production, water is used from the centralized domestic drinking water supply system. According to Rospotrebnadzor and our long-term studies it was found that the content of chloroform increases in natural water in the spring-summer period [1]. In addition, chloroform, dichloroethane and trichlorethylene are formed as by-products after the treatment of natural water with chloragents. The classical technology of water treatment does not provide the complete purification of water from organic substances [2]. According to the Department of Natural Resources and Ecology of the Kemerovo Region in Kuzbass in 2011–2016, the proportion of water samples from the centralized water supply distribution network that did not meet hygienic standards for sanitary and chemical indicators was 9.4–13.8% [3].

For deep water purification at the enterprises of beer and alcoholic beverage industries corresponding to the modern level, reverse osmosis plants are mainly used [4]. However, it should be noted that the studies of foreign scientists have found that chloroform, trichlorethylene and dichlorethane are not practically retained even by highly selective reverse osmotic membranes [5, 6].

Chloroform, according to the classification of the International Agency for Research on Cancer (IARC, Lyon, France), which is part of WHO, is assigned to a 2B group, i.e. to the substances possibly carcinogenic to human (potentially dangerous substances). Earlier on [7–11], the effect of chloroform on human health was
noted. In recent years, the direct correlation of consumption of chlorinated water with the increased risk of oncological diseases (of rectum, bladder, urinary tract and brain) has been found. Thus, according to the scientists at Columbia University in the United States, those people who consume water that contains halogen-organic compounds have a 44% increase in the risk of gastrointestinal and bladder cancer [12–14].

Trichlorethylene refers to hazard category 1 substances. Back in 2012, WHO recognized the compound as carcinogenic. The latest research suggests that trichlorethylene increases the risk of cancer of the liver, kidneys, esophagus and cervix. Trichlorethylene is the strongest narcotic drug. It differs from other narcotic drugs in the fact that it provides strong analgesia at a small concentration. A relationship has been found between the regular exposure of trichlorethylene to human and the subsequent development of Parkinson's disease. Constant contact with the chemical increases the risk of the disease by 6 times.

Dichloroethane refers to hazard category 2 substances. When intaken, it causes damage to the central nervous system, the liver, kidneys and the cardiac muscle. It has a narcotic, toxic and carcinogenic effect. The pattern of intoxication by dichloroethane is similar to that of intoxication by chloroform [15].

These substances reduce the safety of water. According to the Hygienic Standard HS 2.1.5.2280-07 "Maximum permissible concentration (MPC) of chemicals in water bodies of drinking and cultural and community use" (Additions and amendments 1 to hygienic standards HS 2.1.5.1315-03), MPC of chloroform in the water of economic-drinking and cultural-domestic water objects is 0.060 mg/dm³, trichlorethylene is 0.005 mg/dm³ and dichloroethane is 0.003 mg/dm³.

In this regard, the studies of the effect of the priority organic contaminants formed in the process of water treatment in the chlorination stage (chloroform, dichloroethane and trichlorethylene) and capable of provoking oncological diseases on the quality of food products are relevant and timely. The study aims at the effect of chloroform, dichloroethane and trichlorethylene on the stability of the main formulation components of beverages during production and storage.

**STUDY OBJECTS AND METHODS**

The subjects of the study were the aqueous solutions of the formulation components of beverages (sucrose, vanillin, sodium benzoate, citric acid, dyes and tarragon) and the aqueous solutions with the addition of organic substances (chloroform, dichloroethane and trichlorethylene); mass consumption beverages – nectars from raspberry, strawberry, sea-buckthorn berries, black currant, cherry and orange and apple fruits prepared on the water basis without organic impurities and aqueous solutions containing chloroform, trichlorethylene and dichloroethane.

The concentration of sodium benzoate in the samples was 0.1441 mg/dm³, dyes (brilliant blue, brown chocolate, green apple, yellow sunset, tartrazine, carmoisine, Ponso 4R) – 100 mg/kg according to TR TS 029/2012 "Safety requirements for food additives, flavorings and technological aids". The concentration of sucrose was 342.3 mg/kg, citric acid – 5 mg/dm³ according to TR TS 023/2011 "Technical regulations for fruit and vegetable juice products". The concentration of organic contaminants in the studied systems was 10 MPC, which corresponds to the maximum possible increase in the content of contaminants in the water of the domestic drinking water supply system in different seasons of the year [16].

