ISSN 2310-9599

КемТИПП

FOODS AND RAW MATERIALS

Vol.4, №1, 2016

The Ministry of Education and Science of the Russian Federation

Kemerovo Institute of Food Science and Technology (University)

FOODS AND RAW MATERIALS

Vol. 4, No. 1, 2016

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Published twice a year.

Founder:
Kemerovo Institute of Food Science and Technology (University), KemIFST
Stroiteley blvd. 47, Kemerovo, 650056 Russian Federation

Editorial Office, Publishing Office:
office 1212, Stroiteley blvd. 47, Kemerovo, 650056 Russian Federation,
phone/fax: +7(3842)39-68-45

http:frm-kemtipp.ru

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Printing Office:
office 2006, Institutskaya Str. 7, Kemerovo,
650002 Russian Federation,
phone: +7(3842)39-09-81

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

The Edition is registered by Federal Service for Supervision in the Sphere of Telecom, Information Technologies and Mass Communications (Media Registration Certificate PI no. FS77-61610 dated April 30, 2015)

The Journal is included in the International Databases: Emerging Sources Citation Index (Web of Science Core Collection), Scopus, Agris, CAS, FSTA, EBSCOhost, ResearchBib, Agricola, Ulrich's, Google Scholar, OCLC WorldCat, BASE.

The journal is included in the SQC list of leading peer-reviewed journals

The Edition "Foods and Raw Materials" is included in the Russian index of scientific citation (RISC) and registered in the Scientific electronic library **eLIBRARY.RU**

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Signet for publishing
June 27, 2016
Date of publishing
June 27, 2016
Circulation 300 ex.
Open price.

Subscription index:
for the unified «Russian Press» catalogue – 41672,
for the «Informnauka» catalog – 40539

Kemerovo Institute of Food Science and Technology (University), KemIFST, Stroiteley blvd. 47, Kemerovo, 650056 Russian Federation

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FOOD PRODUCTION TECHNOLOGY

REASONS FOR THE WAYS OF USING OILCAKES IN FOOD INDUSTRY

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Received April 28, 2015; Accepted in revised form December 18, 2015; Published June 27, 2016

Abstract: Using of secondary raw material resources from the oil and fat industry enterprises for new foodstuff development is an important task giving the chance of products range expansion, which are enriched in many indispensable components. The compliance with quality analysis of oilcakes from several types of olive raw materials, traditional and non-traditional for Russia, to fundamental technical requirements of the normative documents of the Russian Federation, determination of the list of quality and safety indicators of the oilcakes and reasons for the ways of processing them into food became the research purposes. Objects of researches were the oilcakes received in the conditions of specialized enterprises of Altai Krai from the appropriate types of olive raw materials. They are oilcakes made from: Siberian cedar pine kernels, walnut kernels, seeds of Cucurbita pepo, sesame seeds, black cumin seeds, flax seeds, milk thistle seeds, amaranth seeds. By research results, the list and standards of the regulated organoleptic and physical and chemical quality indicator of olive cakes are set; the permissible level of safety indices are justified and the conditional gradation on the prevailing macrocomponents defining the oilcakes nutrition value, their technological properties and the food use potential areas is recommended: 1) the composition is dominated by “proteins and lipids” (oilcakes from sesame seeds and pine nuts kernel) – mayonnaise, dairy and vegetable products; 2) “proteins and carbohydrates” (oilcake from amaranth and pumpkin seeds and oilcake from walnuts kernel) – dairy, vegetable, meat and cereal products, sugary and flour confectionery, food concentrates; 3) “proteins and alimentary fiber” (oilcake from milk thistle and flax) – bakery and flour confectionery.

Keywords: oilseed cakes, oilcakes nutritional value, oilcakes quality and safety factors, oilseed cakes using, processing algorithm of oilseed cakes

DOI 10.21179/2308-4057-2016-1-4-12 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 4–12.

INTRODUCTION

The struggle for customer and sales market stimulates a modern manufacturer to be engaged in sold products range expansion through new types of raw materials attraction and/or giving the functional directivity to worked-out production. It is connected to the fact that in recent years many enterprises of the domestic oil and fat industry process raw materials, rather various in chemical composition and technical characteristics. Walnuts, pine nuts, sesame, thistle, flax, pumpkin, water-melon, amaranth, black cumin seeds, grape and apricot seeds, sea-buckthorn fruit and other types of traditional and non-traditional for Russia olive raw materials are used.

For the last 10–15 years the considered branch has developed rather actively. It has involved a lot of new businessmen. Being guided by Internet-sources data, it is possible to say that today the number of the enterprises registered in this sphere is to one hundred. Chemical composition distinctions of raw materials allow vendors to work out a line of the vegetable oils oriented on a wide range of customers. Distinctions in technical characterristics and, respectively, in aerodynamic properties of raw materials cause the necessity of technology equipment mobile realignment that is more easily feasible in case of small outputs. As a result, preferentially small enterprises of narrow specialization are involved in the considered field of

activity, many of which work according to the diagram given in Fig. 1.

Processing of raw materials by small batches is one of the advantages of the similar enterprises, as it allows to obtain products with very low measure values of oxidizing damage, which is often cannot be achieved in the conditions of large-capacity production.

In Altai Krai oil is get from cultivated commercially flax and nuts, pumpkin, sesame, milk thistle, black cumin and amaranth seeds delivered from other regions.

Walnuts (*Júglans régia*) and Siberian cedar pine nuts (*Pinus sibirica L.*) appear to be a valuable source of oil, rich in tokoferola and indispensable fatty acids ω-6 [1, 2]. The protein which is no less important remains as a part of oilcakes almost without any changes, as walnut and pine nuts oil is produced by technology of a one-fold cold extraction.

Flax seeds (*Linum usitatissimum L.*) are considered by the modern nutritionists not only as a source of the food oil rich with α-linolenovy acid (to 57% as a part of oil), but also as an additional source of protein in soluble and insoluble alimentary fiber [3, 4]. The growing popularity of their use and researches activity as food raw materials are connected to the marked properties of flax seeds [5].

The peculiarity of a milk thistle (*Silybum marianum*) nutrition value is silimarin flavonoid content which is conducive to liver cells regeneration [6, 7, 8] and keeping in oilcakes and oilmeals after allocation from oil seeds. Nowadays thistle-and-flax-seeds flour is massively used only in flour production [9, 10].

Sesame and pumpkin seeds have a certain likeness according to content and greasy oil composition. Sesame (*Sésamum índicum L.*), seeds of which contain about 60% of oil and to 20% of protein, is traditionally applied in seeds, paste or oil form in bread baking and Eastern cuisine cooking due to a pleasant smell and taste. In physiological value sesame is compared to flax seeds [11]. Tocopherols and lignan belong to the main antioxidants responsible both for physiological value and for oxidizing damage of sesame seeds, oil and oilcakes preventing [11, 12]. Modern clinical researches are focused on allergic response to sesame and products of its processing studying [13].

Pumpkin seeds (*Cucurbita pepo L.*), depending on a botanical sort of pumpkin, contain up to 28% of protein and 30–50% of the oil rich in carotinoids and tokoferola [14]. Reduced cellulose content in gymnospermous sorts facilitates technological tasks solution of pumpkin oilcakes use remaining after allocation as food raw materials [15, 16].

Amaranth and black cumin seeds are being researched most actively in recent years. Amaranth (*Amaránthus*) "grain" is appreciated for protein [17, 18] and the increased content of squalene, phytosterols and other minor components in oil [19]. Amaranth flour from is used in national and non-traditional flour products baking [20, 21].

Black cumin seeds oil (*Nigella sativa L.*) possesses the expressed toning and antibacterial properties. This fact is connected to fat-soluble vitamins, sterols, benzopyrones and terpenes composition [22, 23]. Increased content of linoleic acid in black cumin seeds oil (up to 58%) at unusually high content of minor antioxidant components explains the growing popularity of its processed products.

In spite of the fact that today oilcakes and oil meal of oil-bearing crops, including the discussed in this work, are applied as high-protein components of vegetable feed [17, 24] the perspectives of their wide food use are connected to a possibility of giving functional properties to new products not only at the expense of proteins [25], but also at the expense of food fibers [3, 11, 26], lignin [12, 27, 28] and other components [9, 29, 30].

The characteristic peculiarity of the worked-out oilcakes is absence in scientific literature of the accurate systematized data on their chemical composition, especially that within species different botanical sorts of oil-bearing crops can initially be characterized by certain distinctions, both in coloring/form of seeds and in principal components content [5, 14]. The equipment used by the operating enterprises can significantly differ on the operating characteristics, defining production with different residual content of lipids. Marked, as well as state standards absence, regulating figures of all range of olive cakes quality as food raw materials complicates the determination of perspective areas of their industrial food processing. The purposes of the work are the analysis of quality compliance of industrially produced oilcakes made from non-traditional olive raw materials types to fundamental technical requirements of regulatory documents (RD), determination of the regulated figures list of researched oilcakes quality and safety and reasons for their industrial processing in food.

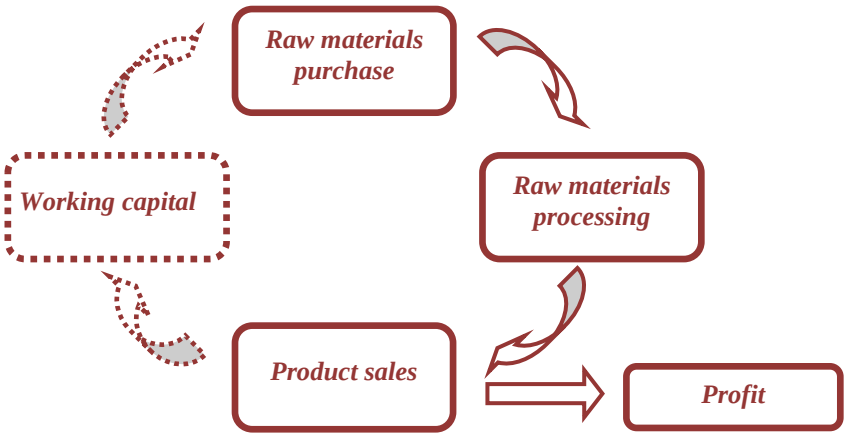

Fig. 1. Basic model of purchase, processing and selling structure of non-traditional types of olive raw materials.

OBJECTS AND METHODS OF STUDY

Tasks of the research are:

– assessment of the macrocomponents content in oilcakes composition;

– determination of the quality and safety regulated figures list;

– oilcakes systematization considering chemical composition and indices of safety data, reasons for the industrial processing in food.

Objects of the research are:

– regulatory documents on oil-bearing crops cakes of industrial value;

– oilcakes received at two specialized enterprises of Altai Krai (LLC Spetsialist, Biysk; LLC Sibirsky produkt, Barnaul) from the appropriate types of olive raw materials: oilcakes from Siberian cedar pine kernel (*Pinus sibirica Du Tour*), walnuts kernel (*Júglans régia*), pumpkin seeds (*Cucurbita pepo L.*), sesame seeds (*Sésamum índicum L.*), black cumin seeds (*Nigella sativa*), flax seeds (*Linum usitatissimum L.*), milk thistle seeds (*Silybum marianum*), amaranth seeds (*Amaránthus*).

For the theoretical material analysis methods of the comparative analysis and systematization of scientific publications and regulatory documents, periodicals and Internet resources were used. Results of own experimental studies are received with the use of standard organoleptic and instrumental methods of researches accepted in oil and fat branch.

Color, smell, look and existence of dark inclusions in oilcakes are determined by organoleptic methods.

The mass fraction of moisture and volatiles were determined by tests drying 5 g weight to constant mass at 103 ± 2°C temperatures, with the subsequent weighing and calculation of a measure value in %.

The mass fraction of a crude protein, in % of oilcake dry substance, was enumerated on the content in oilcakes of the proteinaceous nitrogen determined in hinge plates 2 g weight by Kjeldahl's method.

The mass fraction of crude fat in hinge plates 10 g weight was set by exhaustive extraction of lipidic fraction hexane method with the subsequent weighing and fraction calculation of the extracted fat in % of solid oilcake.

The mass fraction of crude cellulose was determined by Genneberg and Shtoman's method: serial processing of a hinge plate of cake by acid and alkali solutions, with subsequent combustion and quantitative fossil determination by a weight method, with index calculation in % of the dry unoiled oilcake substance.

The mass fraction of ashes was determined by tests calcinating of 5 g weight at 700°C temperature to the complete combustion (set on achievement by the crucible with a hinge plate of constant mass), with the subsequent weighing and measure value calculation in percentage of the dry unoiled oilcake substance.

The acid number of fat was determined by oilcake titration extracted from test crude (etanolo-chloroformic extraction) 0.1M KOH solution in fenolftalein presence.

The peroxide value of fat was determined by oilcake titration of the crude fat extracted from test (in the form of a mixture of acetic acid and chloroform) 0.01M $Na_2S_2O_3$ solution.

All the studies were conducted on the example of six oilcake batches of each name, in three-fold frequency for each index. Results of the researches were processed by statistic analysis method.

RESULTS AND DISCUSSION

Excerpts from operating state standards on the regulated physical and chemical indices of oil-bearing crops cakes used as raw materials for industrial production of compound feeds and foodstuff and realized as “Food oilcakes and oilmeals” are given in Table 1. It should be noted that in Russian normative documents there are no unified approaches to the list of the regulated quality figures:

– ashes is either regulated, or is excluded from the mandatory list;

– different norms of moisture, crude protein, fat and cellulose content are set;

– insoluble ashes content norms set depending on existence/absence of seed coats in oilcake are most justified.

Table 1. The regulated physical and chemical indices of olive cakes of industrial value according to regulatory documents' requirements valid in the Russian Federation

Index name	Oilcake index norm					
	sesame	sunflower	rapeseed	flax	peanut	soy
M.f. of moisture and volatiles, %	6.0–10.0	No more than 8.5	6.0–9.0	6.0–8.0	6.0–8.0	6.0–8.0
M.f. of crude fat in terms of solid, %, no more than	9.5	10.0	9.0	10.0	6.5	8.0
M.f. of crude protein in terms of solid, %, no less than	40.0	38.0	37.0	34.0	52.0	44.0
M.f. of crude cellulose in terms of the dry unoiled substance, %, no more than	6.0	20.0	16.0	9.0	5.0	5.5
M.f. of ashes in terms of the dry unoiled substance, %	–	6.2–6.8	No more than 7.0	No more than 10.0	–	–
M.f. of insoluble ashes in terms of the dry unoiled substance, %, no more than	1.0	1.0	1.5	1.5	0.2	0.6
Pass through a sieve with holes	–	–	–	–	1 mm in diameter, no more than 5%	15 mm in diameter, no less than 100%

Herewith according to normative documents' requirements quoted in table 1, any kind of oilcake which is industrially made from olive raw materials, regardless of the estimated direction of its further processing (fodder or food production) should be characterized by rather low content of fat: mass fraction (m.f.) of crude fat has to be no more than 6.5–10.0% in terms of dry solid. However when receiving oil with the help of one-fold cold molding without raw materials grinding oil output includes only free lipids fraction and the received oilcakes, as a rule, are characterized by naturally high residual oil content [31].

Oilcakes studied in this work are produced by the enterprises in two forms:

– in the form of the granules or flakes received after oil extraction;

– in the form of the powder of a certain dispersibility received after grinding of these granules or flakes. In the second case the size of particles is important to provide equal distribution of cakes in prescription compound.

All the researched oilcakes possess flavor and smell peculiar to the initial raw materials.

The olive raw materials used for oilcakes production has essential distinctions in anatomo-morphological structure therefore it is logical to expect certain discrepancies in the content of principal components of oilcakes chemical composition.

Value ranges of the regulated oilcakes quality figures are given in table 2. Moisture is in rather narrow, typical for all the studied oilcakes range (5.1–8.6%), which is caused by identical approach in oil allocation technology from raw materials. Therefore, it is possible to introduce a single standard for this index for again developed rules on oilcakes from non-traditional types of oil-bearing crops – no more than 9.0%.

On the main foodstuff content the analyzed oilcakes are characterized by the considerable variations. The crude protein content in different batches of different oilcakes types changes from 18% to 54.7% (in terms of solid matter); for six of eight oilcakes names the minimum value of this index is at least 30%. At this level it is possible to set the lower limit of crude protein content in oilcakes intended for processing as protein-bearing raw materials, including concentrates and protein isolates. Thistle oilcake which seeds are initially not rich with protein has the lowest protein content.

It is also important to note that various types of oilcakes differ among themselves on the crude fat content: in the researched batches from 5.3% to 23.9% (in terms of solid matter), in case of the main value range of 12–20%. The highest fat content among the tested samples is characterized by sesame seeds and pine nuts oilcake, which appears in lower flowability of these oilcakes as compared to other species. As oil percentage of cakes is directly linked to their technological qualities, this index norm should be set with restriction in greater party – no more than 25%: oilcakes with higher fat content oxidize quicker and turn rancid, and when grinding stick together in viscous pastelike mass and badly mix up with other free-flowing components such as flour, powdered milk, egg powder and other.

The cellulose making a basis of plant tissue cell wall is one of oilcakes macrocomponents. Several types of raw materials (cedar and walnuts) are exposed to cladding separation of seed/fruit coat or are initially almost deprived of it (gymnospermous pumpkin seeds). The result is rather low content of cellulose in the received oilcakes (from 2.2% to 5.0% for the dry unoiled substance). The content of cellulose, unrepresentative high for walnuts kernel, found in the received oilcakes (to 7.0%), is caused by complexity of these raw materials separation from husk near the kernel before oil allocation. Other types of raw materials are supplied on press without separation of a seed coat, giving oilcakes with more dark color and with higher cellulose content – up to 13.0–27.6%.

Taking into account that in recent years cellulose is considered as one of the major ingredients to develop products of functional purpose, oilcakes with the increased content of cellulose can be entered into new compounding as a source of insoluble food fibers.

For sesame oilcake the content norm of the ashes is not set by valid documentation; for sunflower oilcake the content of ashes depends on pod particles content in the oilcake; for flax oilcake which is produced without seed coat separation, the legal limit of ashes content is 10%. At the same time, for food assignment oilcakes of – soy and peanut cakes – the ashes content is not regulated (Table 1).

Oilcakes as research objects differ among themselves on the ashes content as significantly as on the remaining macrocomponents maintenance. In general, levels of ashes content correlate with existence seed coat (pumpkin seeds oilcake is an exception), but there is no correlation between the content of ashes and insoluble ashes. On the content of insoluble ashes (0.29–0.92%) all analyzed types and batches meet requirements of valid regulations to sesame and flax oilcake, and this index values do not go beyond 1% (Table 2).

Thus, on the basis of requirement analysis of the valid regulatory documents and results of own researches it is possible to designate a row of mandatory organoleptic and physical and chemical indices which can be generalized and controlled in each batch of olive cakes of food assignment. They are appearance, color and consistence, smell and favor, mass fraction of moisture and volatiles (no more than 9%), mass fraction of crude protein for cake from kernel cedar and walnut, pumpkin seeds, flax and sesame (at least 30% in terms of solid), mass fraction of crude fat (no more than 25% in terms of solid) for all types of cakes.

In our opinion, it isn't expedient to regulate in studied cakes the content of cellulose and general ashes – indices, personal for each type of grain of oil-bearing crops and, respectively, for the made cakes, but these cakes which aren't reflected in technical characteristics and making the positive contribution to formation of their nutrition value. Taking into account that on ashes it is also possible to judge existence in production of mineral impurity, as the regulated index it is necessary to enter mass fraction of ashes, insoluble into 10% solution of hydrochloric acid, and reflecting generally the content of mineral impurity, – no more than 1% in terms of solid.

Table 2. Organoleptic and physical and chemical figures of the researched olive cakes quality

Index name	Index value/Oilcake name							
	Cedar nut oilcake	Walnut oilcake	Pumpkin seeds oilcake	Sesame seeds oilcake	Black cumin seeds oilcake	Flax seeds oilcake	Milk thistle seeds oilcake	Amaranth seeds oilcake
Appearance and consistence	Whole – pieces or the dense flake granules of irregular shape. Ground – homogeneous in weight free-flowing powder with less than 0.3 mm dispersibility.							
Color	From cream to yellow and light brown, motley		From brown to brownish-green	From cream to grayish and light brown	From grayish-black to black	Different shades of brown	From brown to brown with a greenish shade	Light brown
Smell	Typical for the appropriate type of olive raw materials, neutral, without mold, musty, pro-rancid and other foreign smells							
Flavor	Typical, sweetish; the insignificant smack of bitterness typical for the husk near the kernel and not interrupting the primary flavor		Typical, insipid, sweetish		Typical, spicy and hot	Insipid, neutral	Insipid, with moderate bitterness	Typical, insipid and slightly bitter, spicy
	Without mold, musty, pro-rancid and other foreign smells							
M.f. of moisture and volatiles, %	6.0–8.5	6.0–8.2	5.5–8.2	5.8–8.0	5.4–6.4	6.9–8.6	5.1–8.3	6.7–8.2
M.f. of crude fat in terms of solid, %	16.4–21.2	12.4–18.2	11.7–19.4	16.1–23.9	18.7–19.7	10.9–19.0	14.1–16.9	5.3–11.4
M.f. of crude protein in terms of solid, %	30.2–45.3	32.2–46.4	45.6–52.3	34.9–54.7	31.9–32.9	32.6–38.1	18.0–21.1	24.7–29.6
M.f. of crude cellulose in terms of the dry unoiled substance, %	3.5–5.0	5.2–7.0	2.2–4.8	9.4–13.0	3.4–4.4	5.7–7.4	21.2–27.6	5.1–6.7
M.f. of ashes in terms of the dry unoiled substance, %	3.8–4.8	4.5–4.7	8.1–8.7	6.3–6.6	6.7–6.9	4.8–5.0	5.0–5.6	3.5–4.3
M.f. of insoluble ashes in terms of the dry unoiled substance, %	0.29–0.43	0.36–0.68	0.44–0.63	0.58–0.82	0.57–0.84	0.54–0.79	0.67–0.92	0.42–0.56

ISSN 2308-4057. Foods and Raw Materials, 2016, vol. 4, no. 1

According to microbiological standards of safety oilcakes conform to requirements of TR TS 021/2011 regarding the Application 1 and Application 2 (item 1.8 "concentrates of vegetable proteins (food) and soy flour"), on hygienic requirements of safety – regarding the Application 3 (item 9 "food meal and flour from seeds of bean, oil-bearing and non-traditional crops"), on the allowed levels of radionuclides – regarding the Application 4 (according to the item 15 "flour").

The unregulated course of the destructive, hydrolytic and oxidizing processes inherent in the destroyed cells of any olive raw materials is peculiar to oilcakes. Taking into account that the considered cakes differ in the content of quickly oxidized polyunsaturated fatty acids, additional introduction to the list of the regulated indices of safety of indices of oxidizing damage – acid and peroxide number, normed taking into account the residual content of oil in cakes is necessary.

Analysis results of the oil selected from cakes with an etanolo-chloroformic compound are provided in Table 3. According to the experimental data, for the considered row of cakes it is difficult to reveal accurate single-digit correlation between measure values of oxidizing damage of fat and composition of fatty acids. It is obvious that in case of almost equally high content of the amount of polyunsaturated fatty acids and existence of a personal set of natural antioxidants – tokoferol, terpenes and others – the speed of oxidizing processes in lipids of cakes and values of controlled indices will be preferentially defined by quality of the used raw materials (periods and storage conditions before processing) and the modes of thermal/moisture thermal treatment of raw materials provided by the exploited technology of separation of oil.

Table 3. Indices of the researched olive cakes oxidizing damage

Index name	Index value/Oilcake name							
	Cedar nut oilcake	Walnut oilcake	Pumpkin seeds oilcake	Sesame seeds oilcake	Black cumin seeds oilcake	Flax seeds oilcake	Thistle seeds oilcake	Amaranth seeds oilcake
Acid number, mg KOH/g fat	0.5–2.1	0.8–2.7	0.9–1.8	0.7–3.5	0.6–1.8	1.3–2.4	0.6–1.4	0.6–3.3
Peroxide value, millimole of active oxygen/kg fat	1.0–4.2	3.6–6.2	2.5–4.8	4.7–6.5	1.5–3.7	3.2–4.3	1.8–2.9	1.4–4.8

Fundamental factors in formation of quality of foodstuff are the raw materials and technology. In our case the choice of foodstuff group into which composition cakes can be entered should be based on data on their chemical composition.

According to literary data, cakes of several types of oil-bearing crops may contain the so-called anti-nutrients restricting their food application and capable to cause allergic responses [32]. Availability of such substances is marked, in particular, in products of processing bean and crucial cultures. However at seeds of non-traditional oil-bearing crops of the majority of sorts of the modern selection (in particular, flax, sesame and amaranth) similar components are present at trace quantities, not critical at aspect of food use of cakes. In kernel and walnuts and pumpkin seeds such components are absent.

Taking into account the experimental data, to justify food use of olive cakes the following algorithm of actions can be offered (Fig. 2).

Step I consists in complex research of cake chemical composition, with determination of the quantitative maintenance of the principal food components, the characteristic of qualitative and quantitative composition biologically of the active and anti-nutritious components, levels correlation of contents food and biologically active agents with the recommended norms of their consuming for preliminary reasons for foodstuff group.

Step II is based on results of the researches conducted at the first stage: data on the maintenance of the prevailing components of a chemical composition are used in case of a choice of the list of physical and chemical figures of merit and determination of their regulated values. Indices of toxicological and microbiological safety are set in compliance with valid TR TS 021/2011 [33], taking into account branch accessory of the considered food raw materials – regarding the Application 1 and Application 2 TP TS 021/2011 (item 1.8 "concentrates of vegetable proteins (food) and soy flour"), on hygienic safety requirements – regarding Application 3 TP TS 021/2011 (item 9 "food oilmeal and flour from bean seeds, oil-bearing and non-traditional crops").

Step III is aimed at research of oilcake influence on structural and mechanical properties of prescription masses and finished goods and, first of all, should include such operations as oilcake functional and technological properties study and value limits determination of its technical characteristics (flowability, bulk density, etc.) for the purpose of a technology equipment choice and oilcake introduction modes to prescription mass composition.

Justifying the oilcake introduction stage is carried out taking into account data about its chemical composition: regarding the maintenance of the components subject to corrupting under the influence of water and the increased temperatures, and regarding the maintenance of components, emulsifying, water and capable to retain fat properties of cake and prescription mass with its involvement. The regularities analysis of structural, mechanical and rheological

properties change of prescription mass after oilcake introduction is necessary for timely adjustment of oilcake dosage and varied parameters of technological process guiding.

This step finishes researches complex directed to justifying foodstuff group and type of as any modification connected to change of a compounding of traditional foodstuff as a result of this or that type of cake introduction will be followed by nutrition value change and the regulated figures of finished goods quality and safety. Therefore, each specific example requires not only theoretical reasons, but also the experimental confirmation of a possibility of considered oilcakes use in new foodstuff names production, with special approaches development to support technological properties of semi-finished products and a nutrition value of received production.

Step IV, the last one, consists in oilcake dosage, compounding and technology of a new product approval, design and approval of the specifications and technical documentation.

Scientific literature analysis and own experimental data show that as a part of the studied oilcakes three options of components couples can prevail:

– proteins + lipids (sesame seeds and pine nuts kernel oilcakes);

– proteins + carbohydrates (amaranth and pumpkin seeds and walnuts kernel oilcakes);

– proteins + food fibers (thistle and flax seeds oilcake).

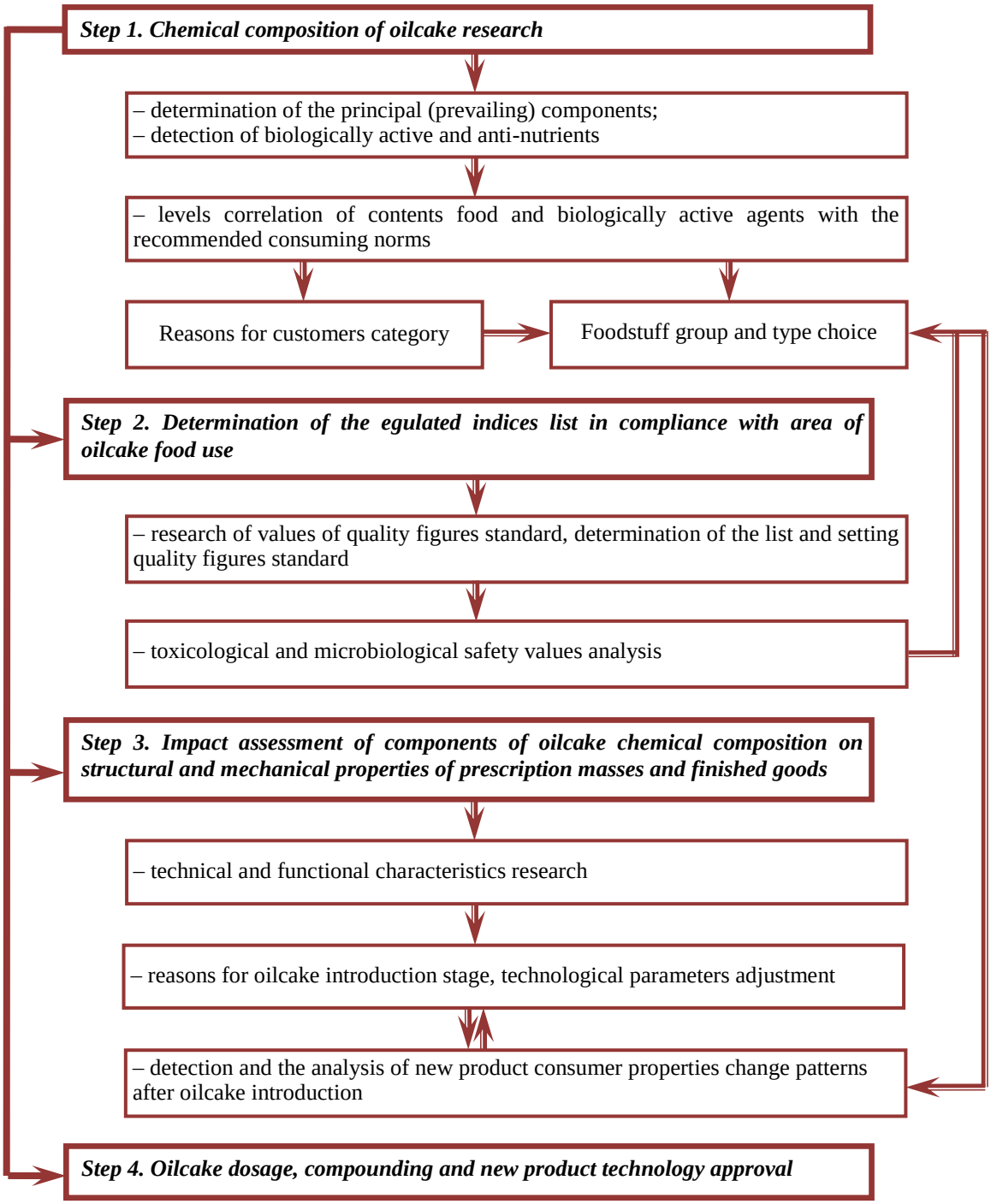

Fig. 2. Algorithm-sequence of actions in case of justifying the ways of industrial processing of oil-bearing crops cakes in food.

There are no bases to exclude that even within one type of olive raw materials for all batches of cakes the quantitative dominance of the marked couple of components will be absolute. So, for example, for black cumin seeds oilcake from a batch to a batch the prevailing couple of components will be either "proteins + lipids", or "proteins + food fibers" as black cumin seeds are processed without separation of seed coats; for pumpkin seeds oilcake and oilcake from walnuts kernel – either "proteins + carbohydrates", or "proteins + lipids". The marked inconstancy in olive cakes nutrition value of should be connected both to technological factors (used press power, initial olive raw materials preparation features), and to natural factors (in particular – maturity level of the nuts prepared for processing and oil-bearing crops seeds). foodstuff properties formation the following can be offered as the potential directions of food use of the studied oilcakes:

– in case of "proteins + lipids" dominance – mayonnaise and dairy and vegetable products. In this combination oilcake proteins will show essential for mayonnaise and dairy and vegetable products emulsifying, water and capable to retain fat properties, polyunsaturated fatty acids of cakes – to enrich products composition, and the hindering influence of carbohydrates and food fibers will be less expressed;

– in case of "proteins + carbohydrates" dominance oilcake can include some dairy and vegetable (ice cream, desserts, pastes, cottage cheese products) and meat and cereal products (for example, pasty), sugary and flour confectionery, food concentrates. In this case oilcake won't be considered as the enriching ingredient any more, only as variety of a power-intensive filler;

– in case of "proteins + food fibers" dominance the most perspective way of oilcake production use will be bakery and flour confectionery for which introduction of food fibers sources is the traditional way of compounding modification for the purpose of new product names production.

CONCLUSION

(1) The study of oilcakes chemical composition produced on the basis of new to Russia oil and fat industry, allows to create the conditional gradation taking into account the prevailing macrocomponents of a chemical composition defining a nutrition value and technological properties of oilcakes:

– proteins + lipids (oilcakes from sesame seeds and pine nuts kernel);

– proteins + carbohydrates (oilcakes from amaranth, pumpkin and walnuts kernel);

– proteins + food fibers (oilcakes from thistle and flax seeds).

By parity of reasoning other types of olive cakes can be studied and systematized.

(2) By results of fundamental consumer properties researches and a chemical composition the list and standards of the regulated organoleptic, physical and chemical figures of oilcakes from cedar, walnuts, pumpkin, sesame, black cumin, flax, milk thistle and amaranth seed the allowed levels of data security indices are justified.

(3) On the basis of that the raw materials are considered to be fundamental factors in consumer

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Please cite this article in press as: Bochkarev M.S., Egorova E.Yu., Reznichenko I.Yu., and Poznyakovskiy V.M. Reasons for the ways of using oilcakes in food industry. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 4–12. doi: 10.21179/2308-4057-2016-1-4-12.

THE PHYSICAL AND CHEMICAL CHANGES OF WATER AND THE HYDRATION OF THE PROTEIN COMPLEX IN CHEESE DURING FREEZING

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Received March 16, 2015; Accepted in revised form November 27, 2015; Published June 27, 2016

Abstract: The use of low temperatures is one of the available factors to inhibit the over-ripening of cheeses and the preservation of their quality. This study reveals the patterns and physical and chemical nature of the phase transition of water into ice, and the state of bound water, when freezing semi-hard cheeses in the range of ultra-low temperatures (-20 ... -50°C). The authors research the cheese's resistance to freezing based on the water retention capacity of the proteins. They study the factors of product stability during storage in the frozen state conditioned by a change in state of tightly bound water in the protein complex during freezing to different temperatures. The paper examines three main subclasses of semi-hard cheeses with a high, low temperature second heating which, based on fat content in dry matter, are considered fat and semi-fat cheeses. The research conducted tests to obtain the basic patterns of rapid freezing at different air conditions. The air velocity in the cooling zone was up to 10 m/s. Samples of the finely packaged cheeses weighing up to 0.2 kg were being frozen at a given volume-average temperature of -20°C. The tests allowed to obtain the data about water phase transformation into ice, depending on the values of the low temperature. The kinetics of the process has shown a gradual transition of heterogeneous water into ice in accordance with its binding energy in descending order. Based on the analysis of the experimental data, the phase diagram of water states, depending on the final volume-average temperature of frozen cheese, has been created, and the data on the degree of hydration of the protein complex in the temperature range of -20 to -70°C has been obtained.

Keywords: freezing, tightly bound moisture, low temperatures, cheese, casein, hydrophilicity, frozen water

DOI 10.21179/2308-4057-2016-1-13-18 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 13–18.

INTRODUCTION

Modern innovative technologies in the dairy industry allow to extend dairy products' shelf life and to resolve urgent challenges of the competitive production of dairy products with the highest quality and the lowest cost. The domestic and international practice has accumulated experience and has the means to prolong the shelf life of products in the technological chain, providing minimal changes in their quality parameters [1, 2]. It is important to maintain a valuable set of consumer properties in food products, their high organoleptic properties and satisfactory appearance. The use of low temperatures is one of the practically available factors to inhibit the over-ripening of cheeses and the preservation of their quality. Storage efficiency is determined by how effectively it slows down the micro-biological, biochemical and physical-chemical processes in cheeses.

The peculiarity of Russian cheese-making is that most companies produce semi-hard rennet cheeses with a low temperature second heating, mainly in the framework of the "low-cost" market segment. The most popular cheeses in the Russian market are "Rossyisky", "Gollandsky", "Poshehonsky", etc. One of the factors limiting their consumption, is brief shelf life, generally not exceeding 3–4 months.

Freezing is a solution for extension of the long-term storage of semi-hard rennet cheeses. The methods of refrigerated processing allow the creation of a long-term reserve of cheeses and to transform the supply system, thus solving the problem of preserving their quality and satisfying the needs of the Russian dairy market [2, 3, 4].

Storage at low positive temperatures does not not ensure the quality of the cheese during long-term preservation, because it does not sufficiently slow down the microbiological processes in dairy products. As of today, freezing is the best method in many respects and a promising way of extending the shelf life of food products. Ultra-low temperatures can significantly slow down the rate of the microbiological and biochemical processes that lead to product quality deterioration. Freezing has a number of benefits for the conservation of the original, natural properties of the product; it is also beneficial from an economic and energy consumption viewpoint. The low-temperature processing and storage ensure longer food preservation up to a year or more.

The results of extensive use of low positive temperatures in the food technology have become the basis of studies on the effect of negative temperatures on possible qualitative changes in cheeses and their storage in the frozen conditions. Researchers' opinions differ about it; however, the idea of long-term storage of ripened cheeses in frozen conditions prevails as the possibility of the recovery of their structure after thawing is satisfactory [1, 2].

A number of Russian scientists in the 30–50's years of the twentieth century attempted to freeze semi-hard cheeses, but it had not become a basis for the development of the detailed methods and the technology of the low-temperature food storage. In some countries of the southern regions (Greece, Turkey, Italy, Spain) the research succeeded to develop the technology of the deep freezing and the storage of their traditional cheeses and curd made from cow, goat and sheep milk [3].

The analysis of the research on the rapid freezing and storage of frozen dairy products showed that the possibility of long-term storage of frozen semi-hard cheeses is least studied. A number of Russian researchers today (Moscow, Uglich) froze hard rennet cheese, and their research findings allowed them to infer the rational regime parameters of its freezing and thawing [6].

Kemerovo Institute of Food Science and Technology (University) has been conducting a study on the low-temperature storage of dairy products, in particular cheeses of different types, over the past 25 years.

When freezing, the modes and methods of refrigerated treatment are crucial in preserving the original properties of the products. The phase transition of water into ice can damage the original internal structure of the cheese, and thus reduce its qualitative characteristics.

The purpose of this study is to develop the theoretical foundations of the physical and chemical nature of the freezing of semi-hard rennet cheeses in order to increase their shelf life.

OBJECTS AND METHODS OF STUDY

We studied three main subclasses of semi-hard cheeses with a high, low-temperature second heating and cheddaring curd of a fat and semi-fat type, depending on the mass fraction of the fat in the dry matter. The geometrical dimensions met the standard to ripen cheeses of different shapes. To get finely packaged cheeses, we cut ripened cheese bars and heads into portions weighing 0.1–0.2 kg before testing.

To study the freezing process, a test bench was set up, designed to change and to maintain the air temperature in the chamber to minus 100°C and a flow rate of 10 m/s. The monitoring of the temperature in the chamber, the tunnel and the tested samples during freezing was carried out according to the indications of the automatic electronic potentiometer (PCB-4 with a scale of 40 to -200°C, accuracy class 0.5). As the sensor, we used a Chromel-Copel thermocouple junction with a diameter of 0.3×10^{-3} m.

The cheese samples were packaged in the plastic wraps and bags of a new generation of Cryovac VV3U type.

The freezing was carried out at various ambient air conditions in the range -20 ... -50°C. The air velocity in the cooling zone was measured by a hot-wire anemometer (Testo 405-v1) with a measuring range of 0..15 m/s, and a scale of division of 0.1 m/s. The test samples were placed onto freezer shelves simulating a commercial freezer. The samples were frozen down from an initial temperature of 20°C to a pre-set volume-average temperature of -20°C and -12°C. Thermal images of the freezing were the main testing instruments during the heat-exchange experiments. They helped to determine the basic indicators of the process – the duration and the average freezing rate. The air temperature at 0 to -3°C in the refrigerator served as control storage conditions.

We have been examining the quality of the tested samples before the freezing and during the refrigerated storage over 18 months with a sampling frequency of every 3 months. Prior to sampling, the samples were thawed out at a room temperature in air of 0 ... 3°C.

To evaluate the properties of the original product at all stages of its low-temperature storage, we determined a set of quality indicators. We used the conventional and original research methods, including the physical-chemical, microbiological, biochemical and other methods.

RESULTS AND DISCUSSION

When considering the factors of product stability during its storage in a frozen form, it is necessary to single out the content and the properties of the protein fraction in cheeses, as the most significant component. A high proportion of milk protein in their composition (from 23 to 29%) leads to the high water absorption and water-binding properties of the curd [5]. The hydrophilic properties of the casein determine the steadiness of the protein particles to freezing. Unwanted loss of water upon freezing and during the storage can lead to protein aggregation at low temperatures.

The water absorption properties affect the structural and mechanical characteristics of the product's consistency; in this connection, the degree of hydration of the protein complex is one of the most important physical and chemical factors in assessing the impact of the cold on the frozen product. Low protein hydration is one of the causes of the texture's defects: not elastic enough, crumbly, powdery [5]. For frozen products, the preservation of the degree of the hydration of the curd and consequently, of its satisfactory texture, is a matter of paramount importance.

The amount of bound water is a criterion of the changes in the cheese protein complex. The polar groups of the protein molecules -COOH, -OH, >CO-NH<, NH_2-, -SH, and others are laid in several layers around the hydrophilic centers of the protein molecules, forming a so-called hydration (water) shell (Fig. 1). This tightly-bound portion of the water is strongly compressed at the surface of the protein, and therefore it is very difficult to remove. The cold

destroys the adsorbed layers of water, especially those that are at a greater distance from the surface of the molecule. Thus, casein micelle stability in frozen cheese will be determined by the strength of the hydration shell.

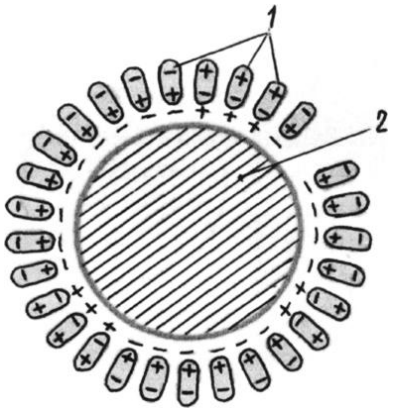


Fig. 1. The chart of the hydration shell: 1 – the water molecules; 2 – protein particles.

In determining the hydration characteristics of a combining form of the casein complex, we used P.A. Rehbinder's classification scheme, R.I. Ramanauskas' methods and recommendations, as well as the graphic differentiation of the thermal images of drying [7, 8]. We determined the equilibrium binding energy (E) in the dependence on the temperature of the frozen cheese. For the calculations, we used the D.G. Ryutov's formula. It is known that the kinetics of the freezing process is a gradual transition of the heterogeneous water into ice in accordance with its binding energy in descending order of [1].

The data analysis showed that due to the temperature lowering in cheeses in the frozen system, a portion of the water remains unfrozen, and it has a high binding energy with the dry substances (Table 1).

Table 1. The binding energy of water in frozen cheeses

Temperature of the product, ºC	Parameters	
	L, кJ/кg	E, кJ/кg
-5	323.5	5.92
-10	313.4	11.40
-20	300.5	21.80
-30	271.0	29.76
-40	256.1	37.13
-50	229.2	41.95
-60	222.3	48.80
-70	206.4	55.00
-80	191.5	58.60
-90	177.4	68.30
-100	163.2	76.00

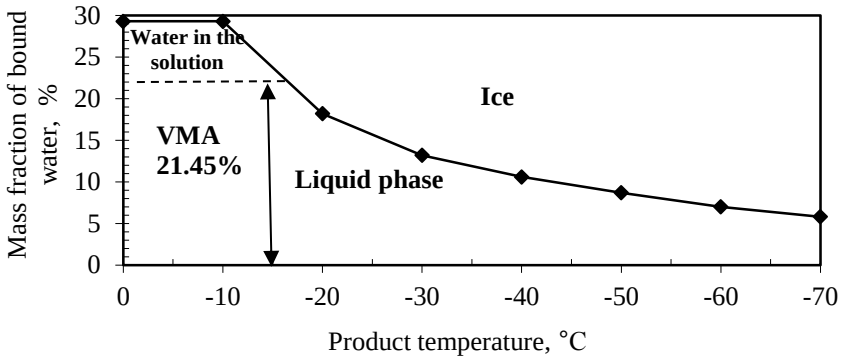
The process of water freezing goes from low to high-energy forms of the water bond. Low temperatures do not break the chemical bond between the product and the moisture, and so it is hard to completely remove the moisture. At temperatures below minus 70÷80°C the chemical bond between the moisture and the proteins is the strongest, and thus it does not turn into ice. We studied the resistance of a combining form of casein complex to freezing by its ability to bind moisture. While the proportion of bound water in the protein matrices structure remains the same, the stability of the protein molecules in the frozen cheese increases. We considered the degree of water freezing based on its unavailability for chemical reactions and on the preservation of the monomolecular layer around the protein macromolecules [9]. The results of the research on the change in the water states in the cheeses at low temperatures were used to design the phase diagram of the water solutions in cheeses.

Fig. 2 shows the effect of the freeze modes on the change of the amount of the most tightly bound forms of the water with proteins, i.e., on the hydration of the cheeses' combining form of casein complexes. The amount of moisture of the mono- and multilayer adsorption in the non-freezing water phase served as a criterion of change in the cheeses' protein complex. The kinetics of the bound water's slow freezing is shown as the product's temperature decreases. The freezing mechanism is such that at -10°C the amount of bound moisture has not changed due to the removal from the cheese of unbound moisture, loosely kept together by proteins (capillary moisture). Thus, the water modifies its physical state (transition into ice) in a relatively loose form (micro capillaries and coarse cheese pores) with a low binding energy (E = 11.4 kJ/kg) with the dry cheese matter.

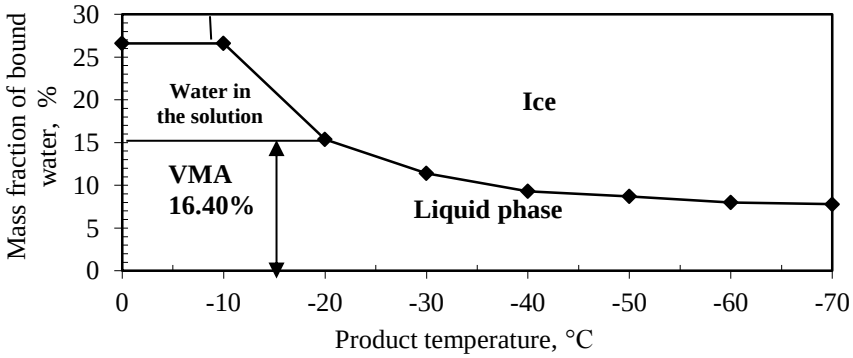
At the stage of the cheese freezing to -20°C, the moisture of the various binding forms transforms into the crystalline form, except for the tightly-bound moisture of the mono- and multi molecular adsorption. Under these conditions, the freezing energy of water binding with the polar groups of proteins is higher than the energy released during the crystallization and turning into ice.

For example, in the cheese "Sovetsky", 18.2% of the liquid phase was recorded at that temperature, and this quantitatively corresponds to the degree of the cheeses' hydration in the form of bound by absorption moisture. However, we noticed the beginning of the decrease in the amount of that water, which could be due to the crystallization of the diffusion layer of the water of multilayer adsorption.

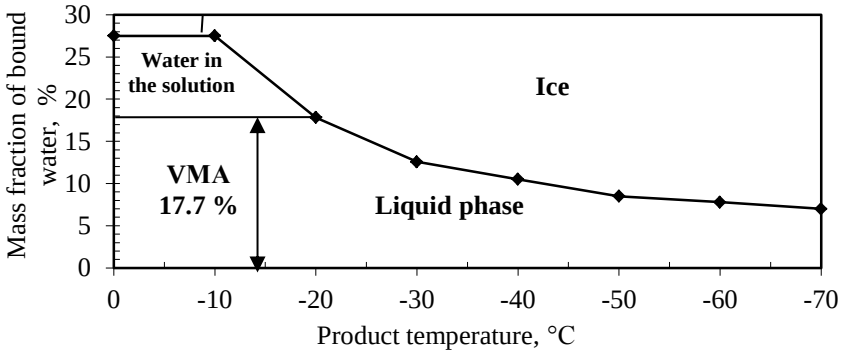
Based on the energy images of cheese "Sovetsky", P.F. Krasheninin, N.I. Gamayunov and V.P. Tabachnikov noted that 15% of the moisture in the cheese mass corresponds to the moisture's maximum binding energy at which the water molecules are unable to separate from the protein mass [7]. Apparently, in this connection, when the temperature in the centre of the frozen product reached -30°C, 13.2% of the water remained unfrozen, and it retained the hydrate shell around the micelles of casein (Fig. 2). In the subsequent phases of the freezing, the water tightly bound by the proteins in the form of MMA (moisture-monomolecular adsorption) was gradually freezing out, turning into ice. Further lowering of the product temperature below -30°C (-40°C, -50°C, etc.) leads to a quantitative reduction of the liquid phase of the bound water (VMA) and thus dehydrates the cheese protein complex, especially in the semi-hard cheeses with a low temperature of second heating.



(a)



(b)



(c)

Fig. 2. Change in the amount and physical condition of the bound water in the cheese depending on the freezing temperature (BMA – related moisture adsorption): (a) Group of "Sovetsky" cheeses, (b) Group of "Rossyisky" cheeses, and (c) Group of "Gollandsky" cheeses.

In these cases, the protein macromolecules lost retained water, and they were willing to participate in the new intermolecular bonds. In such a state intermolecular protein aggregation is possible. It seems like this happened in our experiments, when at low temperatures (below -40°C) we observed structural changes in cheese consistency. Thus, freezing the product at the average volume temperature of -18÷-20°C allowed for the best preservation of the hydration of the combined casein complex. The values of the ultimate temperatures for the freezing of the product are sufficient at -20 (± 2)°C, to maintain the hydration of the cheese's proteins without affecting its structure. We observed the same results when we analyzed the changes in the bound water state during the freezing of the cheeses of the "Gollandsky" type. Figure 2 shows that the amount of moisture, tightly bound by the curd proteins, remained unchanged during the freezing down to -20°C. In the other freeze modes the proteins were slowly losing a small amount of water due to its tendency to crystallize. In this case, the amount of bound water or its degree of hydration served as the criterion for evaluating changes in the protein complex of the frozen cheese. Figure 2 shows that the bound by proteins moisture does not crystallize at -20°C, and its physical properties remain unchanged. This temperature suits the maximum level of the conservation of the proteins' structure. The amount of adsorption-bound moisture (BMA) under these conditions is consistent with the maximum hydration of

the protein complex and is equal to: 21.45% – in "Sovetsky" cheese, 12.76% – in "Rossyisky" cheese, 17.7% – in "Gollandsky" cheese.

Research results show that the degree of the crystallization of the water regulates changes in the product. Thus, at the average volume temperature of average -20°C, the proteins' water-holding capacity and the curd's hydrophilic properties were preserved. When freezing to a lower temperature, resulting in an unwanted transition of the micelle-bound water into ice, there were structural changes that led to the appearance of an extra elastic and crumbly consistency of the cheese. An analysis of the data on the cheese's durability in storage showed that products stored at a temperature of -20 (± 2)°C, had the longest shelf life and the most satisfactory consistency. In this freezing condition there were no factors damaging the protein complex (Fig. 3).

The analysis of the graphs in Fig. 3 shows that in this mode the speed of the chemical reactions is significantly reduced. The main factors in this case are: 1) the presence of unfrozen water (18.3%) in the form of a layer of the polarized water molecules in the form of a monolayer adsorption (BMA), which has a low activity and is not available for the microorganisms' activity; 2) the lack of water which is a solvent with a high concentrations of solutes, that causes the denaturation changes in the proteins. In this freezing mode (volume average -20°C) the hydration of the proteins and hence the water-holding ability of the curd is retained in full. We found that the cheeses had defects in the consistency, caused by the dehydration of the protein complex during the freezing to the lower ultimate temperatures (-30 ... -40°C). The cheeses frozen to a temperature of minus 20°C had the best quality. In this mode, the unfrozen water (18.3%) with the 31.2% concentration in the form of the moisture of the physical and chemical bond, was the most tightly-held by the cheese's protein complex.

Because this type of moisture can't be a solvent, we can assume that the freezing conditions, the physical and chemical changes of the aqueous phase (the viscosity, the degree of mobility of the solutes) will not affect the native structure of the proteins during freezing and storage, unlike in other freezing modes. The preserved hydrophilicity of the proteins frozen to -20°C resulted in a cheese with good texture and water binding capacity. It should be noted that this allows for a fairly low water activity of Aw = 0.67–0.70, which is below the minimum required for microbial activity [10]. Strong energy of water interaction with the protein macromolecules characterizes a low mobility of the adsorbed water molecules in the non-crystallized low-temperature phase. Therefore, due to the lack of a source of enzymatic decay during storage the cheeses' durability will increase significantly.

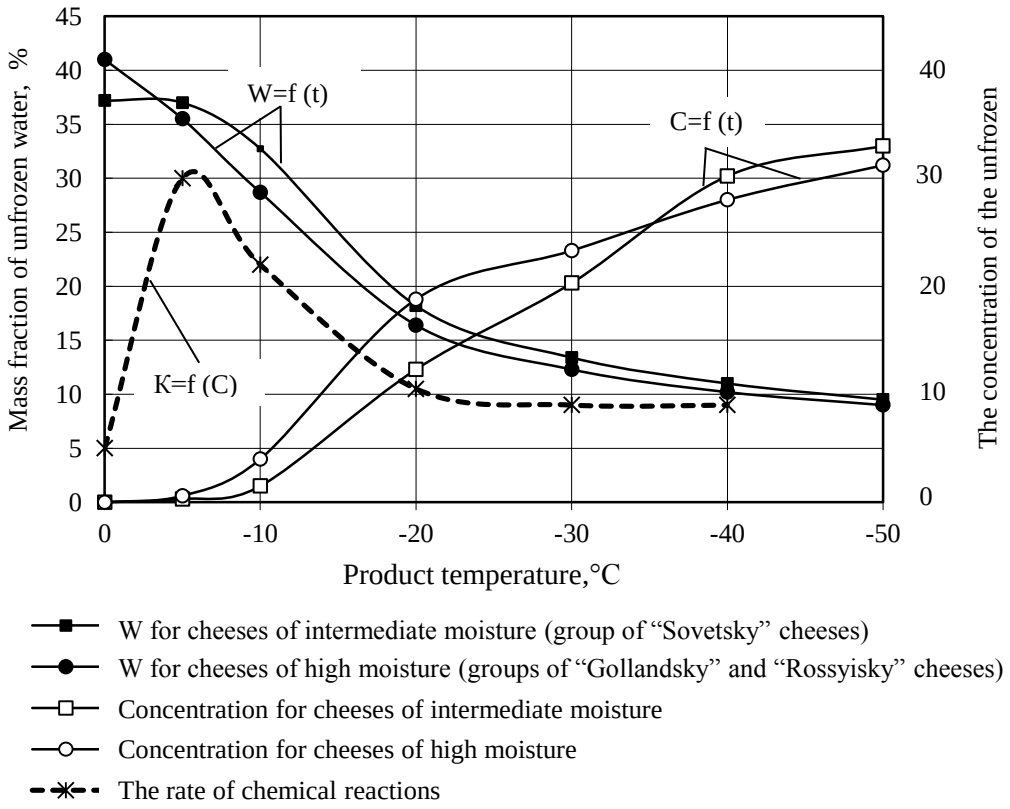


Fig. 3. Factors of stability of deep-frozen cheeses during storage.