The content of chloroform, trichlorethylene and dichloroethane was determined using a standard method (gas-liquid chromatography) using an electron capture detector, the lower limit of 0.01 μg according to GOST R 31951-2012. The following was performed before measuring: the preparation of solutions, the preparation of a chromatographic column, sampling. The concentrations of the solutions analyzed were determined from a calibration graph. The content of sodium benzoate was determined by molecular absorption spectroscopy with hydroxylamine hydrochloride and hydrogen peroxide in the presence of Cu²⁺ ions to form a colored nitrosophenol derivative at a wavelength of 364 nm [10]. The content of citric acid was determined using the method of molecular absorption spectroscopy with the formation of a complex compound with ammonium vanadate at a wavelength of 400 nm in accordance with GOST 28467-90. The intensity of the coloring of dyes and nectars in the samples was also determined using the method of molecular absorption spectroscopy at a wavelength of 400–440 nm. The content of sucrose in the samples was determined by refractometry based on the determination of the refractive indices of a number of standard solutions followed by plotting a calibration curve in accordance with GOST 15113.6-77. B and C vitamins in water were determined by capillary electrophoresis using Kapel-105M (Lumex, St. Petersburg) [17, 18]. The analysis was carried out under the following conditions: the total length of a capillary is 60 cm, the effective length (i.e. the length from the entrance to the detector window) is 50 cm, the working voltage applied to the electrodes is 20 kV, the internal diameter of a capillary is 50 μm, the detection is at 200 nm, the temperature is 200°C, the composition of the reaction buffer is 40 cm³ of sodium tetraborate solution (0.05 mol/dm³), 20 cm³ of boric acid solution (0.2 mol/dm³). The aqueous contaminant containing vanillin solutions were observed using the olfactory method.

The changes in all the indicators were observed for 20 days.

**RESULTS AND DISCUSSION**

**Sodium benzoate.** Regardless of the composition and purpose of a drink (general, special), the most commonly used component of most soft drinks is sodium benzoate. Its content and consumption in beverages are strictly controlled by formulations, sanitary regulations and standards and process instructions.

Table 1 presents a decrease in the content of sodium benzoate in the presence of organic substances.
Table 1. Residual content of sodium benzoate in aqueous solutions, %

<table>
<thead>
<tr>
<th>Number of days</th>
<th>Sodium benzoate aqueous solution without organic impurities</th>
<th>Chloroform containing sodium benzoate aqueous solution</th>
<th>Trichlorethylene containing sodium benzoate aqueous solution</th>
<th>Aqueous dichloroethane containing solution of sodium benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>49.73</td>
<td>45.58</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>100</td>
<td>19.47</td>
<td>7.58</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>100</td>
<td>10.36</td>
<td>4.14</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>100</td>
<td>8.31</td>
<td>3.31</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>100</td>
<td>5.90</td>
<td>2.60</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>100</td>
<td>5.90</td>
<td>2.60</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>100</td>
<td>5.90</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical interaction of sodium benzoate with the priority contaminants contained in water.

Fig. 2. Change in the content of trichlorethylene (1), dichloroethane (2) and chloroform (3) in water in the presence of sodium benzoate over time.

In the presence of chloroform, the concentration of sodium benzoate in water remained unchanged throughout the study period. It has been found that the lowest decrease in the sodium benzoate content in the first days of the study was in the presence of trichlorethylene, and the highest – in the presence of dichloroethane in water. It should be noted that after 10 days of storage the concentration of sodium benzoate decreased in the presence of trichlorethylene by 94.1%, of dichloroethane – by 97.4%. The reduction in the sodium benzoate content, leading to a decrease in the quality of the finished product, is evidently due to its chemical interaction with the priority contaminants contained in water (Fig. 1). Trichlorethylene is characterized by the attachment between the sites of organic acids forming the corresponding organochlorine esters [19]. Dichloroethane interacts with the oxygen of the carboxyl group of benzoic acid forming hydrogen bonds (Fig. 1).

The chemical interaction of organic impurities with sodium benzoate has also been experimentally confirmed by a corresponding decrease in trichlorethylene and dichloroethane in water over time (Fig. 2).

Sucrose (C_{12}H_{22}O_{11}) is one of the main components that make up carbonated beverages and nectars. Given the chemical properties of sucrose and the studied organic contaminants contained in water, there is a possibility of their chemical interaction.

Table 2 presents a decrease in the content of sucrose in the presence of organic substances. It has been established that all organic contaminants, except for chloroform, interact with sucrose, while the quality of the finished beverage decreases. Sucrose interacted most violently with dichloroethane, most weakly – with trichlorethylene.

The chemical interaction of organic substances with sucrose has theoretically been confirmed by chemical equations. Trichlorethylene decomposes in the presence of oxygen dissolved in water forming dichloroacetyl chloride, which can be hydrolyzed to dichloroacetic acid in aqueous solutions (Fig. 3) [20].

Both dichloroacetyl chloride and dichloroacetic acid are active compounds capable of entering into esterification and nucleophilic addition reactions [20–21]. When reacting with the compounds containing an alcohol (phenolic) group, the formation of organochlorine compound esters is possible. For dichloroethane, the presence of electronegative chlorine atoms promotes the redistribution of electron density on the atoms of a molecule and, as a consequence, the formation of hydrogen bonds with the hydrogen of the sucrose alcohol group (Fig. 3).
Table 2. Residual content of sucrose in the studied samples during storage, %

<table>
<thead>
<tr>
<th>Number of days</th>
<th>Aqueous sucrose solution without organic impurities</th>
<th>Aqueous chloroform-containing sucrose solution</th>
<th>Aqueous trichlorethylene-containing sucrose solution</th>
<th>Aqueous dichloroethane-containing sucrose solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>98.33</td>
<td>96.66</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>100</td>
<td>95.00</td>
<td>90.00</td>
</tr>
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<td>5</td>
<td>100</td>
<td>100</td>
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<td>83.33</td>
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<td>100</td>
<td>91.66</td>
<td>73.33</td>
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<td>15</td>
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<tr>
<td>20</td>
<td>100</td>
<td>100</td>
<td>90.00</td>
<td>66.66</td>
</tr>
</tbody>
</table>

The chemical interaction of organic contaminants has also been experimentally confirmed by their corresponding decrease in the presence of sucrose (Fig. 4) and agrees with the data in Table 2.

Citric acid. As an acidity regulator in the production of soft drinks and nectars, citric acid (C₆H₈O₇ · H₂O) is most often used, since it has the mildest taste in comparison with other food acids and does not irritate the mucous membranes of the gastrointestinal tract. There is a possibility of the interaction of citric acid with organic components. In this connection, the effect of organic impurities (trichlorethylene, dichloroethane and chloroform) contained in water on the resistance of citric acid used in the production of beverages and nectars, has been studied.

The experimental data indicate that the content of citric acid decreases in the presence of all organic contaminants except chloroform (Table 3). Fig. 5 presents the chemical reactions between citric acid and the priority water contaminants.
The chemical interaction of organic impurities with citric acid has experimentally been confirmed by a corresponding decrease in the content of trichlorethylene and dichloroethane in the presence of citric acid in water over time, shown in Figure 6, and is consistent with the data in Table 3.

It has been found that citric acid, sodium benzoate and sucrose are characterized by a sharp decrease in their content in the first day of the study, then the concentration of formulation components either decreases slowly or remains at the same level. In connection with a significant loss of sodium benzoate (94.1%) and citric acid (31.6%) in the presence of organochlorine compounds, their additional amount is needed to compensate for losses and preserve the quality of beverages. Given that sodium benzoate and citric acid are expensive components, their excess consumption is undesirable.

**Vanillin.** Vanillin is one the aromatizing agents most often used in the production of beverages. The introduction of a vanilla aromatizer into the formulation makes it possible to improve the organoleptic properties of a drink by giving it an original pleasant harmonious taste and a unique soft vanilla flavor.

Based on the chemical properties of vanillin, we can expect its interaction with organic water contaminants. In this connection, the effect of priority contaminants on the stability of vanillin smell and the change in their content in vanillin containing solutions was studied.