Moreover, the changing of the pH to a level of 5.3–5.5 corresponds to a zone of maximum swelling of the proteins, which suggests saving of the water-binding capacity in the curd's proteins. Other freezing modes resulted in poorer chesses' properties due to the chemical reactions caused by a high concentration of the unfrozen solution and by the freezing out of the bound water. Thus, as the proportion of the bound water in the protein matrix structure remains unchanged, the stability of the protein molecules in the frozen cheeses increases. Therefore, by freezing to -20°C the hydration of the protein complex remains unchanged, and the frozen cheeses have high organoleptic properties.

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Please cite this article in press as: Buaynov O.N. and Buaynova I.V. The physical and chemical changes of water and the hydration of the protein complex in cheese during freezing. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 13–18. doi: 10.21179/2308-4057-2016-1-13-18.

STUDIES OF SOME ASPECTS IN THE PROCESS OF AROMA RESTORATION

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Received April 28, 2015; Accepted in revised form December 18, 2015; Published June 27, 2016

Abstract: The lack of data about the polydisperse nature of distillates and the impact of separate micro particles to recovery of the natural flavor in food still does not have a solution. Such properties of the distillates as dispersion and the size of the micro particles using a Zetasizer Software 7.11 are discussed at the article for the first time. It is established that the hydrodynamic diameter of the particles in the distillate values from 200 nm to 600 nm. Changes of the hydrodynamic particles size in distillate by water dilution confirms the assumptions about their hydrophobic nature and availability of results of such processes as coacervation, hydrophobic hydration, hydrophobic interaction. The differences in sensory characteristics to some extent is confirmed by the differences in the average hydrodynamic diameter of the sample: 150 nm and 190 nm, in the laboratory and industrial respectively. The interrelation between the sensory characteristics of fruit distillates, dispersion and method of heat treatment of fruits in the convective and microwave field is shown. The differences in the shades of the fruit flavours of melon and cucumber in the fruit distillates, manifested in the isomerization of the components of the flavour are given. It is shown that aroma restoration differs in different mediums by pH. In an acidic medium (pH = 3.0) converting of acetals of cucumber distillates to aldehydes leads to the full restoration of the fresh scent, because aldehydes are key components. In subacid medium (pH = 6.0), positive changes of an aroma are made to the components of melon distillate. These results contribute to the economic competitiveness of distillates compared with other types of flavouring materials.

Keywords: aroma, distillate, esters, acetals, dispersion, isomerization

DOI 10.21179/2308-4057-2016-1-19-26 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 19–26.

INTRODUCTION

It is believed that the restoration of flavour – a return of aromatic components lost during the juice concentration [1, 2, 3]. Aromatic components are caught during evaporation of water from the juice or other products, and is collected in the distillate. Flavouring materials in the form of concentrated distillates are added to the final product to restore the original flavours and aromas, as technological processing of food and beverages alter their flavours [4]. Distillates are solutions obtained by separating the liquid components of the aroma by the boiling points or the separation of liquid from substances difficult to evaporate, i.e. distillation [5]. To distillate most of the aromatic substances from pome fruit juices it is necessary to evaporate about 10% of moisture under atmospheric pressure, and from 15% to 85% under vacuum [6]. Concentrated distillates (FTNF, WONF) are in demand in the food industry due to long-term storage and their small volume.

The concentration of aromatic components during diffuse evaporation through the membrane, when only the key aroma compounds, obtained from natural sources, but not all are concentrated, got intensive development in pervaporation processes [7, 8]. The use of such concentrated flavours has such disadvantage as the lack of natural original flavour during the recovery process. This problem is also related to the difficulty of preserving the relative concentrations of various aromatic compounds, their proportions. According to the published facts, none of the existing methods of concentration is not able to accurately restore the original taste and flavour. These methods are improving and, nevertheless, there remains a need to further adjustment of the flavour during restoration process [9]. The lack of data about the polydisperse nature of distillates and the impact of separate micro particles on flavour restoration has led to this study, as a full-fledged restoration of the natural flavour in food products remains the problem, which is not completely solved.

The process of restoring the flavour in fermented beverages with low calorie content is of interest [10]. Ethanol is removed from these beverages by dialysis.

Then, the alcohol is separated from the dialysis liquid by vacuum distillation, and the remaining liquid with flavours is recycled to the main product. The fragrance in end products such as alcohol free wine remains in the initial concentration and is quite impressive as before the distillation. In most cases, the addition of only key components does not solve the problem of aroma restoration, and the use of distillates for this purpose requires further development.

The purpose of the research is to study the dispersion of particles of distillates, their stability and impact on the sensory characteristics. Realization of this goal will let determine the properties of some components of the distillate, the ability of target restoration of key aromas in the different pH mediums and assess the impact of isomerization during the restoration of melon and cucumber aromas.

OBJECTS AND METHODS OF STUDY

Distillates were obtained in the laboratory vacuum plant with microwave heating [11, 12] and the condensation of exhaust gases. The second laboratory vacuum plant is made similar to the first one, but with a convective heating. The process of vacuum distillation was performed at 10 kPa until the volume of homogenates was reduce by 20%. Microwave heating was performed under the power of 0.4 kW, convective heating under the temperature of 40°C. In laboratory plants the distance from the evaporator to the condenser – 450 mm. Concentration of the distillates was carried out by the addition of magnesium sulphate to oversaturation.

Homogenates for obtaining the distillates were prepared from fruit pomace (melon, cucumber), flavour precursors (linoleic and linolenic acid), aqueous extracts of enzymes from plants (soy lipoxygenase), a buffer solution, according to the developed method of the biosynthesis of flavour with shades of fresh fruit [13]. The mechanism of formation of aromatic components from homogenates is described with the help of such reactions as: oxidation of flavour precursor to 9,13-hydroperoxides, hydroperoxides cleavage to alcohols and aldehydes with six to nine carbon atoms, ester formation (from the acids and alcohols), acetals (from aldehydes and alcohols) [14].

The characterization of distillates was carried out according to the following parameters:

– The distribution of size of the colloidal fraction was made on the analyser Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) with a detection angle 173°. All measurements were performed in a temperature-controlled cell by 25°C using ditch/cuvette DTS0012. At least five replicate measurements were made to control results repeatability on each sample. Size distribution in terms of intensity was obtained from the analysis of the correlation functions using an algorithm of General-purpose software analyser Zetasizer Software 7.11.

– The weight content of aromatic substances (was determined by dichromate method according to the quantity consumed by the titration of sodium thiosulfate);

– The concentration of aldehyde (was obtained with the help of spectrophotometer by the number of oxidized 2,4-dinitrophenylhydrazine);

– The number of aroma – the quotient of the mass concentration of the substance on its threshold concentration. The threshold concentration was determined organoleptically;

– Organoleptic, based on the grouping of samples of conventional categories, by the method of distribution. Categories of samples were based on a difference threshold – the minimum perceptible change of the intensity of the stimulus, i.e., availability of flavour shades.

10 women of different age took part in the sensory analysis. Round-table discussions were conducted prior to the beginning of the test to familiarize the panel with the test instructions and protocol. Partitioned booths were located in a temperature-controlled environment (20°C) and equipped with fluorescent lights. Minimal distractions were permitted to interfere with panellist judgments [15].

RESULTS AND DISCUSSIONS

Physicochemical parameters and sensory evaluation of distillates is an important indicator of the processes of production of alcoholic beverages, juices [16]. The distillate, formed during drying, stores volatile components isolated from the raw material in the airspace [17, 18]. Mainly ethers, alcohols, aldehydes, acids, which are transformed during the distillation into the distillate, represent volatile components of melon and cucumber. Due to condensation aldehydes, alcohols, acids dissolve and esters, lactones, diethers of hydrates aldehydes (acetals) in the obtained distillates are in the form of insoluble micro particles. These micro particles are soluble in alcohols, organic solvents and create definite dispersion in aqueous medium. The number of carbon atoms in insoluble micro particles affects their molecular weight, size, and aroma, which can serve as a specific identifier in the studies. Sizing esters and acetals in distillates is a promising direction in the analysis of the processes of separation of volatile components during the distillation process.

Odour of esters, acetals, and lactones depends on the number of carbon atoms in the starting compounds. Aliphatic esters in different combinations play an important role in many fruit aromas and in addition carry the floral-fruit, caramel flavours, and a variety of shades: apple, strawberry, pear, pineapple and others [19]. The major aroma components of the melon distillate are esters of hexyl octanoate hexyl acetate, propyl acetate, ethyl butyrate [20].

The main cucumber odor components are water-soluble aldehydes (E, Z)-2,6-nonadienal and (E)-2-nonenal [21]. Aldehydes are highly reactive components and together with alcohols they create a strong scent of fresh fruitage, vegetables, new-mown grass [22– 24]. However, the share of the ester (cis-3-hexenilacetate) is about 40% of the total number of aromatic components in the new-mown grass odour. Insoluble acetals, which are typical for the scent of cucumber, are represented by (E, Z)-2,6-nonadienal diethyl acetal, di-(Z)-3-hexen-1-yl acetal [25]. Cucumber distillates gain their aromatic

trace due to insoluble ester ethyl 3-(methylthio) propionate and divinyl esters of PUFA.

The chromatographic analysis of melon and cucumber distillates, has showed the presence of complex esters and acetals about 18–20%; their composition was somewhat different depending on the type of fruit, maturity stage and growing conditions. Study of distillates during convective distillation showed the presence of water-insoluble particles with different hydrodynamic diameter (Fig. 1).

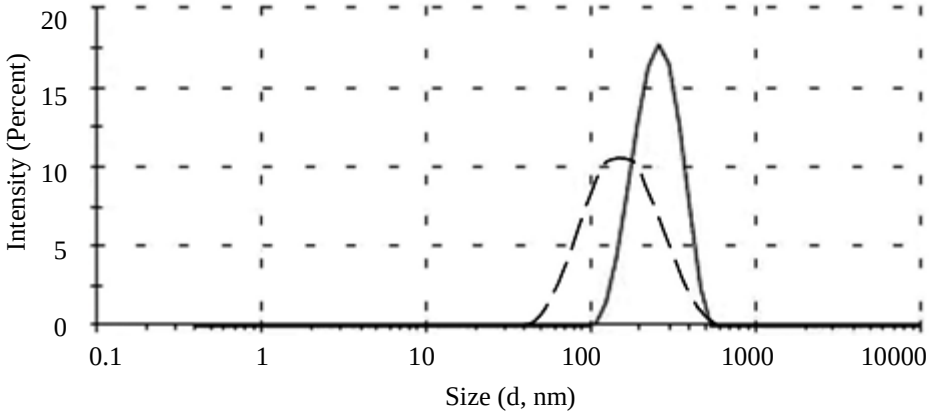

Fig. 1. Distribution of distillates particle by size.

The molecular weight of acetal in the cucumber distillate on average is slightly more than the esters in melon, which is reflected in particles polydispersion characteristics. Hydrodynamic diameter of particles in cucumber distillate is 336 ± 36 nm with a polydispersion 0.436 ± 0.083. Esters in melon distillate have particles sizes 153 ± 2 nm and a polydispersion 0.211 ± 0.022. The molecular weight of acetals (E, Z)-2,6-nonadienal diethyl acetal, di-(Z)-3-hexen-1-yl acetal is 212.3 and 226.4, respectively, and esters in melon on average is 118.2 (Burdock, 2009). Due to the higher molecular weight, most acetals have negligible odour or are odourless. Flavour intensity in the melon distillate was greater than in cucumber, which corresponds to the difference in their polydispersion. It should be noted that, depending on the variety, growing location, maturity, storage conditions and other factors, the numerical values of the hydrodynamic diameter in cucumber and melon distillate could take other values different from the above. Taking into consideration that described the interrelations remains the same.

It was found out that not one, but several peaks and correspondingly different values: 200 nm, 300 nm, 400 nm and 600 nm are observed under repeated measurements of the hydrodynamic diameter of the particles in the distillates. This indicates that the micro particles have an irregular shape in the form of droplets or micelles. Studying the behaviour of micro particles at a dilution in water and alcohol the hypothesis about droplet particle structure and hydrophobic nature is confirm. For example, cucumber distillate stirring on a magnetic stirrer causes a change in the hydrodynamic size from 456 ± 121 nm and a polydispersion 0.546 ± 0.058, to 273 ± 18 nm and the polydispersion 0.454 ± 0.049. These changes are the result of intermolecular interactions and thus depend on the molecular level on the behaviour of the dissolved substance.

Hydrophobic groups added solutes interact weakly with neighbouring water molecules, as if they prefer a non-aqueous medium. However, these weak interactions can have profound structural aftermath [26, 27]. Special structures formation in water near incompatible nonpolar substances is called "hydrophobic hydration" (Fig. 2, Table 1). If there are two isolated non-polar groups, they are incompatible with an aqueous medium and it encourages their association, thereby reducing interfacial surface "water-nonpolar substance". This process is thermodynamically favourable, partly reverse to hydrophobic hydration, and is called "hydrophobic interaction" [28].

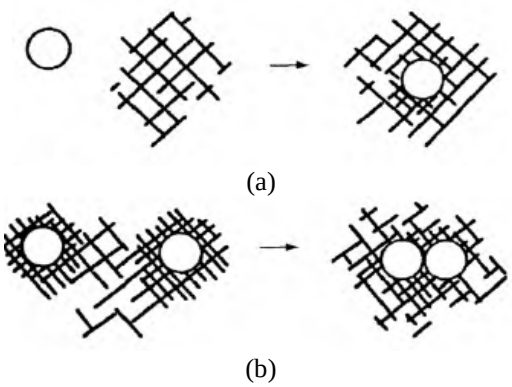

Fig. 2. Schematic map of hydrophobic hydration (a) and hydrophobic association (b). Circles represent hydrophobic groups, and the shaded area – water [29].

Research and visualization of processes of hydrophobic hydration, the hydrophobic interaction of the distillates was carried out at dilution of distillates in bidistilled water at a ratio of distillate: water 1 : 1, 1 : 10, 1 : 20, and 1 : 40 at 25°C (Table 1). Taking into the consideration that the behaviour and size of the micro particles depend on the concentration and nature of the solvent, the samples of distillates were mixed with an alcohol (96% vol) at a concentration of 0.1% under the temperature of 25°C (Fig. 3.1–3.4).

Table 1. Characteristics of Distillates

Name	Melon Distillate	Cucumber Distillate
Size of the particles, nm		
Dilution (water) 1 : 1 Dilution (water) 1 : 10 Dilution (water) 1 : 20 Dilution (water) 1 : 40	283 ± 51 246 ± 31 310 ± 14 390 ± 22	282 ± 20 253 ± 51 260 ± 28 291 ± 31
Odour		
Concentrated distillate (pH = 7)	Melon, with honey shade	Vegetable, with mushroom and cucumber shades
Distillate (pH 6.0)	Fruit with saturated/rich melon shade	Cucumber with vegetable shades
Distillate (pH 3.5)	Melon with extrinsic shades	Fresh saturated/rich cucumber

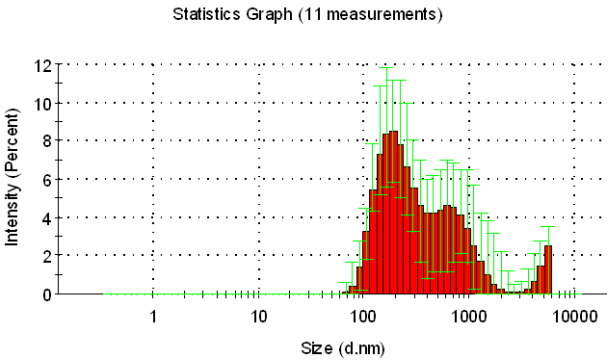

Fig. 3.1. Melon distillate (with an alcohol).

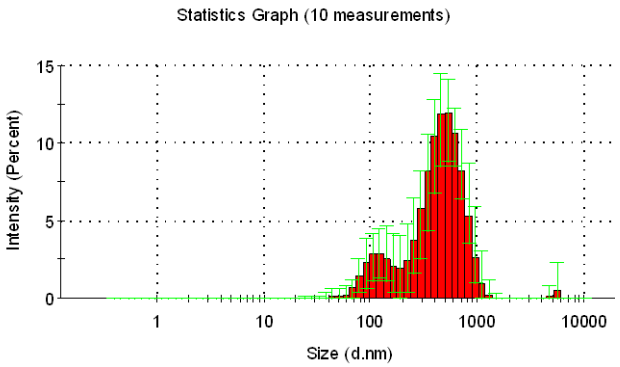

Fig. 3.2. Mellon distillate with changed pH (citric acid solution, 1%).

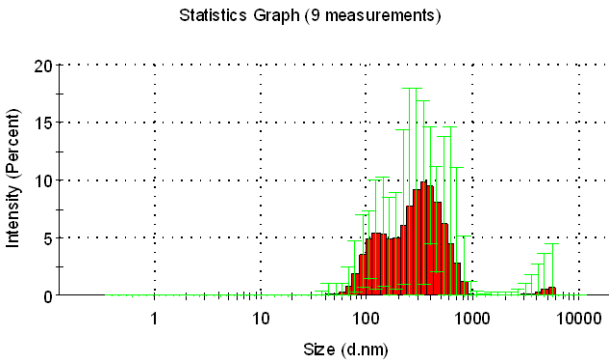

Fig. 3.3. Cucumber distillate (with an alcohol).

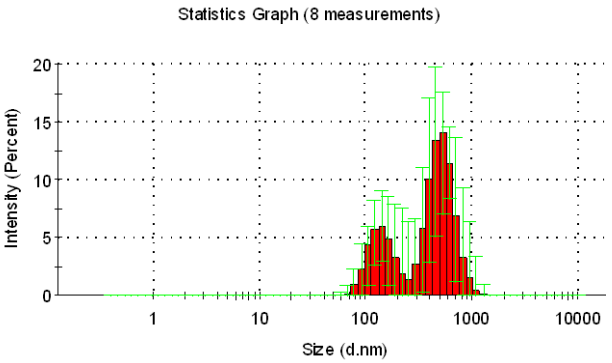

Fig. 3.4. Cucumber distillate with changed pH (citric acid solution, 1%).

Changing of the hydrodynamic size and the polydispersion of the droplet micro particles in an alcoholic solution shown in the figures indicates a coacervation process. The body is separated into two factions with different hydrodynamic size in melon and cucumber distillates. Larger droplets are coacervate – multimolecular complex or drops with a higher concentration of the colloid (dissolved substance) than the rest of the solution of the same chemical composition.

The initial pH of distillates is 7, aroma is conveyed with an intensity similar to the fruit. To analyse the aroma nuances, when changing the pH, the distillate was concentrated, as with different pH values qualitative and quantitative changes of flavour occur. Together with the pH, the perception of the same volatile compounds in different food mediums also changes [30]. Acetals and esters are converted to the starting aldehydes, acids, alcohols in the acid and alkaline medium. The change of the scent was analysed

in mildly subacid medium pH = 6.0 and acid medium pH = 3.5 during the research. Acidity was adjusted with a certain concentration of citric acid. Salts of corresponding acids are formed in an alkaline medium, which bind aromatic components and help to remove the odour from the analysed medium, which contradicts the objectives of the study.

Changes of the hydrodynamic particles size in distillate by water dilution confirms the above assumptions about their hydrophobic nature and availability of results of such processes as coacervation, hydrophobic hydration, hydrophobic interaction. Since water and nonpolar groups exist in an antagonistic relationship, the structure of the water is adapted to minimize contact with the nonpolar groups. Two aspects of this antagonistic relationship are consider: the formation of clathrate hydrates and the association of water with hydrophobic groups [26].

Concentration of distillate allowed feeling better the flavour of samples and giving them a characteristic that serves as a comparison standard. Changing the pH of the medium and the nature of the solvent effect on the organoleptic characteristics of distillate in the distillation [31]. Substantial transformation in acid medium are recorded in cucumber samples. Admittedly acetals (E, Z)-2,6-nonadienal diethyl acetal, di-(Z)-3-hexen-1-yl acetal were converted to the aldehydes from which (E, Z)-2,6-nonadienal, (E)-2-nonenal, and cis-3-hexenal were formed. The changes of pH of the medium in melon distillate also led to a variety of aromatic reactions: in subacid medium melon flavour was more intense and vivid, and in the acid medium is less expressed, with prevalence of non-typical shades.

Liquid melon flavouring material of the firm "GLCS Co" was analysed according to the size of the micro particles in a concentrated form and after dilution 1 : 10 and 1 : 100 as in the previous samples (Fig. 4).

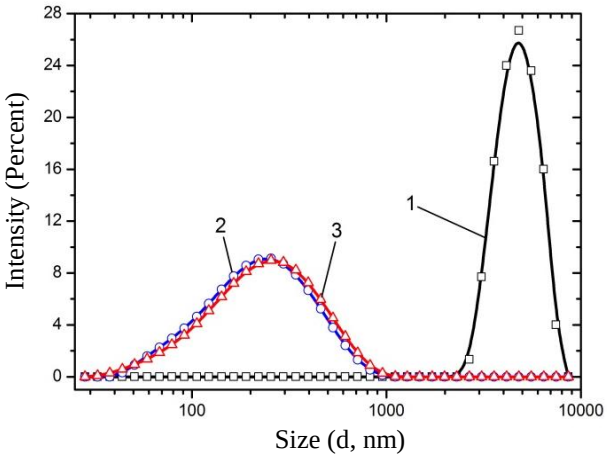

Fig. 4. Changing the size of ether particles in the industry melon flavouring material: 1 – in concentrated form; 2 – at a 1 : 10 dilution; 3 – at a dilution 1 : 100.

Analysis of the obtained results shows that, after dilution 1 : 10 of the concentrate of the melon odour dimensional characteristics has changed downward of the hydrodynamic diameter from 4.138 ± 0.274 mm to 185 ± 2.5 nm, the polydispersion has slightly increased from 0.220 ± 0.057 to 0.253 ± 0.014. Further dilution 1 : 100 in fact does not change the hydrodynamic diameter (196 ± 24 nm), but there was a slight increase in the polydispersion to 0.327 ± 0.148, which confirms the homogeneity of the fractional composition of the samples.

Changes of the hydrodynamic diameter at a dilution 1 : 10 and 1 : 100, can be associated with the presence of the solvent 1,2-propylene glycol in the composition of the flavouring material. Obviously, the dilution of more than 100 times is not desirable from the standpoint of fragrance tangibility and absence of significant changes in the size of the micro particles of the flavouring material.

The scent of the samples of industrial flavouring material in media with different pH is characterized by rich floral and caramel odour, has bright shades, not typical for melon, reminiscent of the odour of quince, pears and flowers. Based on organoleptic analysis the comparison of the aromatic profile of distillates and industrial melon flavouring material showed maximum approximation of laboratory samples to fresh, natural raw materials. The differences in sensory characteristics to some extent is confirmed by the differences in the average hydrodynamic diameter of the sample: 150 nm and 190 nm, in the laboratory and industrial respectively.

Terms of transformation of volatile substances in the distillate during the distillation depends on many factors and determine their diverse composition [32]. Conversion of volatile fractions in distillate during the distillation process implementation depends, among other reasons, on the method of heating the raw materials. Microwave heating differs from convective selectivity with respect to for lipids and hydration components, free fatty acids release from lipids [33]. Free fatty acids in this study are involved in the enzymatic formation of aroma components, so the effect of the microwave heating has been studied in this aspect. The enzyme activity is increased under microwave heating [34] and, consequently, it affects the quantity and quality of the products of biosynthesis of fragrances. Microwave heating increases the mobility of the components, their diffusion, which could affect the probability of effective contacts, and avoid decomposition of thermally unstable compounds. Overheating of a polar solvent and heating of points, which do not contain solvents are the conditions to accelerate the reaction in a microwave field [35]. Changes in the surface strength of solutions were noticed in the microwave field [36]. The processes of cis-trans isomerism can occur in microwave field. Acetals and esters have chiral centres and therefore, the possible stereoisomers. Therefore, analysing the efficiency of the heat mode (convection and microwave) we should take into account the formation of enantiomers (mirror-shaped molecules). Enantiomers (optical isomers) have the same physical properties (boiling temperature, vapour pressure, identical vibrational spectra, etc.), but different aromatic qualities [37]. For example, the smell of carvone, limonene, 1-octene-3-ol, 3-metiltiobutanal in

different spatial configurations have different odour. In the distillates under consideration, acetals and esters may form optical isomers (Fig. 5).

Dimensions of insoluble in water particles of ethers and acetals in distillates do not differ both in convective distillation, and in microwave. Under dilution of samples the nature of changes remains the same at the volumetric heating of, as at the convection. Aromatic distillate profile obtained with microwave heating plant differs from the samples with a convection heating of a suspension (Table 2).

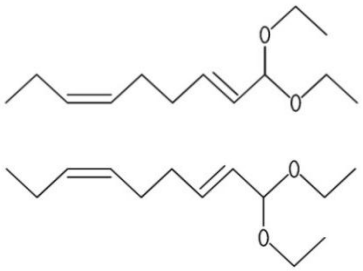

Fig. 5. Cis- and trans-isomers of 2,6-nonadienal diethyl acetal.

Table 2. Characteristics of distillates flavour

Name of characteristics	Cucumber distillate		Melon distillate	
	Convection	Microwave	Convection	Microwave
Characteristics of main aroma	Vegetable aroma, cooked/boiled tone	Fresh cucumber aroma	Melon aroma, fruit syrup tone	Melon aroma, pear tone
Identified shades - (except main aroma)	Grassy, green, vegetable	Fresh, sweet pepper, green rind, fresh pumpkin, kiwi	Carrot, fruit, ether, sweet	Pineapple, wild strawberry, honey, banana
Aldehydes, % mg	0.035	0.084	0.026	0.028
Aroma number (sensory)	2.0	3.1	2.1	2.4

Reaction products of biosynthesis and flavours resulting from these reactions depend on the position of hydroperoxide group the in the fatty acid and enzyme isoform that vary in a microwave field. The cleavage products of PUFA in cucumber suspensions contain double bonds and the intact pentadienoic systems. These systems of double bonds undergo the abstraction of hydrogen atom that results in formation of additional degradation products and intense flavours inherent in cucumbers. For melon flavour the presence of C_6-C_9 carbonyl derivatives is less important than for the cucumber, so the difference in the microwave and convective heating homogenate is little palpable.

Ethers and acetals in distillates affect fruit tones, have a synergistic effect, increase and emphasizes the fullness and complexity of flavours. Their presence contribu.te to improving the quality of distillates, giving bright and fresh shades. Many flavour chemicals can exist in one of several isomeric forms or as mixtures thereof. Similar in structure molecules do not always produce the same shades of odours (such as vanillin and isovanillin). Conversely, there are compounds with similar odour but having different molecular structure. For example, benzaldehyde, and tiglinaldehyde, both having the almond odour. Selective heating in a microwave field allows regulating the composition of the volatile components forming the quality of the aromatic distillate to be obtained. The effectiveness of the isolation and the quality of the aromatic distillate depends on the specific stereochemic positions.

CONCLUSION

These results showed the relationship between the sensory characteristics of fruit distillates, dispersion and method of heat treatment of fruits in the convective and microwave field. Given differences, in shades of odours from the melon and cucumber in fruit distillates, show the advantage of microwave exposure during the distillation of aromatic substances. The results of these studies will be needed for the improvement of pervaporation processes, membrane selection criteria for the concentration of aromatic components, in the technology of microcapsulation of aromatic components.

Restoration of lost aromas consists in adjusting the pH of the medium in which the particles of insoluble distillates are put. In acid medium (pH = 3) the transformation of cucumber distillates acetals to aldehydes leads to full restoration of fresh odour as aldehydes in this case are the key components. In subacid medium (pH = 6.0) positive transformations of the flavour occur with components of melon distillate. These results contribute to the economic competitiveness of distillates compared with other types of flavouring materials.

Technologies that allow getting more saturated distillates in the first stage of extraction of flavours without re-concentration in the preparation of aromatic concentrates with a predominance of C_6-C_9 aldehydes and alcohols are promising. Such technologies are based on the processes of the biosynthesis *de novo* or biotransformation and use of microwave energy. Obtained flavours distillates are made from vegetable material, according to organoleptic characteristics closer to natural raw materials than the existing industrial analogues.

Processes leading to a change in the polydispersion of the particles, isomerization, and formation of enantiomers when extracting aromatic components require further study.

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Please cite this article in press as: Dubova H.E., Sukmanov V.A., Marynin A.I., Zakharevych V.B., and Voskobojnyk V.I. Studies of some aspects in the process of aroma restoration. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 19–26. doi: 10.21179/2308-4057-2016-1-19-26.

PRODUCING OF BACTERIAL CONCENTRATES WITH HIGH CHOLESTEROL LOWERING ACTIVITY

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Received December 30, 2015; Accepted in revised form February 29, 2016; Published June 27, 2016

Abstract: The screening of 8 probiotic strains (*Propionibacterium, Lactobacillus* and *Bifidobacterium*) characterized by high cholesterol-lowering activity was carried out in the scientific research laboratory of East Siberia University of Technology and Management (Ulan-Ude, Russia). In order to research biotechnological potential of probiotic microorganisms and to conduct statistical analysis we used a mix of standard and modern physicochemical, biochemical and microbiological methods. The strains *Lactobacillus helveticus* $3_{5\text{-}1}$, *Propionibacterium shermanii* AC-2503 and *Bifidobacterium longum* DK-100 were found out to have the highest cholesterol-lowering properties. It can be seen that cocultivation of *Lactobacillus helveticus* $3_{5\text{-}1}$ and *Propionibacterium shermanii* AC-2503 strains shows high population density, cholesterol-metabolizing, antimutagenic, adhesive properties and exopolysaccharide biosynthesis. It indicates stable symbiotic bonds between cultures, which served as the basis for making a combined ferment. The analysis of biotechnological potential of *Lactobacillus helveticus* $3_{5\text{-}1}$ and *Propionibacterium shermanii* AC-2503 strains and the study of organoleptic, physicochemical, probiotic properties and cholesterol metabolizing activity of the combined ferment have allowed to establish an optimum culture ratio in the ferment – 5 : 95. Optimum cultivation conditions of the continuous culture *Lactobacillus helveticus* $3_{5\text{-}1}$ and the combined ferment were selected, process parameter of biologically active supplement and frozen ferments of direct loading were substantiated.

Keywords: bifidobacteria, propionic bacteria, lactobacteria, cholesterol-lowering activity, exopolysaccharide, bacterial concentrate

DOI 10.21179/2308-4057-2016-1-27-35 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 27–35.

INTRODUCTION

Nowadays cardiovascular diseases (CVD) are one of the main causes of mortality, disability, social and economic losses. According to World Health Organization (WHO), 23% of early death cases are caused by high blood cholesterol [1].

Increase in cholesterol is connected with the development of the most common disease – atherosclerosis ofarterial vessel. Atherosclerosis and its concomitant diseases rank first in illness distribution and adult mortality worldwide [2, 3].

The analysis of reference data indicates that the following factors can cause hypercholesteremia: exogenous cholesterol load exceeding compensation homeostasis abilities of this sterol in a body, increased cholesterol synthesis in host's organs and tissues, poor transit of cholesterol through digestive tract, poor transformation of cholesterol into bile acids and steroid hormones, poor inclusion of cholesterol into animal cell and microorganism membranes [1, 4, 5].

Reference data indicates that host's microflora is able to participate actively in realization of the processes mentioned above and to control normal blood cholesterol levels [6–10]. Considering an important role of microorganisms in cholesterol maintenance, the use of probiotic bacteria pertaining to intestinal microflora microorganisms for lowering the level of this sterol in blood serum in case of hypercholesteremia is of practical and academic interest.

The changes in blood lipids are always affected by profound microecological disorders in intestine. They present in an increased amount of aerobic bacteria, colibacilli, staphylococci, and fungi alongside with the lowering of lactobacteria in feces. Microecological disorders in a human body should be considered as a releaser of a lipid storage disease [11].

Host's microflora synthesizes, transforms and destroys exogenous and endogenous sterols and participates actively in the process of cholesterol metabolism. A significant amount of data on this topic has been stored for the last years. It let us consider host's microflora as the most important metabolical and regulatory organ taking part in the cooperation with cells in maintaining of cholesterol homeostasis [8, 9, 10, 12, 13].

The perspective of probiotic bacteria use for producing innovative pharma products is stipulated by their ability not only to synthesize different wholesome products but also to dissolve and lower a wide range of poisons.

The analysis of reference data on biologically active compounds produced by probiotic microorganisms revealed that biotechnological potential of anaerobic bifidus bacteria, Propionibacterium, and lactobacteria microorganisms practically hasn't been used so far. Lactic bacteria have been drawing biotechnologists' attention for a long time since they are potentially significant for health, prevention and treatment of many diseases. Meanwhile, it is needless to say that these microorganisms are a new industrial production source of essential metabolites in biobased product. The importance of this research regarding bacterial ecology and the study of cholesterol metabolism by probiotic microorganisms are determined by the need in producing biobased products for public consumption, which can provide good competition with traditional medicinal products in order to perform health care activities.

The mechanism of probiotics medical and preventive effect is really comprehensive and is stipulated not only by high living cells but also by synthesis of extracellular metabolites enhancing probiotic effect.

Exometabolites of probiotic microorganisms inhibit pathogenic flora development and exhibit radioprotective, antitumour, dismutagenic, immune correcting, and cholesterol metabolising properties [8–10, 12–15].

The number of articles on the ability of some lactobacteria strains to show a cholesterol lowering effect has been increasing through the past few years [9, 16, 17, 18]. Nevertheless, the question on producing food with cholesterol lowering activity is still open.

That is why the study of cholesterol metabolism by probiotic microorganisms and producing of biobased products for public consumption in order to provide health care activities is still a promising direction.

The aim of the research is to produce bacterial probiotic concentrates with cholesterol metabolizing properties.

OBJECTS AND METHODS OF STUDY

The targets of the research are the pure cultures of propionibacteria *Propionibacterium freudenreichii subsp. shermanii* AC-2503, *Propionibacterium freundenreichii subsp. freudenreichii* AC-2500, *Propionibacterium cyclohexanicum Kusano* AC-2559, *Propionibacterium cyclohexanicum Kusano* AC-2560, received from the stocks of Institution of Biochemistry and Microbial Physiology (Moscow), the strain *Lactobacillus helveticus* $3_{5\text{-}1}$ and Bifidbacteria cultures *Bifidobacterium longum* DK-100, *Bifidobacterium longum* B379M, *Bifidobacterium bifidum* 8_3, received from All-Russian industrial microorganisms collection of State Research Institute of Genetics and Selection of Industrial Microorganisms and activated by a biotechnological method developed in East Siberia State University of Technology and Management [19].

Bacteria were cultured in growth medium with the following composition: curd whey, microbiological agar, magnesium chloride, trisodium citrate dehydrate, potassium monophosphate, ascorbic acid.

Purified blood serum was used as a source of cholesterol.

Titratable acidity was determined by GOST 3624-92 (all-Union State Standard) by titration 0.1 N NaOH with phenolphthalein and expressed in Turner degrees.

The measure of active acidity was determined by potentiometric method with the help of pH-222.2 device according to GOST 3624-87.

The growth of active biomass was determined according to the extinction of cell-rich fluid with the help of photocolorimetric method on KF-77 upon $\lambda = 550$ nm.

The number of propionibacteria cells was determines by limiting dilution culture method in MH (maleic hydrazide) or HMS according to TS 10-10-02-789-192-95.

The number of bifidobacteria cells was determines by limiting dilution culture method in MH-1 according to MG 4.2.999-000.

The number of lactobacteria cells was determined by limiting dilution culture method in solid agar medium MRS according to TS 10-10-02-789-192-95.

Bacteria morphology was studied through Gram staining and the following microscopic examination in immersion system with 90x zoom. The micropictures of cells were taken with the help of digital microscope USB "BIOR".

The concentration of cholesterol in nutritional medium was measured by a fermentation method [20, 21, 22]. The main principle is that cholesterol ethers degrade into cholesterol and fatty acids under the influence of cholesterol esterase ferment. Then cholesterol affected by cholesterol superoxide dismutase gives coloured compound and hydrogen peroxide. Staining intensity in reactor feed is in direct proportion to cholesterol concentration in a sample. Then we measured the absorbency of a test sample (E) and a calibration sample ($E_к$) against the reagent consisting of ferment mixture at the wavelength 450 nm. The concentration of cholesterol was determined by a computational method. The measurements of test samples of intense green color concede twofold dilution of samples with physiological saline. The data received were divided by 2.

$$C = \frac{E}{E_к} \cdot 4.65, \quad (1)$$

where C is the concentration of cholesterol in a sample, mmole/L; E is the extinction of test samples; $E_к$ is the extinction of calibration samples; 4.65 is the concentration of cholesterol in calibrator, mmole/L.

The selection of cultures in combined ferment was exercised considering cholesterol metabolizing and probiotic properties.

Exopolysaccharide concentration was measured by an anthrone method [23]. The definition is based on the fact that furfural, 5-methylfurfural or 5-hydroxy-methylfurfural when interacting with anthrone give the

product of intensive green or teal color and they are formed in the process of strong sulphuric acid attacking carbohydrates.

Experimental procedure was the following: 1 volume of microbial consortium was added to 4 volumes of deionized water and 10 volumes of freshly mixed anthrone reagent. The mixture was incubated for 10 minutes at 100°C in water bath. The concentration of endoplasmic reticulum was measured by a spectrometric method at the wavelength 620 nm. Glucose solutions of different concentrations were used as a standard.

Ames test was used to determine antimutagenic activity [24]. Test strain *Salmonella enteritidis* was used for determining antimutagenic activity. The principle of this method is that histidine revertants, the number of which reveals mutagenic effect, develop under the influence of mutagen. Subsequently, antimutagenes lower the number of induced revertants.

Experimental procedure was the following: 2 ml of top agar consisting 0.5 mm of histidine/biotin were added to 0.1 ml of fresh culture *S. enteritidis*, 0.1 ml of mutagen under test and 0.1 ml of sample used as a mutagen. The mixture was stirred fast and then powered to the surface of minimal agarized medium (lower agar) into Petrie dishes. Through rapid mixing top agar was equally spread on the surface of lower agar. The dishes were incubated for 48 hours at 37°C. At the same time we put positive control when mixture contained mutagen and no antimutagen, and negative control when there was no mutagen but potential antimutagen was present. The total volume of the mixture was brought to 0.4 ml with the help of 0.2 M of phosphate buffer, pH 7.4. The choice of mutagen concentrates was determined after trial dose-response experiment. Mutagen concentrates from lineal parts of the dose-response curves were used. For sodium azidemutagene it was 3.0 mkg/dish, for nitro-soguanidine – 10 mkg/dish. In some cases we pre-incubated mutagen and antimutagen for 20 minutes at 37°C.

After incubation we counted the number of reverants in the dishes. The experiments were carried out thrice and then statistical data were processed.

Antimutagen activity is determined by the formula:

$$Inhibition(\%) = \frac{(a-b)\cdot 100}{a-c}, \qquad (2)$$

where a is a number of histidine reverants, induced under the influence of mutagen; b is a number of histidine reverants, induced under the influence of mutagen in the presence of antimutagen; c is the number of reverants, developing in the presence of only one antimutagen.

In all cases the number of spontaneous reverants was taken into account and substracted.

Adhesion properties were studied on formalinized red blood cells by a detailed method of V.I. Brilis [25]. A mixture of formalinized human red blood cells O(I) Rh+ blood type and microorganism suspension ($1\cdot10^9$ cells/ml) was incubated at 37°C during 30 minutes and was regularly stirred. Then we prepared a sample, dried, fixed and stained by Romanowsky-Giemsa method. The study of adhesion was conducted under light microscope, the count was kept considering in all no less than 50 red blood cells. In characterizing adhesive properties we used the following criteria: average adhesion score (the average number of microorganisms, having attached to one red blood cell); adhesion index (the percent of red blood cells that have adhesive microorganisms on their surface); microorganisms adhesion index (an average number of microbial cells on red corpuscles). Only those red blod cells that took part in the adhesive process were taken into account. Microorganisms were considered non-adhesive in case of microorganisms adhesion index was from 1.76 to 2.50, middle-adhesive – from 2.51 to 4.00, and highly adhesive in case of microorganisms adhesion index being ≥ 4.1. Red blood cells from only one donor O(I) were used for abidance by the standard terms.

Statistical data were procceded with the help of program package “Statistica 6”. We used Mann-Whitney rank test for comparing independent subsets. The differences were considered significant in case of error probabilityp ≤ 0.05.

RESULTS AND DISCUSSION

At the first step, we studied cholesterol metabolizing activity of different probiotic microorganisms strains in the process of cultivation. The results of the research are presented in Table 1.

Table 1 data analysis shows that all the probiotic microorganisms strains studied in the process of cultivation in growth medium laced with blood serum as a source of cholesterol lower the level of this steroid compound.

Table 1. Cholesterol metabolizing activity of probiotic microorganisms

Strain name	Cholesterol concentration in growth medium, mmole/L							The rate of cholesterol destruction, %
	culture time, h							
	0	4	8	12	16	20	24	
P. Freudenreichii AC-2500	4.92	4.92	4.81	4.48	3.98	3.32	2.92	40.64
P. Kusano AC-2559	4.92	4.92	4.89	4.68	4.10	3.36	2.89	41.36
P. Kusano AC-2560	4.92	4.92	4.86	4.53	3.91	3.21	2.74	44.22
P. shermanii AC-2503	4.92	4.91	4.68	4.25	3.67	2.93	2.64	46.32
B. longum B379M	4.92	4.92	4.90	4.76	4.19	3.68	3.02	38.68
B. longum DK-100	4.92	4.92	4.88	4.61	4.03	3.28	2.85	42.13
B. bifidum 8_3	4.92	4.92	4.91	4.81	4.31	3.87	3.10	37.08
L. helveticus $3_{5\text{-}1}$	4.92	4.90	4.54	4.06	3.36	2.54	2.38	51.70

The *L. helveticus* 3_{5-1} strain has the highest cholecterol lowering activity. This culture binds up to 51.7% of cholesterol. The level of propionibacteria cholesterol degradation is ranged within 40–46% of cholesterol source quantity put into cultivation medium. *P. shermanii* AC-2503 strain has the highest cholecterol lowering activity among propionibacteria. It binds 46.32%. *P. freudenreichii* AC-2500 has the lowest cholesterol metabolizing activity (40.64%). Also it's worth noting that bifidobacteria have high cholesterol metabolizing activity as well (up to 37–42%). *B. longum* DK-100 strain is the best cholesterol destructive (42.13%). *B. bifidum* 8_3 strain is the less active one (37.08%).

As we can see in table 1, the amount of cholesterol for the first 8 hours of cultivation decreases insignificantly, then it is being intensively destroyed and in 24 hours the destruction reaches its peak.

The analysis of the research conducted let us come to a conclusion that all the probiotic microorganism strains analyzed are characterized by reasonably high cholesterol metabolizing properties which depend on species and strains.

At the next step of the research we studied culture association of *Lactobacillus helveticus* 3_{5-1} and *Propionibacterium shermanii* AC-2503 that possesses the highest cholesterol lowering properties.

Numerous studies prove that multiple strain starters are resistant to unfavourable medium factors and have higher biotechnological potential. That's why the top priority in culture selection is to study symbiotic relationship between *L. helveticus* 3_{5-1} and *Pr. shermanii* AC-2503.

Considering high acid forming ability of *L. helveticus* 3_{5-1}, co-culturing was carried out at comfort for propionibacteria growth temperature (30°C).

The choice of microorganism's optimum ratio in the combined ferment was conducted according to biochemical, probiotic and organoleptic properties of cultures and their associations.

The results of the research are presented in Table 2.

The data received indicate rising cholesterol metabolizing activity in case of co-culturing. The highest degree of cholesterol lowering is observed by the ratio of 20 : 80 (67.0%), but in this case hyperacidity is noticed and it has an adverse impact on organoleptic indicator of a combined ferment. The best organoleptic indicators were observed by cultures ratio of 5 : 95. In this case the degree of cholesterol lowering falls insignificantly (63.0%).

The results presented in Table 2 let us regard the observed microorganisms as the potential producers of micronutrients possessing antimutagen and adhesive properties, the concentration of which increases under the conditions of co-culturing.

High population density of *L. helveticus* 3_{5-1} and *Pr. shermanii* AC-2503 was noted. It indicated good compatibility and microorganisms symbiosis. It can be probably explained by high proteolytic activity of *L. helveticus* 3_{5-1} culture which splits protein components with the formation of peptides, amino acids and creates favorable conditions for propionibacteria growth.

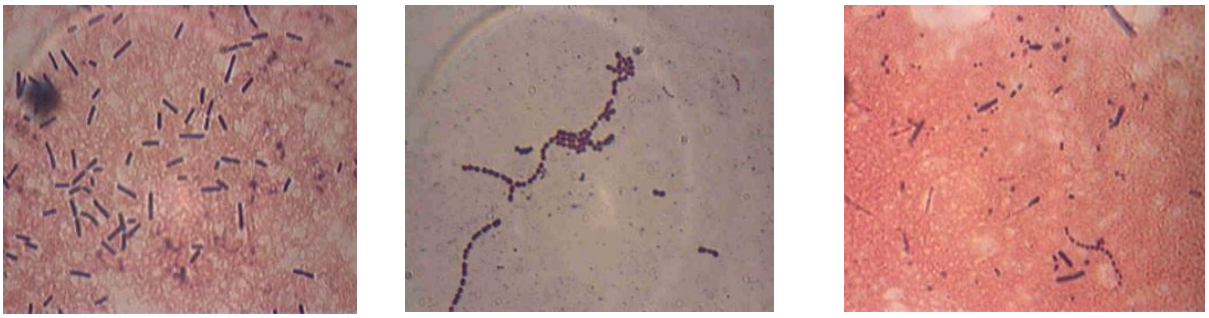
It is worth noting that nowadays, microbiological paradigm is gradually changing. We are moving beyond the idea of microorganisms monocellularity to the idea of microbial colonies being integral epiorganisms. It reflects in the growing interest for microbial colonies form, picture, and marco- and microstructure. That is why the following research is dedicated to bacterial morphology in a combined ferment. The morphology of ferments is presented in Fig. 1.

As we can see on Fig. 1, *L. helveticus* 3_{5-1} cells have a form of single bacilli and *P. shermanii* cells have a form of long and short chains. Cell aggregation can be observed in a combined ferment of propionibacteria and lactibacteria, showing cell cooperation – aggregation (cohesion).

Table 2. The choice of cultures optimum ratio in a combined ferment

Indexes	Characteristics if ferment				
	L. helveticus 3_{5-1}	*P. shemanii* AC-2503	Variant of combined ferment *L. helveticus* 3_{5-1} *: P. shermanii* AC-2503		
			20 : 80	10 : 90	5 : 95
Cholesterol metabolizing activity, %	51.70	46.32	67.00	65.10	63.00
Acidity, °T	93–95	68–70	90–91	88–89	82–83
Activeacidity, pH	4.68	4.98	4.69	4.71	4.72
VFA content, mg/100g	2.8	2.4	3.8	3.9	3.9
Fermentation activity, h	6–8	12–14	6–7	8–9	8–10
Cell count, CFU/cm^3 *L. helveticus* 3_{5-1} *P. shermanii* AC-2503	 $2 \cdot 10^9$ –	 – $1 \cdot 10^9$	 $1 \cdot 10^9$ $2 \cdot 10^7$	 $1 \cdot 10^9$ $1 \cdot 10^8$	 $1 \cdot 10^9$ $2 \cdot 10^9$
Adhesive activity: AAS* ECF**, % MAI***	 4.5 85 5.29	 4.6 85 5.4	 4.7 86 5.51	 4.9 87 5.6	 5.1 88 5.69
Exopolysaccharide biosynthesis, ug/ml	0.578	1.88	1.90	1.92	2.01
Antimutagenic activity (inhibition), %	50.2	50.3	52.5	53.6	53.9

Notes. AAS* – average adhesion score; ECF** – erythrocyte contribution factor; MAI*** – microorganisms adhesion index.



(a) (b) (c)

Fig. 1. Morphology of *L. helveticus* $3_{5\text{-}1}$ ferment (a), of *P. shermanii* AC-2503 ferment (b) and of combined ferment (c) on milk.

From reference data [26, 27] we know that cohesion is not just a number of cells but a special supraorganismal system similar but not identical to multicellular organism. The special feature of such system is the cooperation of certain cells when their common activity targets achieving the same results. The property of such system is cooperation of certain cells when their concerted activity is aimed to achieving one and the same result. One of the cooperation mechanisms is communication (signals and information exchange by means of extracellular metabolites functioning, which regulates bacteria activity). The formation of such cooperation provides adaptative, physiological cell resistance to the influence of ambient negative conditions.

As can be seen from the above, optimum culture balance 5 : 95 that provides high cholesterol metabolizing activity, extracellular metabolites synthesis and also fine organoleptical properties, was chosen.

Starter activity plays an important role in the process of microorganism cultivation. The influence of inoculum doseon cell growth was studied on the next step. *L. helveticus* $3_{5\text{-}1}$ culture, *L. helveticus* $3_{5\text{-}1}$ and *P. shermanii* AC-2503 combined ferments in proportion 5 : 95 were used as inoculum. Inoculum dose varied from 1 to 3%. The received data are presented on Fig. 2 and 3.

As we can see from Fig. 2 and 3, more active microorganisms growth is observed upon stepping up the inoculum dose from 1 to 2%. Further ferment dose increase up to 3% doesn't lead to significant growth.

Then the growth of probiotic microorganisms biomass and the changes of cholesterol level in the process of cultivation were studied next. The results of the research is presented on the Fig. 4.

On the picture 4a we can see that the most active growth of bacterial mass was observed in the process of combined inoculum cultivation, it indicates mutual culture stimulation. It was proved that active cholesterol lowering takes place in the process of cultivation (Fig. 4b). It was also noted that for the first 6 hours the amount of cholesterol decreases insignificantly. This period of microorganisms growth is called lag growth phase, during which the culture adapts to the new environment. Then follows exponential growth phase characterized by the highest speed of culture growth. Cell number dose depends linearly on time. The growth medium peters out as the result of culture growth, metabolism products accumulate and start to lower cholesterol intensively. This period starts after 6 hours of cultivation and lasts up to 18 hours. Phase of growth declining cells regression (stationary phase) is observed after the intensive microorganism growth when spatial delimitation appears upon culture biomass increase and leads to weakening of microbial cell contact with the growth medium [14, 28]. As the result, microorganism cholesterol lowering activity decreases.

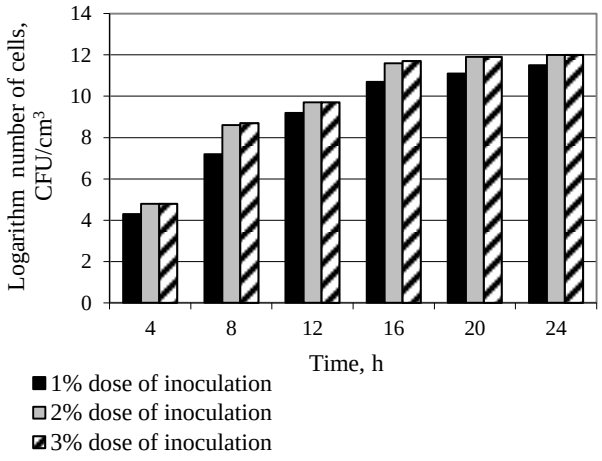


Fig. 2. The influence of *L. helveticus* $3_{5\text{-}1}$ inoculum dose on cell growth.

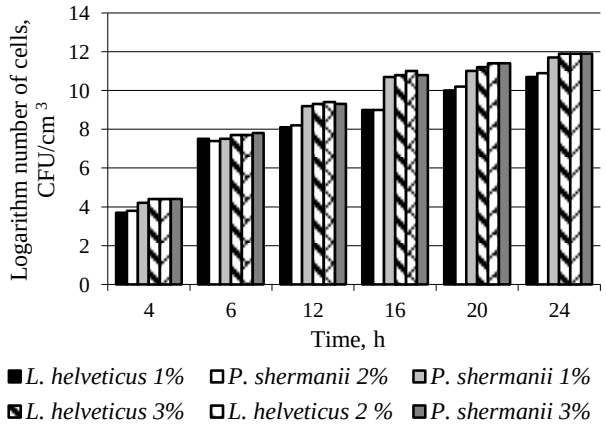
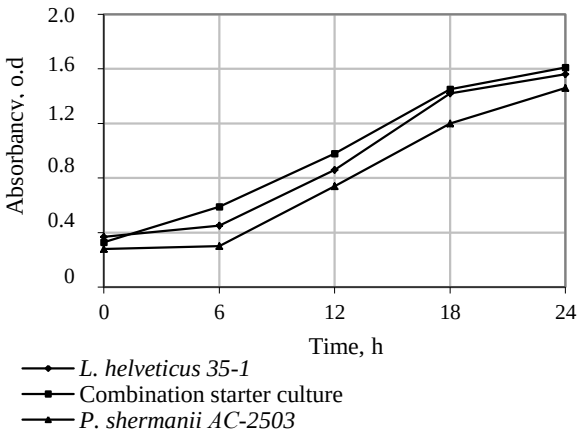
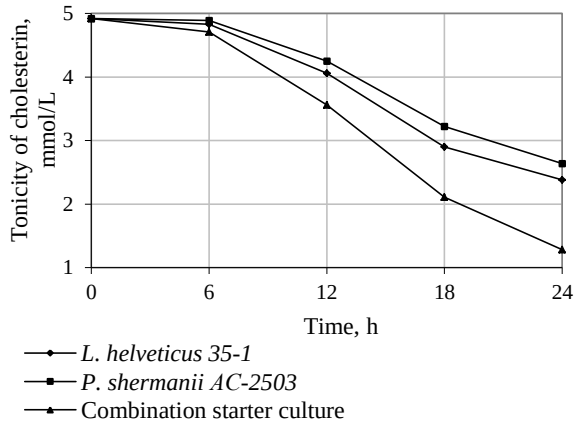


Fig. 3. The influence of *L. helveticus* $3_{5\text{-}1}$ and *P. shermanii* AC-2503 inoculum dose on cell growth.