**Table 3.** Residual content of citric acid in the studied samples during storage, %

<table>
<thead>
<tr>
<th>Number of days</th>
<th>Aqueous citric acid solution without organic impurities</th>
<th>Aqueous chloroform – containing citric acid solution</th>
<th>Aqueous trichlorethylene-containing citric acid solution</th>
<th>Aqueous dichloroethane – containing citric acid solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>100</td>
<td>89.47</td>
<td>84.21</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>100</td>
<td>73.68</td>
<td>68.42</td>
</tr>
<tr>
<td>5</td>
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<td>70.00</td>
<td>63.15</td>
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<td>68.42</td>
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<tr>
<td>20</td>
<td>100</td>
<td>100</td>
<td>68.42</td>
<td>63.15</td>
</tr>
</tbody>
</table>

The observation of the smell of vanillin samples showed that the aqueous trichlorethylene containing chemical solution had a weak original chemical odor after vanillin addition. There was a sharp chemical odor in the dichloroethane containing sample, no foreign odors were found in the presence of chloroform. The appearance of uncharacteristic odors in the samples is due to the chemical interaction of vanillin with the priority contaminants contained in water and the formation of new compounds (Figure 7).
The chemical interaction of organic impurities with vanillin was experimentally confirmed by a corresponding decrease in the content of trichlorethylene and dichloroethane in the samples over the study period (Fig. 8).

Dyes are widely used in the production of beverages and can be used in combination with various food supplements, providing a unique image for a product and giving it the necessary consumer properties. Such dyes as brilliant blue (E133), carmoisine (E122), yellow sunset (E110), brown chocolate (R240), tartrazine (E102), ponceau 4R (E124) and green apple (E100) are widely used in the production of soft drinks (Green Apple, Tarragon, Strawberry, Cherry Flavor, etc.). It has been established that the color intensity of all the dye-containing samples did not change over the entire study period, which is shown in Fig. 9 using the example of a brilliant blue dye.

Tarragon infusion. The results of observations of a change in the color intensity of the tarragon infusions are shown in Fig. 10. The data obtained indicate a slight decrease in the color intensity of the aqueous dichloroethane and trichlorethylene containing solutions.

Coloring agents. Dye-containing berries and fruits are used in the production of common juice beverages (nectars). To a greater extent, the flavonoids that provide mainly a red, blue and violet color and the carotenoids that have a yellow or orange color give color to all fruits and berries. The content of dyes in berries and fruits is shown in Table 4.

In laboratory conditions, according to GOST R 51398-99 "Canned foods. Juices, nectars, juice beverages", raspberry, strawberry, sea-buckthorn, black currant, cherry, orange and apple nectars were prepared. The change in the color intensity in the nectars from all berries (cherry, black currant, sea-buckthorn, raspberry and strawberry) and fruits (orange and apple) in the presence of chloroform was not detected during the whole period of research.

Table 4. Content of the components that impart color to berries and fruits, mg/100 g

<table>
<thead>
<tr>
<th>Substance</th>
<th>Flavonoids (anthocyanins, catechins, leucoanthocyanins), mg</th>
<th>Carotene (provitamin A), mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cherry</td>
<td>300–2500</td>
<td>0.1–0.3</td>
</tr>
<tr>
<td>Black currant</td>
<td>100–130</td>
<td>0.173</td>
</tr>
<tr>
<td>Sea buckthorn</td>
<td>800–1000</td>
<td>11</td>
</tr>
<tr>
<td>Raspberry</td>
<td>600–1300</td>
<td>1</td>
</tr>
<tr>
<td>Apple</td>
<td>300–600</td>
<td>1.1–15</td>
</tr>
<tr>
<td>Strawberry</td>
<td>1500–2000</td>
<td>0.03–0.08</td>
</tr>
<tr>
<td>Orange</td>
<td>70–80</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig. 7. Chemical interaction of vanillin with the priority contaminants contained in water.

Fig. 8. Change in the content of trichlorethylene (1), dichloroethane (2) and chloroform (3) in water in the presence of vanillin over time.
Obviously, the chemical properties of dyes and organic contaminants predetermined the possibility of their interaction, as shown by the example of carotenoids. The interaction of trichlorethylene with carotenoids (polyenes) may lead to pericyclic reactions [22], as a result of which a cyclic chlorine-containing compound can be formed. Dichloroethane is characterized by the formation of hydrogen bonds with the hydrogen of the alcohol group of carotenoids (Fig. 13).

Vitamins contained in the nectars are organic compounds of different chemical nature, so there is a possibility of interaction of the organic impurities contained in water with the vitamins of nectars. The content of single vitamins has been studied in all the samples of aqueous nectars without contaminating impurities and is presented in Table 5.