(a)



(b)

Fig. 4. The growth of probiotic microorganisms biomass (a) and the change of cholesterol level (b) in the process of cultivation.Combination starter culture.

It is worth saying that the biggest amount of cholesterol is destroyed at the end of exponential growth phase after 18 hours of cultivating. This is due to the fact that enough amount of biologically active compounds is accumulated in the growth medium, and the biomass increase reaches its maximum level. The highest cholesterol metabolizing activity is observed upon combined inoculum cultivation (63.0%). At that, the number of living cells in continuous culture *L. helveticus* $3_{5\text{-}1}$ and in combined ferment reaches 10^{12} cm^3.

The active growth of propionibacteria in a combined ferment was elicited (Fig. 5).

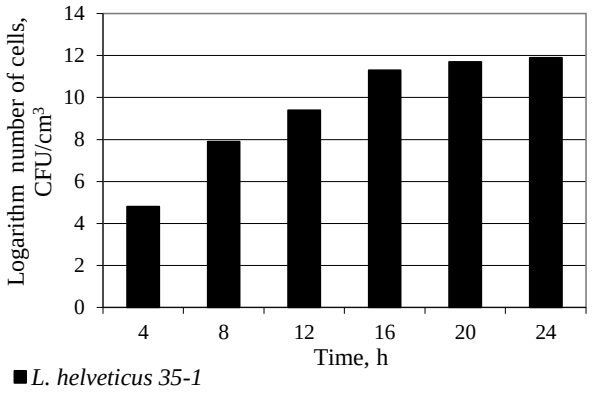
The analysis of the received data indicates that upon chosen cultivation process parameters *L. helveticus* $3_{5\text{-}1}$ and *P. shermanii* AC-2503 strains develop well in the growth medium on the basis of milk whey. On the basis of the conducted research we chose optimum conditions for cultivation of *L. helveticus* $3_{5\text{-}1}$ and combined ferment: inoculum dose – 2%, cultivation temperature – 30°C and the duration – 18–20 h.

Received experimental data revealed that *L. helveticus* $3_{5\text{-}1}$ is characterized not only by marked cholesterol lowering activity, but also by fine probiotic properties that served as a methodological foundation for producing ferments. The research of structure-functional foundation of *L. helveticus* $3_{5\text{-}1}$ and *P. shermanii* AC-2503 cooperation proves the perspectives of combined ferment use in bacterial concentrates production.

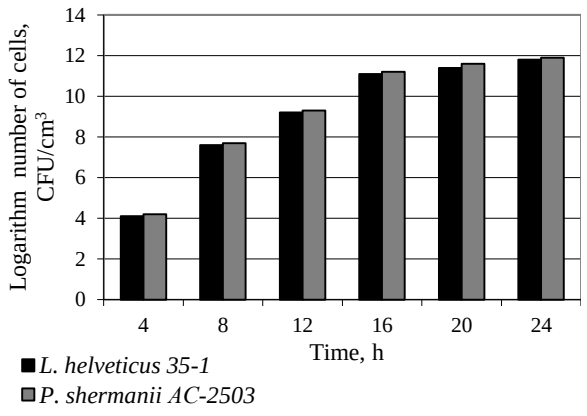
As the result of conducted research we chose optimal technological parameters of getting concentrates. The technology of getting concentrates includes such operations as growth medium preparation, introduction of inoculum and upstream. After the process of cultivation culture liquid is segregated from biomass. This liquid concentrate can be used as biologically active additives. For production of frozen concentrated ferments of direct loading biomass is decanted to the complete segregation of culture liquid and is mixed with the protective medium. The protective medium aims to decrease cell damages in the process of freezing. It consists of water, sucrose and Tri-Sodium citrate dehydrate. The protective effect can be explained by the ability of sucrose to hydrate, to decrease the point of freezing water and to slow down the speed of crystallization.

The researches indicate that after the freezing process the number of probiotic microorganism living cells decreases insignificantly and biochemical activity is preserved. The mixture is flasked, frozen after blending with the protective medium and is kept until realization.

The storage life of BAA (biologically active additives) is 120 days and the storage life of frozen ferments of direct loading is 240 days during which the high amount of living cells and biochemical activity are preserved. Qualitative description of concentrates is presented in Table 3.



(a)



(b)

Fig. 5. Growth rates of *L. helveticus* $3_{5\text{-}1}$ (a) and the combined ferment (b) in growth medium.

Table 3. Qualitative characteristic of bacterial concentrates

Index	Characteristic of biologically active supplement		Characteristic of frozen ferments of direct loading	
	BAA on the basis of *L. helveticus* 3_{5-1}	BAA on the basis of acombined ferment	Ferment of direct loading *L. helveticus* 3_{5-1}	Combined ferment
Flavor and aroma	Clean, slightly sour, tasteless and odorless			
Consistency	Homogenous with subtle whey residuum		A small pillar of frozen suspension	
Color	From white to slightly yellow			
pH limit	4.2–4.4	4.6–4.8	5.4–5.6	5.8–6.0
Fermentation activity, h	–	–	6–8	8–10
Cholesterolmetabolizing activity, %	51.73	63.00	49.45	62.30
Antimutagenic activity (inhibition), %	50.2	53.9	48.3	51.5
Adhesive activity: AAS ECF, % MAI	 4.5 85 5.29	 4.8 86 5.58	 4.1 83 5.21	 5.1 88 5.69
Exopolysaccharide, ug/ml	0.578	2.010	0.569	2.310
Outlet temperature, °C	4–6		Minus 25	
Number of cells, CFU/cm^3 *L. helveticus* 3_{5-1} *P. shermanii AC-2503*	 $4 \cdot 10^{12}$ –	 $2 \cdot 10^{12}$ $4 \cdot 10^{12}$	 $2 \cdot 10^{12}$ –	 $1 \cdot 10^{12}$ $3 \cdot 10^{12}$
The volume of product (cm^3) consisting Coliforms *S. aureus* Pathogenic microorganisms (including salmonella) Yeasts and mold, CFU/cm^3, no more than	 10 10 50 10		 10 10 100 5	

The analysis of data in Table 3 indicates that developed BADS and frozen ferments have high cholesterol lowering ability. BADS are recommended to be used in practical healthcare and frozen cells can be used in the production of fermented milk products tof functional nutrition.

The results received in this study show relatively high cholesterol lowering ability of probiotic microorganism which depend on specie and strain. The change of cholesterol concentration in cultivation process shows the interrelation of cholesterol level and incubation time. The highest cholesterol lowering activity was registered at the end of exponential growth phase when the highest amount of metabolites accumulates. The received results are coherent with reference data on cholesterol lowering activity of probiotic microorganisms [13, 16, 29].

Though the mechanisms of cholesterol destruction are understudied, some data point to the fact that probiotic microorganisms regulate endogenous cholesterol synthesis through the formation of short-chain fatty acids that accumulate in the process of fermentation.

It is known that propionate formed in the process of propionibacteria cultivation is able to lower cholesterol level. Rectal introduction of acetate and propionate to adults in proportion of 3 : 1 is followed by the lowering of cholesterol concentration and the increasing of triglycerides amount in blood serum. Consequently, upon accession of probiotic microorganisms into digestive tract they will synthesize volatile fatty acids and change acetate and propionate pool in a body and regulate cholesterol synthesis [27].

Probiotic microorganisms (bifidobacteria, propionibacteria and lactobacteria) showing proteolytic, hydrolytic, lipolytic or other biochemical activity are able to modify regulatory combinations or lower them controlling cholesterol formation [30, 31].

One more significant result of this work is the creation of combined ferment from *L. helveticus* 3_{5-1} and *P. shermanii* AC-2503 cultures with high biotechnological potential. Optimal culture choice of propionibacteria and lactobacteria increases not only the degree of cholesterol destruction but also biosynthesis of exopolysaccharides, antimutagenic substances and volatile fatty acids. The formation of short-chain fatty acids, as mentioned above, regulates cholesterol synthesis. The increase of adhesive activity, exopolysaccharides and antimutagenic properties in combinations of different microorganisms will contribute to better colonization rates and adaptation of microorganisms in extreme conditions when passing through digestive tract.

In natural cenosis different microorganisms are represented by organized population clusters possessing collective functions and cooperation [32]. Nowadays we have a concept of "biological bacterial consortium" consisting of morphologically and functionally differentiated microorganisms performing a complex of metabolic process on cooperative basis [6, 33].

Adaptation to environmental factors is provided by mechanisms ensuring the stability of microbial consortium. These mechanisms include, for example, cell cooperation – bounds (cohesion) and solid attaching of cells to substrate (adhesion) [26, 30, 34].

According to our sources, morphological cell differentiation of propionibacteria and lactobacteria in combined ferment is stipulated by high exopolysaccharide potential of propionibacteria, which helps to form microcolonies.

As long as mechanisms of cell junction realization and cell attaching to substrate are provided by the same structures [35], our research proves the applicability of highly adhesive propionibacteria and lactobacteria strains as perspective for producing bacterial preparations.

Antimutagenic substances formed in combined ferments, first of all, protect microorganisms from mutations under the influence of different chemical and biological mutagens. Also, practical realization of such concentrates will enable producing of biobased products with antimutagenic properties.

Bacterial concentrate from the mixture of propionibacteria and lactobacteria cultures (*L. helveticus* $3_{5\text{-}1}$ and *P. shermanii* AC-2503) with high biochemical and cholesterol metabolizing activity was developed during this research for the first time. Technological parameters of its preparing for further practical use were also substantiated.

Received in this paper data indicate that cholesterol can modify and degrade by both pure and mixed cultures. They can be used practically for product-line expansion of fermented milk products of functional nutrition.

In conclusion, the received data open wide perspectives for searching new strains of probiotic microorganisms that synthesize essential biologically active substances, and aim at sequential realization of their unique metabolism in biotechnology.

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Please cite this article in press as: Khamagaeva I.S., Tsybikova A.H., Zambalova N.A., and Choi Suk-Ho. Producing of bacterial concentrates with high cholesterol lowering activity. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 27–35. doi: 10.21179/2308-4057-2016-1-37-35.

BEER QUALITY ASSURANCE BY CONTROLLING WORT POLYPHENOLIC CONTENT WITH ADSORPTION METHOD

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Received March 3, 2016; Accepted in revised form May 2, 2016; Published June 27, 2016

Abstract: This research explores feasibility of adsorption method used for regulating polypenols content in wort with a view of improving beer quality. It examines adsorption of polyphenols (quercetin, gallic acid, rutin) from pure substance solutions, their mixtures and beer wort treated by sorbents that differ by origin, making, structure and surface chemical composition. The work determines patterns and specific features of polyphenols adsorption with activated carbons. To describe adsorption mechanism more precisely, we specified structure, surface chemical condition, and calculated adsorption parameters using equations of Langmuir, Freundlich and Dubinin-Radushkevich, and the multilayer adsorption theory (BET model). It is demonstrated that polyphenols adsorption mechanism depends on carbon characteristics and is of physical nature that reveals itself in dispersion interaction in micropores and in specific one with oxygen-containing functional group (OFG) on carbon surface. Polyphenol competitive adsorption in mixture and wort recognized. At polyphenols adsorption from model solutions and wort, carbon sorbents are identified to share sufficiently close sorption characteristics. We performed comparative evaluation of quality characteristics of beer produced from activated carbons treated and untreated worts. It is shown that beer samples produced from unhopped wort filtered through semi-coke adsorption, meet regulatory standards requirements of safety by organoleptic, physical and chemical indicators. Moreover, beer obtained from semi-coke treated wort exceeds control sample in terms of organoleptic and stability ensuring indicators.

Keywords: gallic acid, rutin, quercetin, wort, carbon sorbents, adsorption

DOI 10.21179/2308-4057-2016-1-36-43 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 36–43.

INTRODUCTION

Breweries particularly focus on quality of their product. Lately, there has been observed an increase in demand for beer of higher quality and nontrivial formulation [1]. Polyphenolic compounds are an important component in chemical composition of hop and barley that have a substantial effect on intensity of beer making processes and its quality. They negatively affect stability of aroma, flavor, foam properties, color and colloidal stability; therefore, regulating their content appears to be pertinent problem [2, 3].

In 1950s beer polyphenols became an active area of research all over the world. Emergence of the substances specific adsorbents facilitated a removal of significant amount of polyphenolic compounds substantially improving beer stability. Intensive research revealed polyphenols distinctive properties and structure. In Russia polyphenolic compounds in domestic beers and brewery ingredients became a subject of research in the end of 1970s [4].

To remove polyphenols from beer, activated carbons of the grades BAU-A and BAU-MF are used in production [5].

However, due to the fact that the finished product is largely affected by polyphenolic compounds contained in malt rather than hop, it seems more practical to remove polyphenol through adsorption from wort.

One of the effective means of lowering polyphenol levels might be unhopped wort treatment by activated carbons. There is no reference to such studies in scientific literature.

In the last years the assortment of carbon materials has been expanded by introduction of semi-cokes produced using a new technology. The given technology distinction lies in substitution of traditional two-stage carbonization of source material in inert medium followed by activation, for one-stage process of carbonization/activation by air. It lowers a final cost of sorbent by reducing energy consumption for the production. Use of such adsorbents enhances cost-effectiveness of the beer and non-alchoholic beverages manufacturing process.

In order to study patterns and specific features of polyfphenolic compounds adsorption, main polyphenol components of wort have been selected (quercetin, gallic acid, rutin). Quercetin and rutin represent flavonoids, a large group of natural polyphenols that have significant impact on beer stability during storage; gallic acid being a predecessor of a series of polyphenolic substances. The studied compounds are

characterized by acid-base properties, hydrophobicity and presence of substituents on an aromatic ring [6, 7].

The purpose of this study was to explore adsorption of polyphenols for adjustment of polyphenol component in wort with a view of beer quality improvement.

OBJECTS AND METHODS OF STUDY

The objects of research are the following: water solutions of polyphenols (gallic acid, quercetin, rutin) and wort; activated carbon of the grade AG-OV-1 – granular AC (OJSC "Sorbent", Perm); and semi-coke ABG (OJSCO "Karbonika F", Krasnoyarsk) and "Purolat-Standart" (OJSC "Sintez", Rostov-na-Donu). Prior to the research all activated carbons were rinsed with distilled water to remove the dust particles and dried at room temperature (23 ± 2°C) for 24 hours.

Adsorption from water solutions of polyphenols and wort was studied at room temperature (23 ± 2°C) from limited quantity continuously stirring for 7–9 hours in static conditions within concentration range from 20 to 700 mg/dm^3. Ratio of activated carbon : phenolic components solution was 1 : 100. Ratio of the studied polyphenols in the solution was 1 : 1 : 1 close to their actual ratio in wort. Sorption of polyphenolic compounds from wort was observed in static conditions using activated carbon "Purolat-Standart", phenolic components concentration ranging from 8 to 160 mg/dm^3.

Polyphenols adsorption (Γ, mg/g) was estimated using the equation:

$$\Gamma = \frac{C_0 - C_{eq}}{m} \cdot V_{solut}\ , \qquad (1)$$

where C is the initial solution concentration, mg/dm^3; C_{eq} is the equilibrium solution concentration (following the adsorption), mg/dm^3; V_{solut} is the volume of the solution, dm^3; m is the adsorbent mass, g.

The amount of gallic acid, quercetin and rutin in the solution was measured by spectrophotometric method of bandgap absorption.

To perform the analysis we selected a wavelength based on spectral curve registered by a device SF-46 for a pure substances water solution.

Concentration of pure substances solutions for examined polyphenols under optimal conditions was 20 mg/dm^3, to reference a solution distilled water was used. Based on the results obtained through analysis we have selected the thickness of light absorbing layer to be 10 mm, and wavelength 230 nm for gallic acid, 340 mm for quercetin and 380 nm for rutin.

The relative error in polyphenolic compounds measurement was 4%.

To determine polypenols, the studied solutions where concentration was above 25 mg/dm^3 were diluted, a dilution coefficient selected for each solution individually. Gallic acid content in the studied solution was calculated from the formula:

$$C = X \cdot \frac{V_{mc}}{V_{al}}, \qquad (2)$$

where X is the gallic acid concentration in mg/dm^3, determined from graph; V_{al} is the aliquot of the studied sample, cm^3; V_{mc} is the measuring cup, cm^3.

Structural characteristics of adsorbents were found based on low temperature adsorption of nitrogen with specific surface area analyzer "Sorbtometer M" (Institute of Catalysis SB RAS, Novosibirsk).

We evaluated chemical condition of activated carbon surface that is a number oxygen-containing functional group using Bem titration method

Characteristic energy values (E) and half-width size of slit-like pores (χ) were calculated taking into account the affinity coefficient:

$$E = \beta \cdot E^0, \qquad (3)$$

where E is the characteristic energy of dissolved organic compound adsorption, kJ/mol; E^0 is the characteristic energy of benzene vapors adsorption, kJ/mol; β is the affinity coefficient.

We identified the total number of polyphenolic components in wort and beer using the the Erumanis method. The method implies treatment of samples with the solution containing trilon b and carboxymethyl cellulose (CMC), which if present in alkaline solutions makes polyphenolic compounds to react with iron ions. Then, we calculated the optical density at 600 nm of test solution versus blank.

Polyphenols concentration is determined using the formula:

$$X = A820 \cdot F, \qquad (4)$$

where X is the polyphenols concentration , mg/dm^3, A is the optical density; F is the dilution coefficient [8].

RESULTS AND DISCUSSION

To reveal the patterns, specific features and mechanisms of polyphenols removal with activated carbons, it appeared to be necessary to specify the activated carbons structure (Table 1) and to determine sorbents surface composition (Table 2). The adsorption of polyphenolic compounds was examined.

Based on experimental data produced by adsorbing polyphenols with activated carbons from pure substance solutions, we drew the adsorption isotherm shown in Fig. 1.

Table 1. Structural characteristics of activated carbons

Grade of activated carbon	V_{micro}, cm^3/g	V_{meso}, cm^3/g	V_{macro}, cm^3/g	V_S, cm^3/g
AG-OV-1	0.22	0.24	0.57	1.03
ABG	0.02	0.24	0.73	0.99
"Purolat-Standart"	0.07	-	0.43	0.5

Table 2. Surface composition of activated carbons

Sample	Elements content*		N_{OFG}, mmol(eq)/g (mcmol(eq)/m^2)			
	N+S+O	O_{act}	*-OH*	$-COOH_{strong}$	*-COO-*	*>C=O*
AG-OV-1	2.71	2.28	0.213 (0.312)	0.032 (0.047)	0.078 (0.114)	2.08 (3.05)
ABG	7.05	2.76	0.130 (0.314)	0.020 (0.048)	0.040 (0.097)	3.70 (8.94)
"Purolat-Standart"	9.23	0.86	0.218 (0.700)	-	0.020 (0.064)	0.63 (2.02)

Note. * Percents per organic matter.

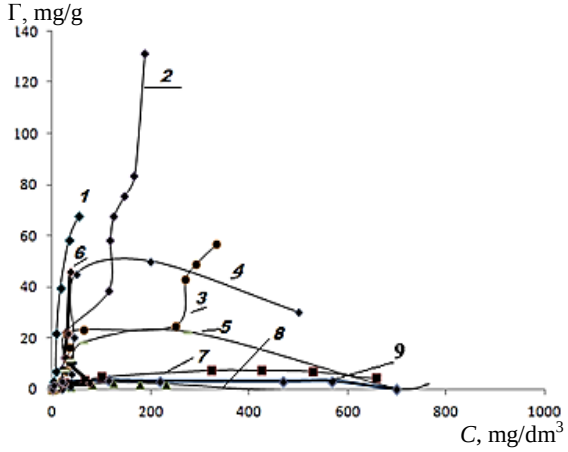

Fig. 1. Isotherm of polyphenols adsorption from pure substance solutions: quercetin with the sorbents grades ABG (1), "Purolat-Standart" (2) and AG-OV-1 (3); gallic acid with the sorbents grades "Purolat-Standart" (4), AG-OV-1 (5) and ABG (6); rutin with the sorbents grades ABG (7), AG-OV-1 (8) and "Purolat-Standart" (9).

Patterns of the studied organic components removal vary and depend on the grade of the sorbent used.

The shapes of quercetin adsorption isotherm drawn based on removal of the studied flavonoid from standard test solutions with carbon sorbents grades ABG and "Purolat-Standart", belong to S-type isotherms according to Giles classification. Curve knee and further rise of the isotherm is observed, which is typical of the sorption in meso- and macropores [9]. The shape of adsorption isotherm exhibiting quercetin removal with microporous activated carbon AG-OV-1, can be described as L-type according to Giles classification. The isotherm curves at higher concentrations and becomes flat. Drawing upon the graphs of adsorption isotherms it can be suggested that adsorption has a physical nature, which with activated carbon grade AG-OV-1 largely reveals itself in dispersion interaction; and with sorbents ABG and "Purolat-Standart" – in specific interaction.

When removing quercetin with semi-cokes, monomolecular adsorption of the studied organic component prevails on the surface of carbon sorbent at the initial stages of the process; then followed by removal at the expense of the formed secondary adsorption centers due to adsorbate-adsorbate interaction, which explains the isotherms inflections. Adsorbed molecules inter-molecular forces promote further removal revealing cooperation where separate molecules loose their individuality in complexes [10]. Probably, the complexes formation occurs due to hydrogen bond between quercetin molecules (Fig. 2).

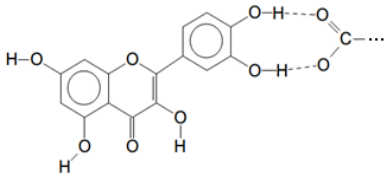

Fig. 2. Suggested bonding structure of quercetin and oxygen-containing surface groups of carbon sorbent.

Isotherm of gallic acid adsorption with activated carbon AG-OV-1 is of a classical shape and belongs to L-type isotherms according to Giles classification, which may indicate physical nature of adsorption. Isotherms of adsorption with sorbents grades "Purolat-Standart" and ABG belong to the S-type. It can be concluded from the sorption isotherms that interaction forces between solute and adsorbent are less than adsorbed molecules interaction forces, which might be explained by a formation of hydrogen bonds. Based on gallic acid properties and structure hydrogen bonds can form both with water and between the molecules of gallic acid itself (Fig. 3 a, b).

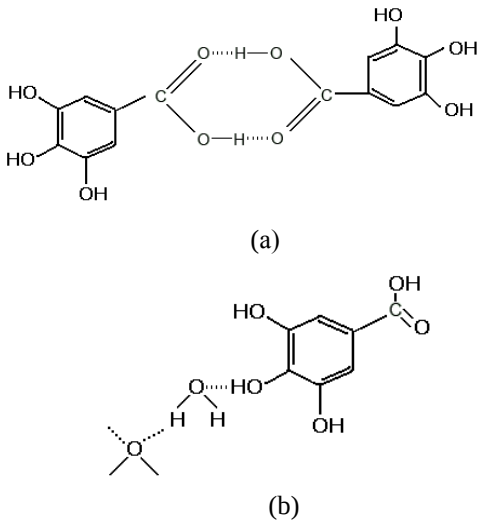

Fig. 3. Suggested hydrogen bonds formation schemes in gallic acid – water system: (a) between gallic acid molecules; (b) between molecules of gallic acid and water.

The shape of initial part of experimental isotherm of rutin adsorption with the sorbens grade ABG and AG-OV-1 (Fig. 1) belongs to the L-type, and with the

sorbents grade "Purolat-Standart" it can be classified as H-type by Giles. Isotherms of the given types describe the adsorption where removed molecules practically fail to interact between each other. Besides, L-type adsorption isotherms suggest physical adsorption. Chemically, adsorbed particles maintain their molecular nature and remain unchanged. The H-type isotherm of the sorbent grade "Purolat-Standart" suggests that the removal occurs by chemical bonding between oxygen-containing functional groups (OFG) from carbon sorbent surface and adsorbed molecules. Steep rise of the adsorption isotherm illustrates a high rate of removal from low concentration solutions when rutin is adsorbed with semi-coke "Purolat-Standart", which can be attributed to the interaction of adsorbents active centers with flavonoid molecule.

Table 3 shows the adsorption parameters calculated values.

Table 3. The parameter of polyphenol adsorption from pure substance solutions with the studied carbon sorbents in static conditions Langmuir, Freundlich and Dubinin-Radushkevich, and the multilayer adsorption theory (BET model)

Carbon grade	Equation type							
	Langmuir		Freundlich		BET model		Dubinin-Radushkevich	
	-G, kJ/mol	Γ_{max}, mmol/g	1/n	b	Q, kJ/mol	Γ_{max}, mmol/g	Γ_0, g/g	E_0, kJ/mol
Quercetin								
AG-OV-1	29.36	0.16	0.88	$3.6 \cdot 10^{-4}$	2.47	0.15	0.115	7.17
ABG	27.33	0.59	1.51	$4.6 \cdot 10^{-5}$	2.48	0.56	0.940	4.86
"Purolat-Standart"	27.59	0.58	1.30	$1.3 \cdot 10^{-4}$	2.49	0.55	0.699	4.57
Gallic acid								
AG-OV-1	30.72	0.15	1.41	$6.1 \cdot 10^{-5}$	13.00	0.15	0.023	8.54
ABG	29.41	0.12	4.27	$8.8 \cdot 10^{-9}$	12.50	0.12	0.024	4.60
"Purolat-Standart"	30.19	0.16	8.08	$6.6 \cdot 10^{-16}$	7.00	0.19	0.030	3.05
Rutin								
AG-OV-1	29.08	0.04	0.53	$1.8 \cdot 10^{-4}$	9.92	0.038	0.006	11.23
ABG	32.35	0.01	0.53	$3.2 \cdot 10^{-4}$	11.86	0.014	0.004	10.55
"Purolat-Standart"	41.54	0.05	0.12	$1.7 \cdot 10^{-3}$	16.96	0.051	0.004	25.35

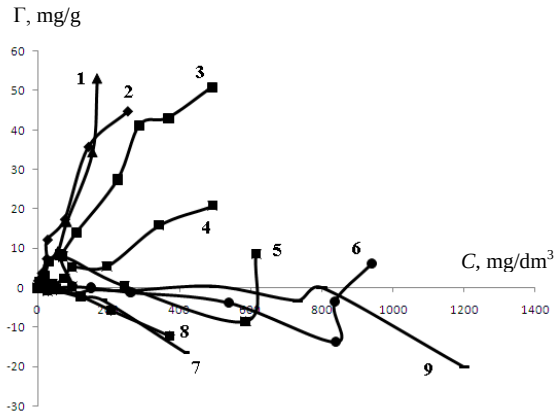

Fig. 4. Isotherms of polyphenols adsorption from mixture: quercetin with sorbents grade ABG (1), "Purolat-Standart" (2) and AG-OV-1 (3); gallic acid with sorbents grade "Purolat-Standart" (4), ABG (5) and AG-OV-1 (6); rutin with sorbents grade ABG (7), "Purolat-Standart" (8) and AG-OV-1(9).

We examined adsorption from mixtures of polyphenols in order to specify the components mutual influence. Fig. 4 displays equilibrium study results-based isotherms of polyphenols mixture adsorption. The isotherm shapes at the origin for quercetin with carbon sorbents grades ABG and "Purolat-Standart" belong to the L-type isotherms by Giles classification which is evident of physical adsorption.

During the studied process interaction is insignificant between adsorbed molecules; and surface filling degree has no impact on activation energy. Quercetin adsorption isotherms with activated carbon grade AG-OV-1 belongs to C-type. The initial linear portion characterizes ongoing distribution of solute between solution and adsorbent, which is typical for removal with microporous sorbents (the given activated carbon incl.). At lower concentration adsorption occurs in micropores, and at higher concentration – on carbon sorbent surface. The obtained results suggest that as the active centers fill and provoke the emergence of new secondary adsorption centers, the surface available for adsorption grows in proportion to the amount of removed from solution substance.

We observed negative adsorption (Fig. 4) for gallic acid sorption with activated carbons grades AG-OV-1 and ABG. However, with the concentration increase, isotherm climb and transition to positive area become noticeable, the reason for this probably being a concurrent interaction of quercetin with OFG and secondary activation centers formation, gallic acid included. Reasoning from the polyphenols molecules structure it appears possible that removed at their high concentration flavonoid molecules promote gallic acid sorption by forming complexes where specific interaction between hydroxyl groups of quercetin and those of gallic acid tend to change molecules individuality. When gallic acid is removed with semi-coke "Purolat-Standart", the shape of the initial section is concave relative to isotherm concentration axis belongning to L-type (Fig. 4). The acid removal pattern

differs due to structural characteristics of activated carbons.

Negative adsorption of rutin is indicative of mutual effect polyphenols mixture components have on each other, and results from the specific structure and the size of phenolic compounds molecules.

The comparative evaluation of isotherms of phenolic compounds adsorption from pure substance solutions and their mixture provides evidence that at polyphenols removal from pure substance solutions rate of adsorption is higher than at polyphenols removal from solutions containing phenolic compounds mixture. This is consistent with a Freundlich and Mazius mutual exclusion rule at organic components mixtures adsorption removal. At this, the phenolic compound to be adsorbed at a higher rate is the one that is better absorbed from a pure substance solution. The isotherms describing gallic acid adsorption with activated carbons ABG and "Purolat-Standart" clearly shows that pure substance solutions removal rate is 5 times higher than removal rate for mixtures. Experiments proved that quercetin removal rates values with carbon sorbents are higher both for mixtures AND pure substance solution when compared to other studied compounds. Quercetin is adsorbed stronger than gallic acid, the latter having a higher solubility value. Rutin sorption removal from pure substance solution was lower compared with other polyphenols, on account that their molecules are too large for sorption in micropores. Rutin negative adsorption from mixture might be explained by phenolic compounds competing for adsorption centers. Rutin and quercetin compounds are structurally related both containing bulky disaccharides substituents.

Calculated adsorption parameters values are shown in Table 4.

Table 4. Parameters of polyphenols adsorption from mixture with the studied carbon sorbents in static conditions

Carbon grade	Type of equation							
	Langmuir		Freundlich		BET		Dubinin-Radushkevich	
	-G, kJ/mol	Γ_{max}, mmol/g	1/n	b	Q, kJ/mol	Γ_{max}, Mmol/g	Γ_0, g/g	E_0, kJ/mol
Quercetin								
AG-OV-1	27.28	0.00026	0.86	0.00027	2.48	0.00036	0.064	7.64
ABG	27.99	0.00026	0.66	0.0011	2.48	0.00029	0.087	7.93
"Purolat-Standart"	31.61	0.000056	0.92	0.010	2.482	0.000055	0.073	8.09
Rutin								
"Purolat-Standart"	33.8	0.000051	0.685	0.00019	6.11	0.00014	0.078	6.62

Isotherm shape and calculated parameters indicate physical nature of adsorption. Phenolic compounds are sorbed due to Van der Waals forces in micropores and specific interactions resulting in formation of hydrogen bonds with surface OFG, this being supported Gibbs energy values (-G, Table 4).

Coefficient b values obtained using Freundlich equation, relate to the nature of carbon sorbent. Increase in its value at adsorption with activated carbon grade ABG may be attributed to intermolecular interaction increase.

Based on the research carried out with model solution we chose semi-coke "Purolat-Standart" to further study polyphenols behavior under static conditions in wort.

Polyphenols adsorption from wort was studied under static conditions with activated carbons within concentration range from 8 to 160 mg/dm^3. It is worth noting that apart from the studied polyphenols there are other polyphenolic compounds groups present in wort.

With a view to examine impact of substances of different nature we conducted correlation of experimental data obtained from polyphenols adsorption from the model solutions and wort.

As it may be seen from Fig. 5 the isotherm of polyphenols mixture adsorption from water solution belongs to L-type according to Giles classification, whereas the isotherm of polyphenolic compounds adsorption from wort is S-shaped.

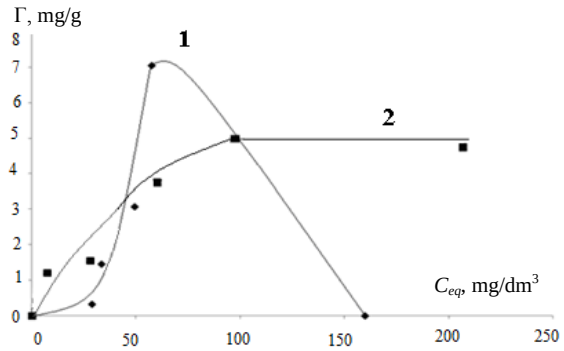

Fig. 5. Isotherms of polyphenolic compounds adsorption from wort (1) and their mixture in the model solution (2) with carbon sorbent grade "Purolat-Standart".

Isotherm 1 (Fig. 5) as opposed to isotherm 2 has an obvious maximum point which may be attributed to the presence of wide range of polyphelic compounds and substances of a different nature in wort.

Low polyphenolic content promotes individual molecules to be sorbed. Increase in polyphenols concentration causes molecular aggregation. It is a transition of molecular aggregations into carbon surface that can explain the significant increase in adsorption. Macromolecular and supermolecular structures interaction in the solution becomes stronger leading to the emergence of continued three-

dimensional mesh in solution, which blocks macromolecules from transiting to the carbon surface. Consequently, having passed its maximum adsorption falls to zero.

Sorption parameters were found from Langmuir, Freundlich and Dubinin-Radushkevich equations (Table 5).

Table 5. Polyphenols adsorption parameters with carbon sorbent "Purolat-Standart"

Langmuir	Dubinin-Radushkevich		Freundlich	
-G, kJ/mol	Γ_0, mg/g	E_0, kJ/mol	1/n	b
mixture				
27.85	5.1	7.3	0.54	$9 \cdot 10^{-4}$
wort				
27.18	7.8	6.14	0.55	$1.9 \cdot 10^{-4}$

When comparing parameters of polyphenols adsorption from model solutions and wort, sorption characteristics of carbon sorbent were found to be sufficiently close (Tables 3, 4). Maximum adsorption capacity value for polyphenols removal from wort is higher, which may be explained by its components interaction with each other and activated carbon.

Comprehensive studies of polyphenols adsorption from pure substance solutions, their mixture and wort revealed that polyphenols adsorption behavior in pure substance solutions and mixtures have varying characteristics. When the studied compounds being removed from mixture the isotherm shape and steepness change is observed, which may be attributed to change in interaction nature between components in the solution and carbon adsorbent surface. Polyphenols adsorption from wort (taking into account its more complex composition compared with the studied polyphenols mixture) will proceed somewhat differently. Experimental data indicate that the process of association of molecules of various nature actively proceed in the solution during polyphenols adsorption from wort; and it is molecular aggregates and not individual molecules that are sorbed on activated carbons further transforming into the active adsorption centers and thus, polyphenols adsorption amount increases.

To reveal semi-cokes application potential in the process of improving beer quality, we compared quality properties of beer produced from semi-coke treated and untreated worts. To make beer we selected samples of untreated wort (control sample) and the wort treated by adsorption on pilot unit with a capacity of 0.8 m^3/h using semi-cokes ABG and "Purolat-Standart" (Table 6).

Table 6. Physical and chemical indicators of unhopped wort

Indicators	Wort control sample	Wort treated by sorbent	
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"
Color, color units	1.2 ± 0.02	0.93 ± 0.02	0.85 ± 0.01
Polyphenols content, mg/dm^3	224 ± 1.50	180.40 ± 1.20	175.89 ± 1.25
pH	4.87 ± 0.02	4.93 ± 0.03	4.9 ± 0.02

After that the obtained wort samples boiled for an hour with addition of granulated hops and suspended solids were removed. Characteristics of the obtained hopped wort are shown in Table 7.

Table 7. Physical and chemical indicators of hopped wort

Indicators	Wort control sample	Sorbent-treated wort	
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"
Color, color units	1.7 ± 0.02	1.6 ± 0.02	1.4 ± 0.02
Polyphenols content, mg/dm^3	271 ± 1.20	225.5 ± 1.25	221.4 ± 1.20
pH	4.92 ± 0.02	4.99 ± 0.03	4.96 ± 0.02
Dry solids mass fraction, %	12 ± 0.01	12 ± 0.01	12 ± 0.01

Hopped wort fermentation took place in laboratory environment at temperature 10 ± 1°C. Laboratory samples underwent secondary fermentation for 21 days at temperature 2°C followed by tasting and analysis of the drink.

Tables 8, 9 display main indicators of the beer control sample, and the beer produced with semi-cokes grades ABG and "Purolat-Standart".

Table 8. Physical and chemical indicators of light beer

Indicators	Beer control sample	Beer produced from sorbent-treated wort		GOST (State Standard) 31711-2012
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"	
Color, color units	1.55 ± 0.02	1.45 ± 0.03	1.15 ± 0.02	0.20–2.50
pH	4.64 ± 0.02	4.72 ± 0.02	4.70 ± 0.02	3.80–4.80
Acidity, acid. units	3.00 ± 0.01	2.60 ± 0.01	2.70 ± 0.01	lesser than 3.20
Dry solids mass fraction, %	5.00 ± 0.02	4.60 ± 0.03	4.50 ± 0.03	lesser than 5.00
Alcohol by volume, %	4.50 ± 0.02	4.81 ± 0.03	4.85 ± 0.03	more than 4.50
Foam head, mm	40.0 ± 2.0	50.0 ± 2.5	60.0 ± 2.0	more than 40.0
Foam stability, min	4.00 ± 0.15	7.00 ± 0.15	8.00 ± 0.20	more than 3.00
Carbohydrates, g in 100 g of beer	4.4 ± 0.02	4.10 ± 0.02	4.05 ± 0.02	lesser than 4.70

Table 9. Controllable physical and chemical indicators

Indicators	Beer control sample	Beer made from sorbent treated wort		Recommended values [4, 10]
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"	
Polyphenols content, mg/dm^3	256.08 ± 1.00	207.51 ± 1.15	201.36 ± 1.20	180–220
Sedimentation limit	13.00 ± 0.05	14.00 ± 0.04	15.00 ± 0.04	more than 15.00
A protein fraction, mg/100cm^3	10.00 ± 0.02	9.55 ± 0.02	9.20 ± 0.02	lesser then 14.00

Table 10. Comparative organoleptic indicators of wort treated by adsorption with semi-cokes ABG and "Purolat-Standart" and untreated wort

Indicators	Control beer	Beer made from sorbent-treated wort	
		Grade ABG	Grade "Purolat-Standart"
Clarity	Clear, no brilliance	Clear with brilliance, no haze particles	Clear with brilliance, no haze particles
Color	Matches beer type, at mid level	Matches beer type, at mid level	Matches beer type, at mid level
Aroma	Fresh, pronounced, fermented, malty, hop, matches given type of beer, no off-odors	Fresh, pronounced, fermented, malty, hop, matches given type of beer, no off-odors	Fresh, pronounced, fermented, malty, hop, matches given type of beer, no off-ordors
Flavor	Clean, pronounced, fermented, malty, hop, matches given type of beer	Excellent flavor, full, clean, well-balanced, fermented, malty, hop bitter, soft, matches given type of beer, no off- flavors	Excellent flavor, full, clean, well-balanced, fermented, malty, hop bitter, soft, matches given type of beer, no off- flavors
Head (foam) and carbon dioxide saturation	Rich, stable, compact, adhesive, 40 mm depth and 4 min retention with slow and abundant bubbling	Rich, stable, compact, adhesive, 50 mm depth and 6–8 min retention with slow and abundant bubbling	Rich, stable, compact, adhesive, 60 mm depth and 7–10 min retention with slow and abundant bubbling

As compared with beer control sample, alcohol volume fraction increases in beers produced from semi-cokes treated wort, and is associated with yeast life function intensification due to reduction in polyphenolic compounds content. The obtained data show that samples of beer made from wort filtered by sorption with semi-cokes contain less polyphenolic compounds and have a higher sedimentation threshold limit value of subsidence compared to control sample. Beer made from wort treated by sorbent grade "Purolat-Standart", has indicators of higher quality.

Loss in value of A protein fraction content (Table 9) in comparison with control sample may be explained by its removal due to interaction with adsorbed polyphenolic compounds. Beer sample from wort treated with activated carbon grade "Purolat-Standart" has the smallest A protein fraction in agreement with its higher adsorption capacity towards polyphenolic compounds.

Tasting analysis of samples was undertaken; organoleptic indicators determined for compliance with GOST (State Standard) requirements 31711-2012 "Beer. General technological conditions" (Table 10) [11] In addition, evaluation was performed quantitatively on a scale system from 1 to 25 (Table 11).

As it may be seen from the tasting analysis result, beer containing smaller amount of polyphenolic compounds is soft flavored, light with brilliance, and has stable and deep head.

Table 11. Organoleptic estimate of beer samples

Indicators	Beer control sample	Beer made from sorbent-treated wort	
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"
Clarity	4.5 ± 0.2	4.9 ± 0.1	4.9 ± 0.1
Color	3.5 ± 0.2	3.5 ± 0.2	4.0 ± 0.1
Aroma	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Flavor	4.0 ± 0.2	4.5 ± 0.1	4.5 ± 0.2
Head (foam) carbon dioxide saturation	4.0 ± 0.2	4.5 ± 0.1	4.5 ± 0.1
Total	21.0 ± 0.1	22.5 ± 0.1	23.0 ± 0.1

Beer samples were studied for compliance with Technical Regulations of Customs Union 021/2011 "On food safety" in terms of toxic elements content and safety microbiological indicators (Tables 12, 13) [12].

The obtained experimental data prove that the beer complies with the technical regulations of Customs union in terms of toxic elements content (Table 13).

As it is evident from Table 12 by the 11th day beer samples exhibit growth in number of mesophilic aerobic and facultative anaerobic microorganisms in control sample to a greater degree; however their quantity is below minimum standards.

Table 12. Microbiological indicators of beer safety

Indicators	Beer control sample		Beer made from sorbent-treated wort				TR CU requirement 021/2011
			Sorbent grade ABG		Sorbent grade "Purolat-Standart"		
	1 day	11 days	1 day	11 days	1 day	11 days	
QMAFAnM, CFU/cm^3	10	440	10	400	10	320	lesser than 500
Product volume, cm^3, containing:							
Coliform bacteria	—*	—	—	—	—	—	not allowed in 10 cm^3 of product
Pathogenic (salmonella incl.)	—	—	—	—	—	—	not allowed in 25 cm^3 of product

Note. * not found.

Table 13. Toxic elements contained in beer

Indicators, mg/kg	Beer control sample	Beer made from sorbent-treated wort		Technical regulations requirement, lesser than
		Sorbent grade ABG	Sorbent grade "Purolat-Standart"	
Lead, Pb	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.3
Arsenium, As	0.04 ± 0.001	0.04 ± 0.001	0.04 ± 0.001	0.2
Cadmium, Cd	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.03
Mercury, Hg	less than 0.001	less than 0.001	less than 0.001	0.005
Nitrosamines	less than 0.001	less than 0.001	less than 0.001	0.003

Thus, control beer complies with the GOST (State Standard) requirements 31711-2012 and TR CU (Technical regulations of Customs union) TP TC 021/2011 in microbiological indicators for unpasteurized beer.

It may be concluded from the analysis that beer from wort treated with semi-coke "Purolat-Standart" exceeds other samples both in organoleptic and colloidal stability predictive indicators.

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Please cite this article in press as: Krasnova T.A., Gora N.V., and Golubeva N.S. Beer quality assurance by controlling wort polyphenolic content with adsorption method. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 36–43. doi: 10.21179/2308-4057-2016-1-36-43.

STUDY OF CHEMISRTY AND HYDROLYSATES DRYING PARAMETERS OF FEATHER-DOWNY RAW MATERIAL

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Received February 07, 2016; Accepted in revised form March 20, 2016; Published June 27, 2016

Abstract: The article describes chemical and amino acid composition of feather-downy raw material. It determines the mass fraction of crude protein, crude fiber, ash, calcium, phosphorus, sodium in the samples of feather-downy raw material. It is stated that the waste from poultry processing obtained from hens of all the studied species are characterized by a high content of crude protein and low in crude fiber and ash. The most valuable feather-downy raw material regarding protein is waste containing keratin obtained from the Lohmann Brown hens. We have studied the composition of the peptide fractions of feather-downy raw materials by polyacrylamide gel electrophoresis according to Laemmli in order to understand chemistry of feather-downy raw material better. The obtained results show the presence of a wide variety of protein fractions with different molecular weights in tested keratin raw material. Half of all the proteins are fractions with a molecular weight of 60.0–56.0 kDa. It is found that feather and downy raw material has a sufficient number of low-molecular peptides. It is proved that this fraction corresponds to alpha-keratin. An important indicator for animal feed is their amino acid content, so we studied the quantitative content of essential and nonessential amino acids in feather-downy raw materials. The results of research indicate that poultry processing waste is rich in sulfur-containing amino acids such as cysteine and methionine. It is proved that the data on the physical and chemical composition of feather-downy raw material obtained from three different breeds of chickens, allow recommending the processing of poultry waste as a promising object for high-protein feed for farm animals with a balanced content of macro and microelements.

Keywords: Feather-downy raw materials, mass fraction of protein, fiber, ash, waste from poultry processing

DOI 10.21179/2308-4057-2016-1-44-50 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 44–50.

INTRODUCTION

The most interesting of all the waste from poultry processing is feather-downy raw material as it constitutes 30% of all waste weight. For its processing it is necessary to study the structure and origin of the raw materials in details and to find an alternative source of its degradation [1, 2, 3]. O.F. Chernova, who works at A.N. Severtsov Institute of Ecology and Evolution, in her article, gives the broad and substantive review of hypotheses of raw materials origin containing keratin, such as feathers and hair. Moreover she makes a list of similarities and differences in their structure, analyzes some signs of convergence [4, 5].

In this study physical and chemical properties of the feathers are of special interest for the selection of the optimal conditions of total hydrolysis. Feathers and hair are developed from the follicle with the ectoderm and mesoderm, they have multi-layered keratin structures capable of regeneration. Regeneration is provided by stem cells located within the follicle [6, 7].

It was found that the development of feathers and hair is governed by a set of similar proteins which work in uniform signaling cascades. Protein SNN is the alarm center, Noggin is responsible for the division of feather and hair cells, WNTβ-11 catenin determines the differentiation of stem cells in buds. The appearance of skin structures is determined by gradients of signal proteins. It is shown experimentally that all the skin appendages appear as a result of the interaction of ecto- and mesoderm. The degree of participation of ecto- and mesoderm in this case may differ, i.e. the development and differentiation of skin appendages are “different variations” [8, 9, 10].

The similarity of functions and a hypothetical common origin of feathers and hair suggest a lot of similarities in their structure. Under the common origin we understand participation of the same tissue structures in the production of feathers and hair. It means some common histological features and similar biochemical regulation.

Feathers and hair evolved separately. It is seen from different composition of feathers and hair keratins. Feathers and scales of modern reptiles are composed by two types of keratins: α- and β-keratins, including the specific form of β-keratin – φ-keratin (analyzed by the appropriate tissue of an alligator). Modern mammals' hair have only α-keratin [11, 12].

Keratins (from Greek *keras*, Gen.case *keratos* – a horn) are structural fibrous proteins consisting of parallel polypeptide chains which have the conformation of α-helix or β-structure (the structure of the pleated sheet). α- Keratins (often simply called keratines) are the main type of protein, which form the outer protective coating of vertebrates. Keratin is a typical representative of fibrous proteins. It is found in the tissue of epidermal origin that is wool, hair, horns, claws, feathers, hooves, whalebone, and others [13]. All of the above tissues are complex multi-component biological formation composed of separate cells which form their various histostructural elements [14].

The aim of this research is to study the chemistry of feather-downy raw material of chickens of various breeds and the development of technological parameters of hydrolysates drying of feather-downy raw material.

OBJECTS AND METHODS OF STUDY

The object of the research is feather-downy raw material obtained from hens of different breeds: the French breed F-15 from LLC "Breeding Poultry State Fram Kolmogorov" (Kemerovo region, Yashkinsky district, village Kolmogorovo); the Lohmann Brown breed from JSC "Kuzbass Poultry Factory" (Kemerovo region, Novokuznetsk district, village Stepnoi); The Lohmann LSL-Classic from Ltd "Inskaya Poultry Factory" (Kemerovo region, village Inskoi).

At different stages of work we used the following materials and reagents: distilled water (GOST 6709-72); sodium chloride (GOST 4233-77, 99.8%, reagent grade); soluble starch (GOST 10163-76, 98.0%, analytical grade); acrylamide ("Sigma", USA); N, N'-methylene-bisacrylamide ("Sigma", USA); ethidium bromide (Sigma, USA).

At different stages of work we used the following equipment: a spectrophotometer UV 1800 (Shimadzu, Japan), ultracentrifuges Beckman J2-HS (Beckman, USA), a nitrogen analyzer Rapid N Cube (Elementar, Germany), a liquid chromatograph LC-20 (Shimadzu, Japan), an amino acid analyzer ARACUS (Analytical Systems Gmb, Germany), the camera for vertical electrophoresis and power source PowerPack HC (Bio-Rad, USA), a UV-transilluminator TCP-20M (Vilber Lourmat, USA), gel documentation system Doc XR plus (Bio-Rad, USA), a fermenter Biostat A plus MO, 5 l, Sartorius (Sartorius, Germany), a refractometer HI 96801 (HANNA, Romania), a centrifuge CV-50 (ELMI, Latvia), analytical balance AND HR-202 i (A & D, Japan), a pH-meter Sevew Compact (Mettler Toledo, USA), a laboratory microbiological incubator ILM-170-01 "Laminar-C" (JSC "Laminar systems" Russia), freeze-drier "HOARFROST-6M" (Russia).

Theoretical and experimental studies were carried out in accordance with modern research methodology of complex phenomena by means of conventional, standard and original methods of biochemical, physical, chemical, structural and mechanical analysis.

RESULTS AND DISCUSSION

The results of the mass fraction of crude protein, crude fiber, ash, calcium, phosphorus, sodium in the samples of feather-downy raw materials are shown in Table 1.

Table 1 shows that the waste of poultry processing, obtained from chickens of all studied breeds, are characterized by a high content of crude protein (79.53–90.11%) and a low crude fiber content (less than 0.87%) and ash (max 0.20%). Most valuable feather-downy raw material regarding protein content are keratin-wastes obtained from the Lohmann Brown hens (mass fraction of crude protein is 90.11%).

The results of studying microelement composition of feather-downy raw material suggest that test samples satisfy the requirements of normative documents for poultry feed regarding calcium (0.90–0.97%), phosphorus (0.70–0.71%) and sodium (0.16–0.31%) content.

The obtained data indicate that poultry processing waste can be used as a promising feedstock for producing high-protein feed for agricultural animals.

In order to understand chemistry of feather-downy raw material better we studied the composition of the peptide fractions by polyacrylamide gel electrophoresis according to Laemmli. The results are shown in Table 2.

Table 1. Chemistry of feather-downy raw materials obtained from hens of different breeds

Indicator	Indicator values of raw material from hens of different breeds		
	"F-15 Isa"	The Lohmann Brown	The Lohmann LSL-Classic
Mass fraction of crude protein, %	79.53 ± 5.33	90.11 ± 5.56	89.08 ± 5.51
Mass fraction of crude fiber, %	0.87 ± 0.04	0.49 ± 0.02	0.69 ± 0.03
Mass fraction of ash. insoluble in hydrochloric acid, %	0.20 ± 0.02	0.31 ± 0.02	0.30 ± 0.02
Mass fraction of calcium, %	0.90 ± 0.10	0.97 ± 0.10	0.89 ± 0.10
Mass fraction of phosphorus, %	0.66 ± 0.05	0.71 ± 0.05	0.57 ± 0.04
Mass fraction of sodium, %	0.16 ± 0.02	0.31 ± 0.02	0.20 ± 0.03

Table 2. Molecular weight distribution of proteins in feather-downy raw material

Molecular weight range, %	Relative content of fractions, %		
	"F-15 Isa"	The Lohmann Brown	The Lohmann LSL-Classic
71.0–66.0	4.1	4.1	3.9
66.0–60.0	3.6	3.9	4.1
60.0–56.0	48.6	50.1	49.1
56.0–46.0	16.1	15.6	13.9
46.0–36.0	5.5	4.7	4.9
36.0–30.0	15.9	13.8	14.1
30.0–15.0	9.7	6.8	9.2
less than 15	3.4	2.0	3.1

The results presented in Table 2 indicate a wide variety of protein fractions with different molecular weights in the test raw material containing keratin. Half of all the proteins are fraction with a molecular weight of 60.0–56.0 kDa.

Feather-downy feedstock has a sufficient number of low-molecular peptides with molecular weight less than 10 kDa. According to the literature, this fraction corresponds to the α-keratin. In addition, the electrophoretogram identified protein fractions with a molecular weight of 43.0–33.0 kDa which are probably representatives of β-keratins.

An important indicator for animal feed is its amino acid composition. Therefore, we studied the quantitative content of essential and nonessential amino acids in feather-downy raw material. The obtained data are shown in Fig. 1–3.