In the apple nectar, a decrease in vitamin B1 in water in the presence of trichlorethylene by 38%, dichloroethane by 32%; vitamin B2 in the presence of trichlorethylene by 50%, dichloroethane by 46%; vitamin B4 in the presence of trichlorethylene by 55%, dichloroethane by 70%; vitamin C in the presence of trichlorethylene by 26%, dichloroethane by 16% has been detected.

In the cherry nectar, a decrease in vitamin B1 in water in the presence of trichlorethylene by 14%, dichloroethane by 9%; vitamin B2 in the presence of trichlorethylene by 29%, dichloroethane by 22%; vitamin B4 in the presence of trichlorethylene by 44%, dichloroethane by 38%; vitamin C in the presence of trichlorethylene by 37%, dichloroethane by 15% has been detected.

In the orange nectar, a decrease in vitamin B1 in water in the presence of trichlorethylene by 12%, dichloroethane by 19%; vitamin B2 in the presence of trichlorethylene by 39%, dichloroethane by 18%; vitamin B4 in the presence of trichlorethylene by 42%, dichloroethane by 36%; vitamin C in the presence of trichlorethylene by 33%, dichloroethane by 23% has been detected.
The change in the content of vitamins in the nectars in the presence of chloroform was not detected during the entire study period. For all the studied nectars, the regularity of a decrease in the vitamin content during the first 2–3 days of the studies has been noted, and then the content of vitamins practically does not change.

The chemical interaction of the priority contaminants (trichloroethylene and dichloroethane) contained in the water used for the production of nectars and vitamins has been confirmed by chemical equations (Figures 14–17):

Trichloroethylene is able to enter into nucleophilic addition reactions with heterocyclic amines [23]. Dichloroethane, in addition to the formation of a hydrogen bond with the hydrogen of the alcohol group of thiamine, is also able to form a hydrogen bond with the nitrogen of the aromatic ring of vitamin B₁.

Since the studied bioactive substances are polyfunctional compounds (for example, thiamine, riboflavin, and pyridoxine contain both amine nitrogen and an alcohol group), the reaction with dichloroacetyl chloride formed by the oxidation of trichloroethylene with the oxygen dissolved in nectars may result in the following reactions shown in Fig. 15. The chemical interaction of riboflavin with dichloroethane occurs due to the formation of hydrogen bonds with the nitrogen of the aromatic ring and the hydrogen of the alcoholic group of vitamin B₂.

The study of a change in the content of organic contaminants in nectar showed that there is a relationship between a decrease in the content of vitamins and their molecular masses and the molecular masses of the priority water contaminants: there is a greater decrease in the concentration of vitamins with a decrease in the molecular weights of vitamins and an increase in the molecular weights of the priority organic contaminants.
Fig. 14. Chemical interaction of vitamin B₁ with the priority contaminants contained in water.

Fig. 15. Chemical interaction of vitamin B₂ with the priority contaminants contained in water.
Fig. 16. Chemical interaction of vitamin B₄ with the priority contaminants contained in water.

Fig. 17. Chemical interaction of vitamin C with the priority contaminants contained in water.
CONCLUSIONS

The carried out study made it possible to establish that chloroform does not change the content of formulation components due to lack of the functional groups of beverages that are capable of interacting with beverage components. At the same time, dichloroethane and trichlorethylene have a significant effect on the resistance of the main components of non-alcoholic carbonated beverages, with the exception of dyes, and also on the color intensity and resistance of vitamins C, A, group B vitamins and nectars, interacting with them chemically, as confirmed by chemical reactions, and reducing the quality characteristics of beverages (the content of sucrose, vanillin, sodium benzoate, citric acid, tarragon infusion, vitamins and the resistance of color). Moreover, the process is most active in the initial period, their concentration does not practically change during further storage (10–20 days). This reduces the quality and duration of storage of beverages. To maintain the quality of products, it is necessary to add the additional amount of formulation components, which results in the over-consumption of raw materials and increases production costs. In addition, the presence of chloroform, trichlorethylene and dichloroethane in the beverages, which have toxic and cancerogenic properties, reduces the safety of drinks.

The carried out study showed that to provide the quality and safety of beverages, the water used for the production of non-alcoholic carbonated beverages and nectars must be previously purified of organic contaminants [24].

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REFERENCES


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