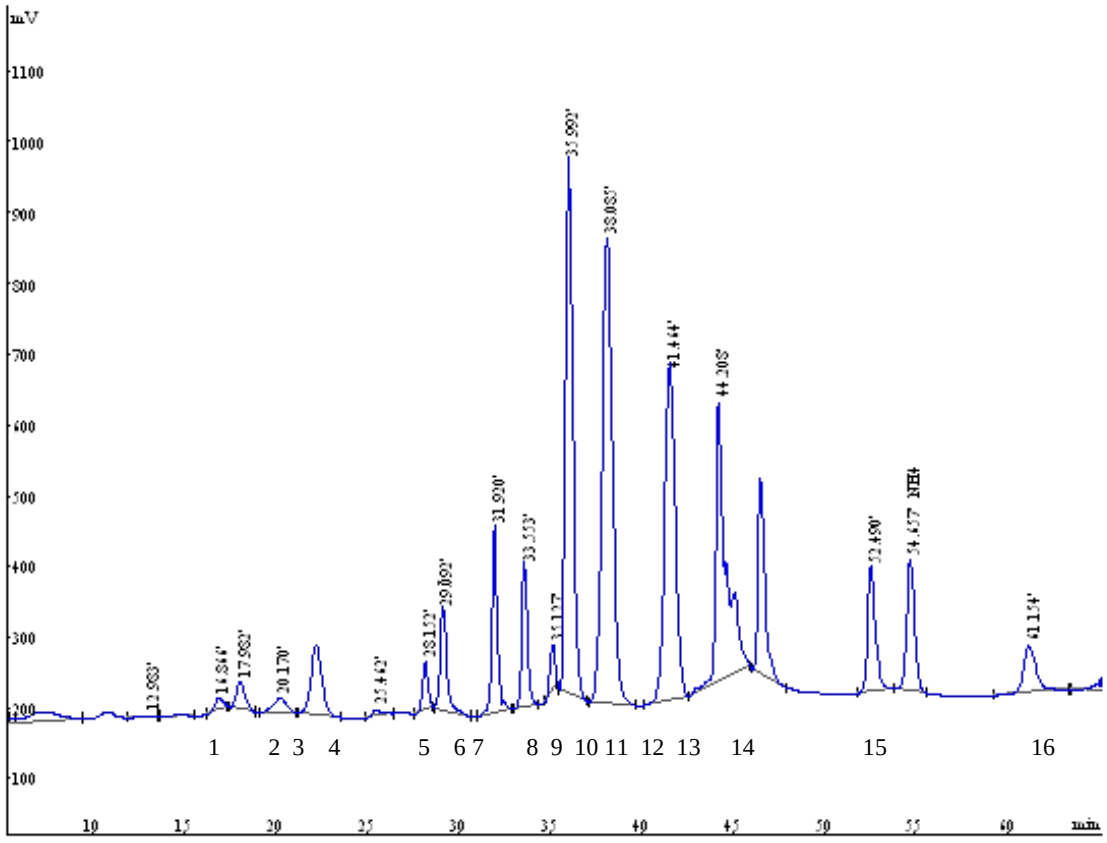

Fig. 1. The chromatogram determining amino acid composition of feather-downy raw material obtained from the breed "F-15 Isa": 1 – aspartic acid; 2 – serine; 3 – threonine; 4 – glutamic acid; 5 – proline; 6 – glycine; 7 – alanine; 8 – cysteine; 9 – methionine; 10 – isoleucine; 11 – leucine; 12 – tyrosine; 13 – phenylalanine; 14 – histidine; 15 – lysine; 16 – arginine.

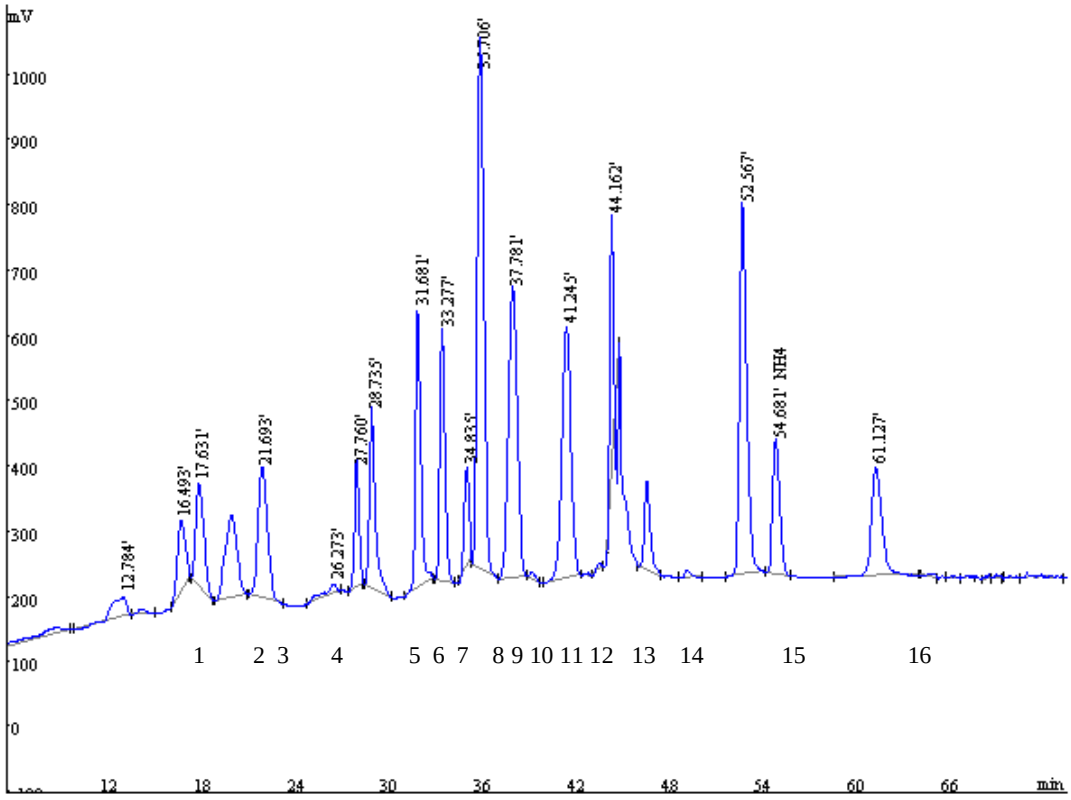

Fig. 2. The chromatogram determining amino acid composition of feather-downy raw material obtained from the Lohmann Brown breed: 1 – aspartic acid; 2 – serine; 3 – threonine; 4 – glutamic acid; 5 – proline; 6 – glycine; 7 – alanine; 8 – cysteine; 9 – methionine; 10 – isoleucine; 11 – leucine; 12 – tyrosine; 13 – phenylalanine; 14 – histidine; 15 – lysine; 16 – arginine.

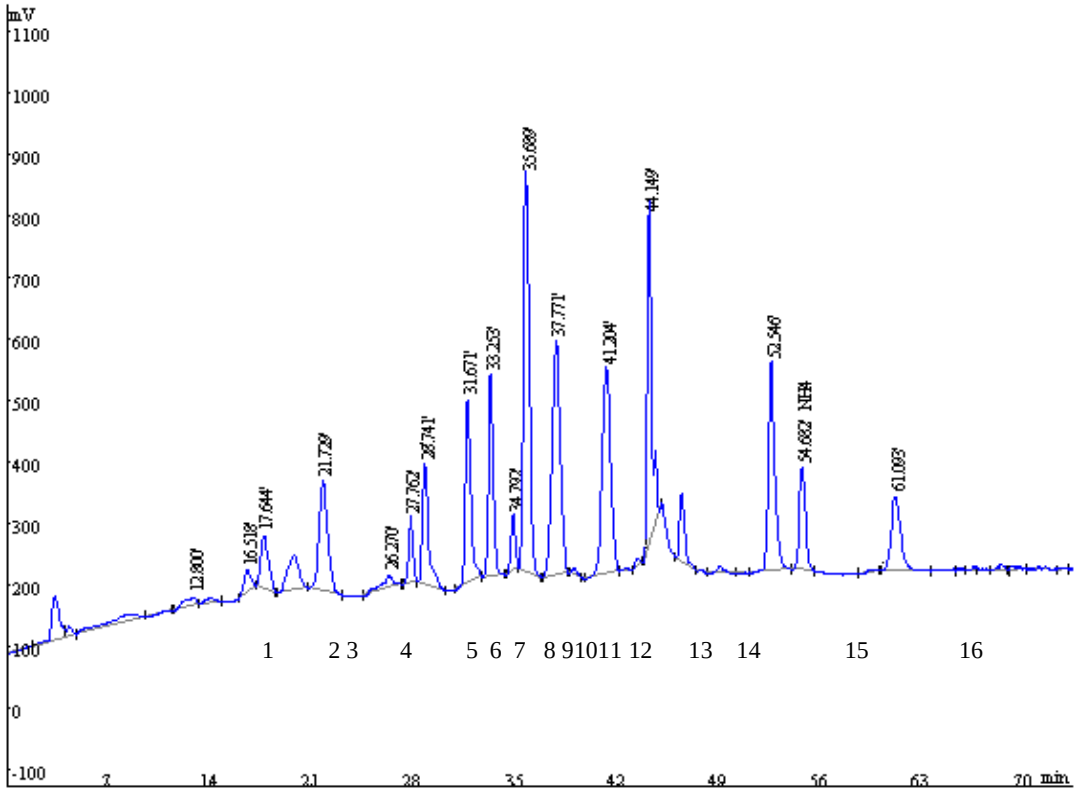

Fig. 3. The chromatogram determining amino acid composition of feather-downy raw material obtained from the Lohmann LSL-Classic breed: 1 – aspartic acid; 2 – serine; 3 – threonine; 4 – glutamic acid; 5 – proline; 6 – glycine; 7 – alanine; 8 – cysteine; 9 – methionine; 10 – isoleucine; 11 – leucine; 12 – tyrosine; 13 – phenylalanine; 14 – histidine; 15 – lysine; 16 – arginine.

The obtained results of amino acid composition of feather-downy raw material indicate that poultry processing waste is rich in sulfur containing amino acids such as cysteine and methionine. The cysteine content is at the average 9.14 mg / 100 g of sample, the methionine content is 12.48 mg / 100 g of sample. As for other amino acids, samples had a high content of aspartic acid (6.88 mg / 100 g), serine (5.39 mg / 100 g), glycine (6.51 mg / 100 g), alanine (5.36 mg / 100 g), leucine (6.24 mg / 100 g), tyrosine (5.14 mg / 100 g), lysine (5.08 mg / 100 g), arginine (5.98 mg / 100 g).

An important step in the production of feed for farm animals based on hydrolysates of secondary raw materials is their dehydration (drying).

Among hundreds of engineering solutions and processes which are applied nowadays there are two basic directions: atmospheric drying and vacuum drying. Atmospheric drying has a significant drawback: it involves a prolonged contact of the product with a high temperature oxygen ambient air composition. It leads to intense oxidation reactions and, as a result, to low-quality of most dry food. Therefore at present vacuum drying at pressure below the triple point (freeze-drying) or vacuum evaporation are more widespread.

Analysis of modern methods of drying leads to the conclusion that the gentlest method of dehydration of biological objects is freeze-drying (lyophilization).

Lyophilization is dehydration of biological objects in a frozen state under vacuum. When freeze drying method is used the removing moisture is carried out through transition ice - vapor. Most moisture (65–85%) is removed by ice sublimation at temperatures below 0°C, and residual moisture removes only when it is heated up to 50–70°C.

Freeze drying has three stages. The first process step of freeze drying is to freeze biological material. In the process of object freezing 20–25% of the moisture vaporizes through the allocation of ice melting heat when water freezes.

The second period (sublimation) is characterized by the constant speed of object drying. At this stage the bulk of moisture is removed. The third period of removing residual moisture is characterized by falling of drying speed; the object temperature becomes positive. During this period the bound moisture not frozen in the object is removed. The drying speed depends on the intensity of heat input. The object temperature is gradually increased to ambient temperature.

Freeze drying provides a longer shelf life of products (up to 10 years) and the maximum degree of recoverability.

At this stage we set parameters of freeze drying (temperature and layer thickness) of waste hydrolysates of feather-downy raw material in order to produce feed for farm animals.

Scores of the temperature and freeze drying duration of waste hydrolysates of feather-downy raw material were carried out at the drying layer thickness of 6.0 mm and at different drying temperatures: 26 ± 1°C, 31 ± 1°C, 36 ± 1°C and 41 ± 1°C. Curves of lyophilized hydrolysates of feather-downy raw material at different temperatures are shown in Fig. 4.

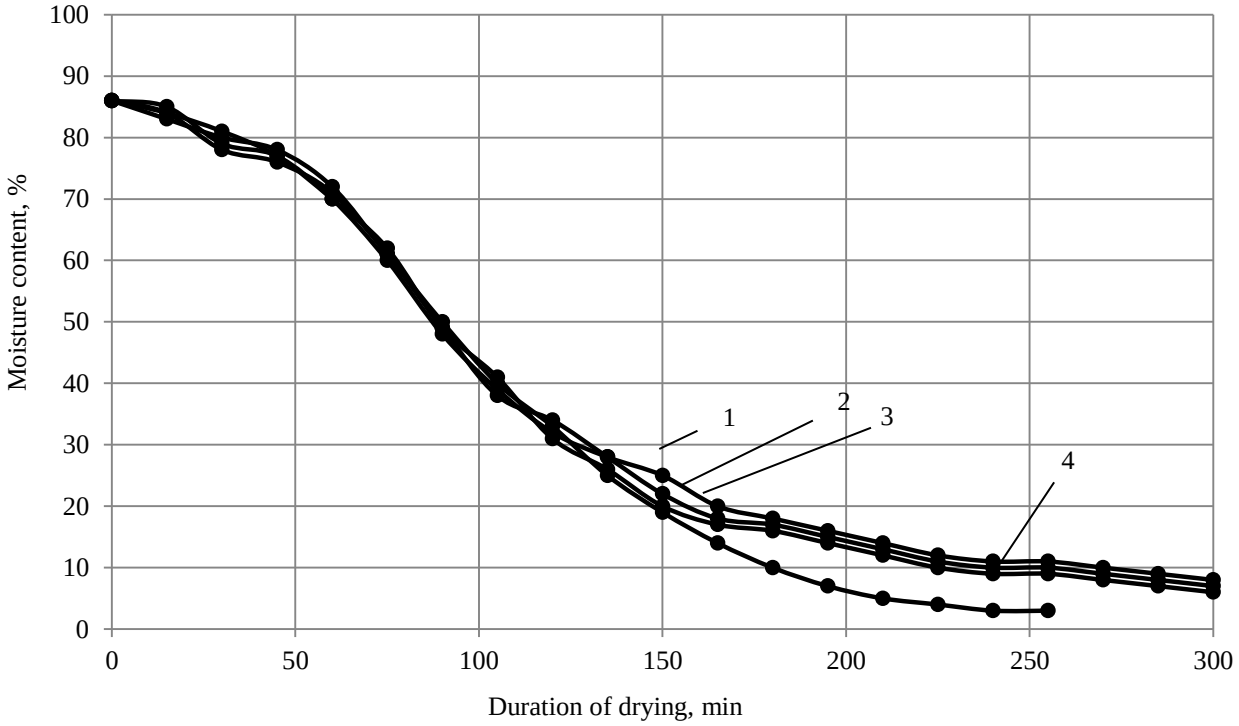

Fig. 4. The curves of lyophilized hydrolysates of feather-downy raw material at different temperatures: 1 – 26 ± 1°C; 2 – 31 ± 1°C; 3 – 36 ± 1°C; 4 – 41 ± 1°C.

Fig. 4 shows that an increase of the heating temperature decreases drying time and moisture content in the hydrolysates at temperatures of 26 ± 1°C, 31 ± 1°C and 36 ± 1°C. The duration of lyophilization is 300 min; the moisture content in the final product is equal to 9.5%. Drying time at temperature 41 ± 1°C is 255 min; the moisture content of lyophilized hydrolysates is 2.5%. Therefore, for further studies we selected temperature 41 ± 1°C of freeze drying of hydrolysates waste from poultry industry and lyophilization duration was 255 min.

An important parameter of freeze drying is the layer thickness. We obtained curves of lyophilization hydrolysates of feather-downy raw material at temperature 41 ± 1°C and with different thickness values (Fig. 5).

Fig. 5 demonstrates that the increase in layer thickness of waste hydrolysates of feather-downy raw material leads to duration of drying and to moisture content in the final hydrolysates. Thus, when a layer thickness is 6.0, 9.0, 16.0 and 21.0 mm the length lyophilization is 240, 270, 300 and 300 minutes, and the moisture content is 2.5, 3.5, 5.5 and 7.5%, respectively. 9.0 mm is selected as an optimal drying layer thickness.

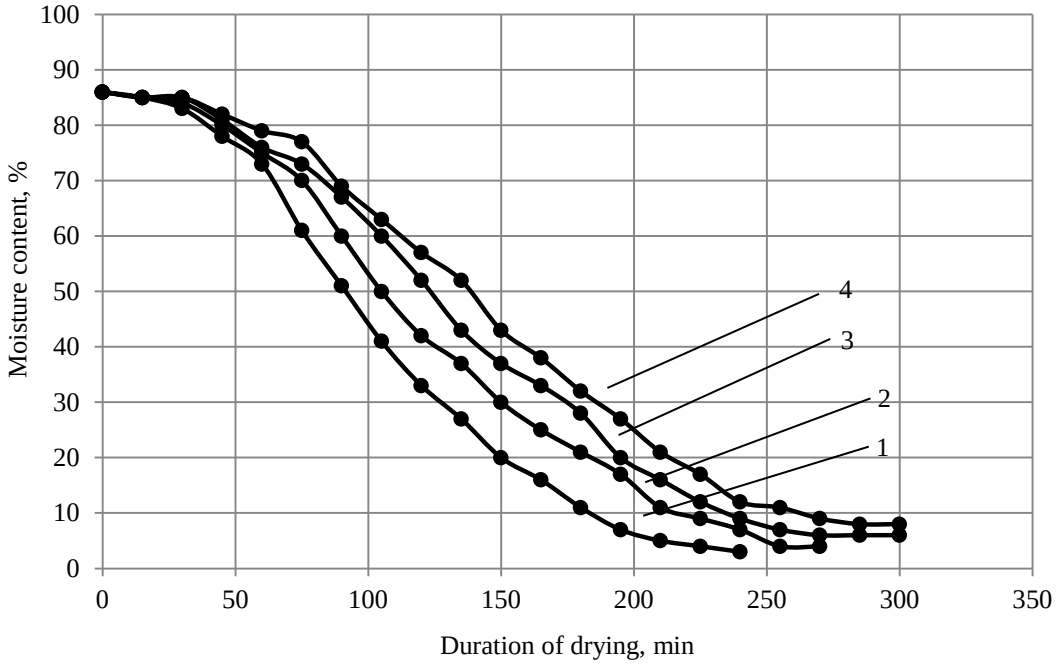

Fig. 5. Curves of lyophilization hydrolysates of feather-downy raw material at temperature of 41°C and of products with different layer thickness: 1 – 6.0 mm; 2 – 9.0 mm; 3 – 16.0 mm; 4 – 21.0 mm.

CONCLUSION

So, we set the freeze-drying parameters of waste hydrolysates from poultry industry: the heating temperature is 41 ± 1°C; the duration of drying is 4.5 hours; the layer thickness is 9.0 mm.

Thus, the research of physical and chemical composition of feather-downy raw material and the study of freeze drying parameters allow recommending the poultry waste processing as a promising object for high-protein balanced high-quality feed.

ACKNOWLEDGEMENTS

The work was completed under an integrated project funded by the Ministry of education and science of the Russian Federation within the framework of government resolution 218 “Organization of high-tech manufacturing high-protein fodder additives and bio-fertilizers on the basis of complex technology of processing of feather down raw materials and other low value waste of the poultry industry” (agreement № 02.G25.31.0151 from 01.12.2015 year).

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Please cite this article in press as: Lisitsyn A.B., Kriger O.V., and Mitrokhin P.V. Study of chemisrty and hydrolysates drying parameters of feather-downy raw material. *Foods and Raw Materials*, 2016, vol. 4, no. 4, pp. 44–50. doi: 10.21179/2308-4057-2016-1-44-50.

PHYSICAL PRINCIPLES OF PROCESSING OFF-GRADE BUCKWHEAT

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Received January 11, 2015; Accepted in revised form December 03, 2015; Published June 27, 2016

Abstract: Currently, there is deterioration of the quality of incoming buckwheat supply. Intensification of harvesting processes, due to application of new combine harvesters aimed at expanding the moisture range of harvested grain, leads to a grain increase at farms and this bulk is kept under unregulated conditions on large open areas. The lack of centralized storage, conditioning and grain drying leads to the fact that producers have to deal with post-harvest handling and storage, though they do not have grain drying units, cleaning equipment and the required number of granaries. In addition, grain storage requires considerable financial costs; therefore not every producer can meet the necessary technological requirements of grain receiving and post-harvest processing. The incoming grain has another moisture content, hard-separable impurities, filmness and content of germinated grains. Processing of such grains using standard practice is costly or this grain is used for feeding purposes. Off-grade grain batches (which don't conform to the requirements of regulatory documents) collected in the foothills of the Altai Territory were chosen for research. The research was carried out for the most common grain parties: with moisture of 17.0–22.0%, with hard-separable impurities above 2.0%, with filmness not more than 19.0%, and buckwheat containing germinated grains. The results of the research allowed offering technology, the distinguishing feature of which is the absence of preparation grains phase before processing. The proposed technology allows to process off-grade buckwheat in order to produce peeled buckwheat and guarantee profitability. The obtained data prove significant advantage of the proposed technology. Economic efficiency of grain processing with four defects is calculated. It is stated that the cost of processing off-grade grain on the proposed technology is much lower than the standard technology of producing buckwheat according to the requirements.

Keywords: buckwheat, humidity, hard-separable impurities, filmness, germinated grains, off-grade grain, spoiled kernel, drying, steaming

DOI 10.21179/2308-4057-2016-1-51-60 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 51–60.

INTRODUCTION

Among cereal crops buckwheat has a special place [1]. Due to its high nutritional and biological value [2–5], the products produced from buckwheat are widely used not only in public catering, but also in baby and healthy foods [6]. Peeled buckwheat, crushed buckwheat and buckwheat flour are produced from buckwheat grains (steamed or not steamed). Peeled buckwheat is the main and most valuable product of processing. However, the quality of peeled buckwheat does not always meet the requirements. Processing efficiency is determined by the characteristics of process, physical, mechanical and technological properties of grain. Quality of processing grain is controlled by the requirements of regulatory documents. Analysis of the enterprise JSC "Biysk elevator" in the foothills of the Altai Territory demonstrated that the proportion of off-grade buckwheat, grain which don't meet the requirements, supplied to the processing for the period from 2000 to 2013 in some years was up to 50%.

To meet the regulatory requirements in the process of preparing this grain to the processing is quite difficult and is not always worthwhile. Processing of such grains leads to the production of substandard buckwheat groats or low grade buckwheat and reduces its nutritional value and eating qualities. The key to the success in the first place is the high quality of products.

Adverse effects on the grain may occur:

– during its cultivation (resulting from edaphoclimatic conditions, affected grains, dead-ripe stage or overwintering in the field);

– during the harvest (as a result of mechanical damage, prolonged storage in heaps, waiting for post-harvest treatment);

– during post-harvest handling or storage because of pests and microorganisms.

Extraneous impurities and high humidity greatly reduces grain value, deteriorates its technological advantages and physical properties. In order to achieve high efficiency of processing buckwheat it is necessary to improve its initial properties to the specified

requirements. Such preparation provides the stabilization of the grains quality produced in accordance with normative documents.

The technological process of buckwheat production currently consists of the following steps: removal of impurities, hydrothermal treatment, sorting into factions, flaking, peeling, separation of peeled products according to the requirements.

This technology allows processing the grain in accordance with the requirements of regulatory documents, however, the use of off-grade grain leads to the production of defective products and unprofitable production.

The aim of this work is to determine basic principles of processing off-grade buckwheat providing obtaining of peeled buckwheat according to the requirements and effectiveness of the proposed technologies.

OBJECTS AND METHODS OF STUDY

Standard methods were used in this research. For test samples we selected a batch of buckwheat grown in the foothills of the Altai Territory in 2012–2014 and a batch imported from China. Both batches did not meet the established requirements according to its characteristics.

The objects of research were:

– batches of grain with moisture content 17.0–22.0%;
– batches of grain containing hard-separable impurities more than 2.0%;
– batches of grain with filmness of not more than 19.0%;
– batches of grain containing germinated grains of buckwheat.

All experimental researches were carried out under factory conditions at the buckwheat producing factory JSC “Biysk elevator” (the Altai region, Russia) with capacity of 100 tons / day.

RESULTS AND DISCUSSION

Buckwheat groats quality indicators are directly dependent on quality indicators of produced grain. Technology analysis enables us to assert that the existing grain processing technology are based on its preliminary preparation to the specified requirements. A distinctive feature of the proposed technologies is the absence of preparation phase before processing. Implementation of the proposed technology allows using off-grade grain to produce peeled buckwheat and guarantee profitability in the processing of defected grain.

Processing of grain with a moisture content of more than 17%

During the processing of grain from JSC “Biysk elevator” with a moisture content of over 17%, buckwheat grain had a high concentration of spoiled and vitreous grain, resulting in the production of low grade buckwheat or substandard products. Separate batches had up to 10% of spoiled grain [7]. Such concentration of spoiled grain does not allow using standard technology for the grain processing, as according to the regulatory documents the amount of spoiled grain mustn’t be higher than 0.2 for 1st grade; 0.4 for the 2d grade and 1.2% for the third grade.

Some grain batches are kept by manufacturers under floor storage on various reasons and the moisture content is up to 22.0%. In such conditions active growth of microorganisms on grains leads to a color change. The initial period is characterized by appearance of "pigmented grains". These grains are not spoiled but the processing leads to the production of buckwheat with high content of spoiled core.

Researches on improving the technology of processing buckwheat with high humidity allowed us to try a new method [8]. It is based on the reduction of the duration of grain preparation for processing. Batches entering the elevator with humidity over 17% and the range of differences not more than 1.0% were distinguished. These batches without pre-drying preparation were sent to a factory for processing. Grain drying up to moisture content 13.5–14.5% was replaced by steaming in this method.

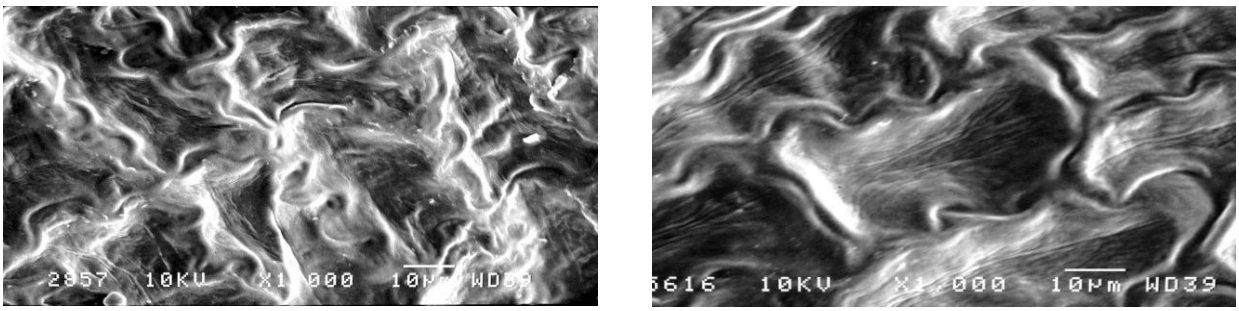
Grain drying is an important step in processing, It provides a quick preparation of grain for necessary conditions, helps equalize the humidity of separate grain components, improves the look and color of the grain, leads to stable storage condition. However, it should be noted that grain drying is costly and energy consuming process. This is due to the fact that convective grain dryers used for drying use liquid fuel for fresh air heating and supply to a drier requires significant power consumption. Moreover, in cases where incoming batches with humidity 17.0–22.0% are processed these direct-flow dryers mustn’t reduce moisture more than 2.0–3.0% per pass of grain.

According to the requirements for drying grain of this quality it is necessary to have a few drying passes until moisture content is 14.0%. Such drying leads to increased fuel consumption and an increase in the cost of grain drying up to necessary moisture content.

The problem of quality and safety of grain is very important in recent years and it is associated primarily with a significant depreciation of existing equipment and increasing of the incoming grain which needs drying.

At the same time a significant damage to grain and the deterioration of its quality happen while passing through the grain elevator equipment, preparation of grain for drying and transporting it. The technological line for acceptance and post-harvest grain processing has more than 30 places of grain damage [9]. Grain damage adversely affect its durability during storage, technological properties and reduces the yield of the end product. Quantitative losses happen due to peeling of buckwheat grain with numerous bumps in the process of transportation and spray of crushed and hulled grains.

A distinctive feature of the wet grain is increasing its size and the surface of deformation respectively. The microstructure of dry and sodden grain surface was examined by photomicrographs obtained on JSM-840 scanning electron microscope (Fig. 1).



(a) (b)

Fig. 1. The surface morphology of buckwheat grain with a moisture content of (a) 14.2%, and (b) 17.8%.

As it can be seen from Fig. 1, the outer surface has a honeycomb structure, in the dry core cell sizes are 10 × 30 microns, in sodden – 70 × 40 mm. This difference is due to the fact that sodden grains are bigger in size.

The proposed method of processing buckwheat with high humidity have optimal conditions for the destruction of microflora from grain surface. Grain, consistently passing through the stages of grain cleaning, was delivered to the steamer of periodic action A9-BIS. Preheating before steaming for 35 minutes up to 20–30°C accelerates the process of hydrothermal grain processing. Steaming is carried out during 6 min at a pressure of 0.55 MPa. The steaming duration is determined by the time from the beginning of steam delivering into the steamer until delivering stops. When grain is taken out from the steamer, it is delivered to the shaft type dryer, and dried by air of 60°C for 20 min until the moisture content is 16.0–18.0%.

Then, the grain goes to the second shaft-type dryer, and dried by air at a temperature of 120–140°C for 50 min. After steaming and drying up to moisture content is 13.5–14.5%, the grain goes to peeling, followed by groats grader and buckwheat production. The proposed technology was applied to research on using grain with following quality indicators (Table 1).

As it is shown in Table 1, for research we used non pre-dried grain before peeling with humidity more than 17.0% for the proposed technology and with a humidity of no more than 14.5% on the recommended technology. The results of comparative tests are given in Table 2.

From the obtained data we can assume that if grain with high moisture content is dried using the recommended technology the Penicillium begins to develop without visible signs of grain damage. Prolonged and repeated drying process stimulates its development and leads to spoiled grains. Temperature steaming mode prevents the Penicillium development thereby reducing the cause of having spoiled grain.

Table 1. The quality of grain used for processing on the recommended and proposed technology

Grain quality indicators	Grain quality according to standard documentation requirements	Actual quality according to recommended technologies	Actual quality according to proposed technologies
Moisture content, %	14.5	13.5–14.0	17.0–22.0
Weight fraction of core, %	73.0	73.0–73.9	73.3–74.1
Weight fraction of hull, %	–	22.3–22.7	22.1–22.6
Weight fraction of impurity, %	2.0	3.4–4.0	3.8–4.1
Weight fraction of grain dockage, %	2.0	1.2–1.5	1.4–1.6

Table 2. Mass fraction of spoiled grain, % in the processing of grain with moisture content more than 17%

Batch of peeled buckwheat	Grain with moisture content more than 17%	
	Recommended technology	Proposed technology
1/03 150 t	0.44–0.78	0.12–0.20
1/05 160 t	0.34–0.64	0.10–0.18
1/05 100 t	0.30–0.38	0.12–0.20
1/07 270 t	0.36–0.52	0.08–0.16
1/09 250 t	0.36–0.42	0.12–0.20

In addition, the temperature and time changes in the proposed method provides a homogeneous core color of six tones [10].

In the proposed technology replacement of grain drying to steaming in a given mode does not increase the cost of peeled buckwheat. According to technological scheme the line capacity is 90 tons / day.

Thus, the processing of grain with high moisture content is possible when steaming is used instead of drying. Obtained products meet the requirements of regulatory documents.

Processing of grain containing hard-separable impurities more than 2.0%

Grain mass except full-value buckwheat contains various impurities, including hard-separable impurities.

Therefore, parent batches of grain are a mixture of grains of various crops, weeds, impurities of mineral and organic origin.

Prior to processing impurities must be removed in order to exclude it from the end product.

However, the presence of impurities especially hard-separable ones (wheat, joint charlock, Tatar buckwheat, field peas and others) complicates greatly the cleaning process and leads to a complex and multi-grain cleaning process. To separate such impurities passes through the grain-cleaning machines are repeated, special grain-cleaning machines are used, the yield of the main grain to waste is increased. Processing of this grain is not always effective. It leads to significant losses in the main grain, producing of substandard groats or off-grade grain and reduces its nutritional value and taste.

In the process of such grain-cleaning and preparation for subsequent processing the content of main grain in wastes is from 10.0% to 70.0% [11].

Recommended equipment does not provide separation of hard-separable impurities without significant loss of main grains and leads to significant economic losses.

Batches of grain with hard-separable impurities up to 10% were subjected to processing.

Traditional grain cleaning method allows to process grains containing hard-separable impurities not more than 2.0%.

Processing of grain containing hard-separable content exceeding 2.0% is not effective.

Loss of normal grain predetermined number of actions connected with a particular monitoring and control of incoming batches.

For the processing of grain containing hard-separable impurities from 2.0% to 10.0% we suggested technological scheme of grain processing, when every fraction of grain and end products are cleaned in two streams 1–3 fr. and 4–6 fr. in addition to traditional cleaning process. To study the work of grain-cleaning line we used grain with quality indicators presented in Table 3.

Installation of frequency converters brand E1-8001 company Vesper on grain cleaners allows to adjust smoothly the frequency and amplitude of oscillation. Changing these settings allows to achieve optimum operating modes of grain cleaning equipment for grain batches of different quality. Test results are summarized in Table 4.

Table 3. Indicators of quality of the processed grain

Grain quality indicators	Grain quality according to standard documentation requirements	Actual grain quality
Moisture content, %	14.5	14.0–14.6
Weight fraction of core, %	73.0	71.8–75.5
Weight fraction of hull, %	–	21.6–23.2
Weight fraction of impurity, including hard-separable, %	2.0 2.0	3.4–5.6 1.8–3.2
Weight fraction of grain dockage, %	2.0	0.8–1.5

Table 4. Comparative analysis of the recommended and proposed grain cleaning

Grain cleaners	Weight fraction of weed impurities in grains on the recommended technology, %	Weight fraction of weed impurities in grains on the proposed technology, %
Two separators BIS-100, gravel separator P3-BCT-100, Trieur Petkus K236	3.4–5.6% (including hard-separable impurities 1.8–3.2%)	3.4–5.6% (including hard-separable impurities 1.8–3.2%)
Impurity contents after recommended machines	1.6–2.4	1.6–2.4
Control of fraction 1 before cleaning After cleaning	no control	0.7–1.8 0.4–0.5
Control of fraction 2 before cleaning After cleaning	no control	0.4–3.6 0.2–0.4
Control of fraction 3 before cleaning After cleaning Control of fractions 4-5-6 before cleaning After cleaning Control of fraction 4 before cleaning After cleaning Control of fraction 5 before cleaning After cleaning Control of fraction 6 before cleaning After cleaning	no control 1.8–3.1 1.0–1.4 no control no control no control	1.7–2.8 0.3–0.4 1.8–3.1 1.0–1.4 1.2–1.5 0.7–0.9 1.5–1.9 0.6–0.9 2.9–4.1 1.7–2.8
Control of peeled buckwheat 1-3 before cleaning After cleaning	no control	0.5–0.7 0.1–0.3
Control of peeled buckwheat 4-6 before cleaning After cleaning	no control	0.7–1.1 0.3–0.5
Content of weed impurities in the end product	1.1–2.3	0.1–0.3
Content of weed impurities according to GOST 5550-74 not more	0.4	0.4

The sequence of grain cleaning with cleaning of every fraction and additional cleaning of grain of 1-2-3 fractions on separator on WLAN-12, 4-5-6 fractions on paddy-machine PM-0.5 allowed to achieve stable results of processing grain containing hard-separable impurities above statutory requirements.

Thus, changing the mode of the equipment and installation of additional equipment allowed to process grain containing weed impurities. At the same time using the proposed technology the content of weed impurities does not exceed the requirements of regulatory documents.

Processing of grain with filmness up to 19.0%

Natural grain features suggest the development of such methods and processing modes, which would guarantee high technological efficiency of a plant.

One of the most important indicators in the processing grains is its geometrical dimensions, namely the degree of development of bran covering. Depending on edges development, buckwheat can be divided into cruise (with a strongly developed edges, while the core does not fully cover the coat, leaving a hollow top) and wingless with rounded edges (rounded grain shape).

Grains having a wingless form of bran covering are characterized by a high degree of plumpness, bran coverings tightly cover the core, edges have a convex shape. It can be described as of medium size, the content of large fractions (1 and 2 fractions) is on average not more than 55%. The weight of 1000 grains varies between 22 and 25 g. This grain has low technological properties which are determined by wingless form of grain, low uniformity, complexity of separation of coverings from the core with its high content of 78 to 82% [12].

The paper studied the processing of wingless buckwheat. An example of this grain is grain grown in the northern provinces of China.

Comparative qualitative parameters of grain processed by JSC “Biysk” obtained from farmers of the Altai Territory and imported from China (Shanxi, Shaanxi and Gansu) are presented in Table 5.

As it is seen from Table 5, qualitative indicators of Chinese and Altai grain differ significantly. For some indicators (total amount of weed impurities, moisture content, weight fraction of the core, filmness) Chinese grain is much greater than qualitative indicators of Altai buckwheat, which will undoubtedly have a positive impact on technological efficiency of the processing and on increasing the output.

However, there is a flip side of Chinese buckwheat processing, namely it is a low uniformity of grain, low content of large fractions, the high content of small fractions (Table 6). Lack of a distinct grain shape of a tetrahedron (more round shape), a firm adherence of bran covering to the core complicates cleaning, fraction division and the process of peeling grains.

Table 5. Quality indicators of Chinese and Altai buckwheat, %

Grain quality indicators	Chinese grain	Altai grain
Moisture content, %	12.6–13.6	13.6–21.0
Weight fraction of core, %	78.2–80.5	72.0–76.5
Weight fraction of hull, %	16.8–19.0	20.0–23.8
Weight fraction of weed impurity, %	0.6–2.9	1.0–5.6
Weight fraction of grain dockage, %	0.2–1.2	0.4–2.8

Table 6. Fraction composition of Chinese and Altai buckwheat

Fraction number	Chinese grain	Altai grain
1	18.0–25.0	25.0–39.0
2	24.0–35.0	47.0–50.0
3	23.0–25.0	12.0–15.0
4	12.0–15.0	5.0–6.0
5	6.0–9.0	1.0–2.0
6	3.0–4.0	0.5–1.0

Identified features of qualitative composition and morphological structure of Chinese grain defined a number of actions which are connected with technological grain processing:

– Selection and changing of cell of screening surface with a simultaneous change in the kinematic parameters of the screening process equipment;

– Changes in the screening surface at stages of grain separation into fractions.

a) *Cleaning of buckwheat grain from weeds and grain impurities*

Recommended technological scheme of processing buckwheat at JSC “Biysk elevator” identified a number of shortcomings in Chinese grain processing. Features of processing are determined by the difference in quality parameters of grain.

Cleaning buckwheat of Chinese production from weed and grain impurities on the recommended technology led to a loss in grain up to 25% of main grains. The low percentage of grain uniformity up to 55%, a high percentage of small fractions significantly reduces the efficiency of the grain cleaners. Therefore, to separate impurities from Chinese grain is necessary to apply the sieves with larger sizes. Thus, separation needs sieves whose dimensions are comparable to or larger than the sixth grain fraction. This leads to the fact that a considerable amount of grain goes into waste. The content of the sixth fraction is up to 5%, so loss of the end product output can be up to 3.5%. To study the work of grain-cleaning line we use grain with following quality indicators (Table 7).

Table 7. Quality indicators of processed Altai and Chinese buckwheat

Grain quality indicators	Established requirements	Actual quality of Altai grain	Actual quality of Chinese grain
Moisture content, %	14.5	13.9–14.8	13.0–13.8
Weight fraction of core, %	73.0	73.1–74.4	79.4–80.1
Weight fraction of hull, %	–	21.8–22.6	18.8–19.2
Weight fraction of weed impurity, %	2.0	3.4–4.7	1.1–1.6
Weight fraction of grain dockage, %	2.0	1.1–1.6	0.3–0.5

According to requirements grain was cleaned from impurities sequentially on two air-sieve separator. It was suggested to change the specifications of separators, namely the frequency range of vibrations and sieve inclination angle for the cleaning of grain with a high content of fines in the separators BIS-100. Therefore, actuators were fitted with frequency converters of brand E1-8001 company Vesper. It allows to smoothly change modes and separators to determine the optimum operating conditions for a given batch.

Optimal sorting sizes and sieve to separate the elongated, short and shallow impurities are determined empirically for each batch of buckwheat (Table 8).

Table 8. Specifications of BIS-100 separators

Characteristics	Recommended for Altai buckwheat	Recommended for Chinese buckwheat
Frequency of circular field oscillations, min	360	230–280
Radius of the circular oscillations, mm	11	7–9
Number of cleaners in the frame, pieces	32	48
Sorting sieve Cleaning sieve	Triangular 7.5:7.0 2.8x20:2.6x20	Triangular 9.0:8.0 2.6x20:2.6x20
Inclination angle of sieve frames to the horizon, gr.	7	5
Content of normal grain in grain wastes, %	25.7–28.4	8.0–10.1

Thus, changes in the kinematic parameters of sieve oscillation and angle of tip increased the efficiency of impurities separation and helped to determine the optimal performance of sieve separators LSI 100 It led to a decrease in the content of normal corn in grain wastes from 25.4 to 10.1%.

b) *Sorting the grain into six fractions*

A distinctive feature of buckwheat processing is its sorting into six fractions before peeling. When this process before peeling is not accurate enough in the subsequent process of sorting grain products (peeled buckwheat, unscoured grains, crushed grains, buckwheat meal, hull) peeled buckwheat groats can have unscoured grain, which have the similar size. It results in substandard production. According to the requirements peeled buckwheat of 1 st, 2 nd, 3 rd grades can have 0.3, 0.5, 0.7% of unscoured grains respectively.

In the recommended production line the process of sorting by size before peeling has two stages. In the process of pre-screening three grain streams of 1, 2 fraction, 3 and 4, 5, 6 fractions are obtained. The final sorting of buckwheat has six factions.

Chinese buckwheat has a more rounded shape and a low uniformity compared with buckwheat grown in Altai. When the recommended technology is used 1 and 2 fractions contain 15% of fine grains due to the high percentage of 3, 4, 5, 6 fractions.

Grain processing using this scheme leads to an increased content of unscoured grains and increased content of crushed core in peeled buckwheat.

For efficiency of fractionating it was necessary to increase the sieve surface to improve the sorting grain size and product quality in the subsequent processing of the grain.

To solve this problem an integrated approach was used, which consisted in changing the kinematic sieving parameters (amplitude and oscillations frequency) (Table 9) and redistribution of sieving surface.

Table 9. Specifications of plansifters RHA-4M on grain sorting

Characteristics	Recommended characteristics for plansifter ЗРШ-4М	Proposed characteristics
Frequency of circular field oscillations, min	220	140–180
Radius of the circular oscillations, mm	41	20–26
Number of cleaners in the frame, pieces	12	16

Sorting of grain by size was carried out in three stages, preliminary it was divided into four fractions fr. 1, fr. 2, fr. 3 and 4, 5, 6 fractions and finally it was divided into six fractions twice.

Grain divided into 6 fractions according to grain size and passed through sorting steps is delivered separately to buckwheat scourers according to fractions.

Recycling of wingless buckwheat on the proposed technology made it possible to change the efficiency of sorting schemes and to get products which meet the requirements of regulatory documents.

Thus, changes in product movement scheme made it possible to separate grain at the preliminary sieving

step not into three, but into four flows and to increase the sieving surface for final sorting of fractions twice.

These changes helped to find the optimal conditions for the subsequent effective peeling of grains.

Batches of buckwheat containing germinated grains

If there is sufficient amount of moisture grain rapidly increases in volume and starts germination. Defects of such grain batches depend on the number of germinated grain and germination time which is connected with the length of the germ. These grains have improved energy, active physiological state and it leads to a change and deterioration of food processing properties.

As there is no data on processing of germinated grain in the literature, there is a need to prove experimentally the value of the maximum permissible content of germinated grains in the incoming batches.

For the research batches of buckwheat containing up to 3% of germinated grain were selected. The sprout length was 3 mm or more. However, during transportation of grain from the producer to the consumer up to 60% of germs were broken, as well as because of technological machines during grain processing (Fig. 2). The absence of sprout made it difficult to sort out germinated grains according to the degree and duration of germination. Therefore, the sprout length in our research was not considered. Batches for research were formed only on the quantitative content of germinated grains; medium samples were taken in seed-preparation facility.

Fig. 2a shows that grain germination leads to visible damage on surface such as cracks sized 3–5 micron, which are arranged along the formed sprout. On Fig. 2b there is no upper part of the edge with the sprout, and there are crack sized 10–15 microns.

We studied the content of spoiled grain in peeled buckwheat depending on the content of germinated grains (spoiled grains and non-germinated grains were not taken into account).

Grain batches were roughly divided into three groups:

(1) with the content of germinated grains up to 1.0%;
(2) containing 1.1–2.0% of germinated grains;
(3) with the content of germinated grains 2.1–3.0%.

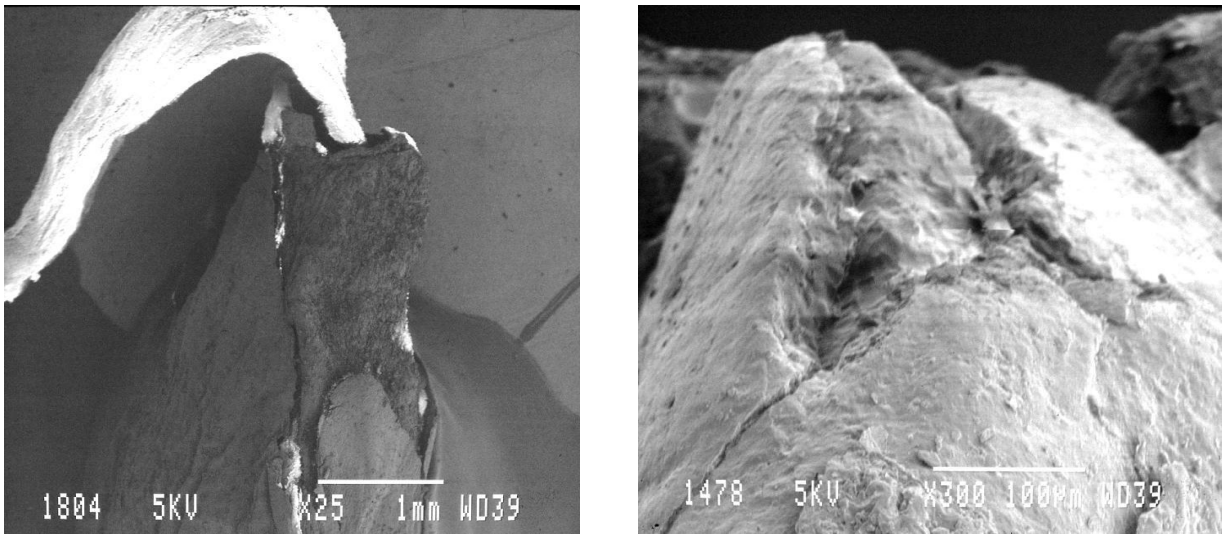
Above divided grain batches were processed, and the contents of spoiled grain was determined according to the requirements. All studies were repeated 3–10 times and processed statistically.

Studies based on the specified requirements demonstrated that peeled buckwheat of first group in terms of spoiled grain belongs to the first class (no more than 0.2%), the second group corresponds to only Class II with the content of spoiled grain of no more that 0.4%, the third group is the third grade (no more than 1.2%).

Non-linear dependence of mass fraction of spoiled grain on the content of germinated grains, is possibly connected with varietal characteristics, long-term keeping of grain in adverse storage conditions, possible sorting of grain producer to the content of germinated grains up to 3%. Grains with more than 3% were not processed. During research it was impossible to trace the length of keeping grain in unfavorable conditions. Moreover, batches were received from the manufacturer and directly from the field.

In order to characterize the quality of germinated grain [13] we determined physical and chemical properties and safety performance. It was shown that the main indicators of normal and germinated grains are comparable. However, germinated grain has an increased content of fat due to possible enzymatic hydrolysis.

To reaserch further the characteristics of the studied group we determined grain indicators fat, fat acid number (KCHZH) and acidity according to conventional techniques. For comparison we used the similar indicators of normal, spoiled and germinated grains. The results are summarized in Table 10.



(a) (b)

Fig. 2. Micrograph: (a) part of surface of unsteamed core with the sprout (from germinated grains) ×25, and (b) part of surface of unsteamed core with the broken sprout (from germinated grains) ×300.

Table 10. Indicators of fat acid number, fat and acidity of the studied groups: normal, spoiled and germinated grains

Sample	Weight fraction		
	Fat acid number, mg KOH/g	Fat, %	Acidity, gr.
Normal grain*	4.1–7.2	1.7–2.0	2.1–2.8
Grain of 1st group	5.0–7.6	1.8–2.2	2.1–2.7
Grain of 2d group	5.1–7.8	1.9–2.1	2.4–2.9
Grain of 3d group	5.4–7.8	1.8–2.0	2.6–3.4
Spoiled grain**	26.4–35.8	1.4–2.0	7.8–12.6
Germinated grain***	28.3–36.4	2.1–2.4	9.8–12.4

Note. * – grain, which has no germinated grains; ** – grain, consisting of spoiled grain; *** – grain, consisting of geminated grains.

Grain of studied groups is comparable to the normal grain, it can be used for the production of peeled buckwheat.

As it is shown in Table 10, the high content of fat acid number and acidity in spoiled and germinated grains is probably due to hydrolysis of fats that occur under the influence of the enzyme lipase forming free fatty acids, decompounding organic phosphorus compounds (phosphatides, phytin, phytosterols), releasing acidic phosphate salts, resolving protein and other substances, increasing amount of acid active compounds.

The increase in the fat content in germinated grain can be associated with the enzymatic hydrolysis, its reduction in spoiled grain, perhaps due to the decomposition of fats by grain microflora.

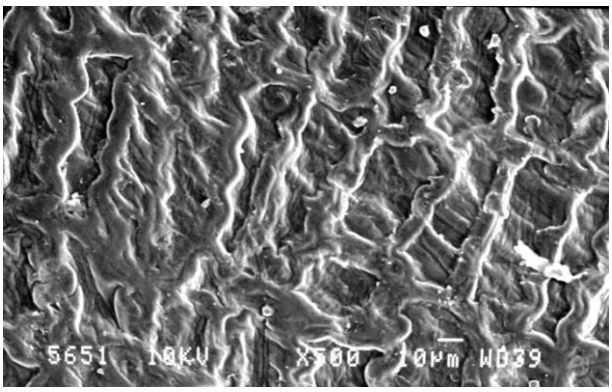
Additionally, we investigated the microstructure of peeled buckwheat surface obtained from normal, spoiled and germinated grains.

The microstructure of the surface of peeled buckwheat surface from normal, spoiled and germinated grains was examined on micrographs obtained from a scanning electron microscope JSM-840. The surface morphology of the samples is shown in micrographs (Fig. 3).

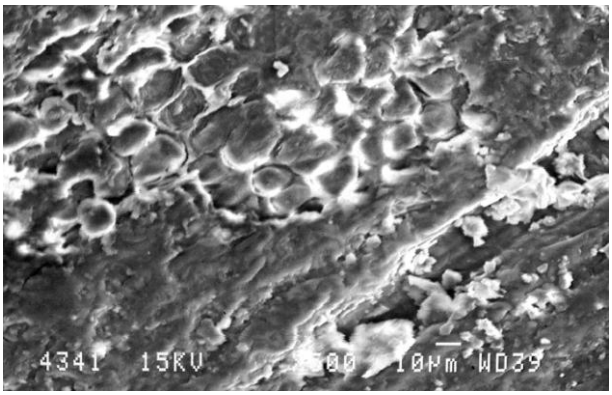
As we can see from figure 3, the microstructure of the outer surface of steamed grain from normal grain has dense cellular structure without damage with uneven distribution of fiber of diameter 5–10 microns. The structure has a polyhedral shape with a mesh size of 10–20 microns up to 70–40 microns. This indicates that steamed grain has surface starch gelatinization and its hardening by heat treatment [14].

The surface of steamed grain produced from spoiled grain has microstructure of discernible densely packed rounded grains of starch sized 5–15 microns. Starch grains are arranged in rows and are connected to the protein matrix, which is consistent with previous studies [15]. The absence of seed coat and the aleurone layer on the core surface is probably due to prolonged exposure to moisture and heat resulting in swelling and deformation of the core and a complete destruction of the structure.

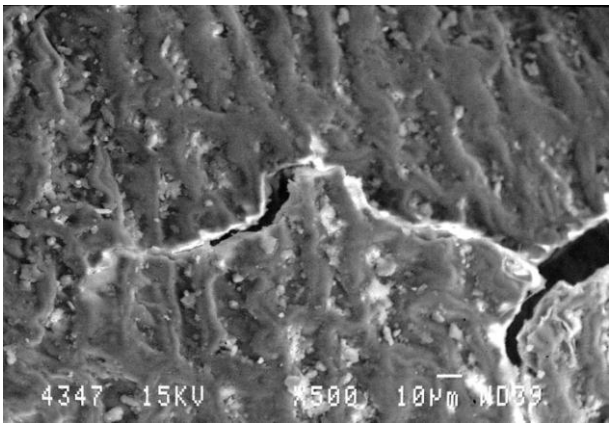
The surface of spoiled grain produced from germinated grain has also a very distinct honeycomb structure with an uneven distribution of fibers of diameter 7–20 microns. The structure has a polyhedral shape with a mesh size of 10–20 microns up to 20–50 microns. This change in the structure of the core surface from germinated grains indicates a higher degree of starch gelatinization in comparison with the normal grain at the same mode of hydrothermal treatment. The entire surface of the core is covered with a grid of cracks having a width of 3–20 mm and length up to 500 mm. Probably, the presence of developed cracks on the core surface lead to a significant increase of the specific surface of the core, contributes to overheating of such grains during heat treatment, greater gelatinization of surface and darkening of core endosperm.



(a)



(b)



(c)

Fig. 3. The surface morphology of buckwheat samples from (a) normal, (b) spoiled, and (c) germinated grains.

The total surface area of germinated and normal grain was determined by BET method at initial step of adsorption isotherm [16]. Measurement of nitrogen adsorption isotherms was carried out on an automatic volumetric vacuum setting "ASAR-2000" at 77.5K (samples were prepared, evacuated in vacuo at 493K before the complete cessation of gas evolution). The results are shown in Table 11.

As it is shown in Table 11, the specific surface area increases 3 times due to germination process.

Analysis of the research results of germinated grain showed that in the process of hydrothermal treatment germinated grains with a developed grid of cracks on the surface are characterized by darkening of the endosperm and considered to be spoiled. The more germinated grain is after the hydrothermal treatment, the more spoiled grain appears.

For the processing of grain containing germinated grains, it is necessary to use a weaker TRP modes to obtain peeled buckwheat of lighter color.

The calculation of the cost of off-grade grain processing was made on the basis of value set in the current period of grains and groats in the standard output of peeled buckwheat 69% and 74% for the Chinese grain. Standard grain was used to compare. Calculation of cost of off-grade grain processing is presented in Table 12.

Table 11. The specific surface of normal and germinated grain

Sample	Specific surface of buckwheat, cm^2/g
Normal grain	1100 +/- 30
Germinated grain	3290 +/- 150

Table 12. Cost of off-grade grain processing

Defect of grain	Cost, 1 ton/ruble	Cost reduction, %	Cost effectiveness, %	Grain price for 1 ton with VAT (ruble)	Groat price of 1 ton with VAT (ruble)
Standard grain	11957	–	10.0	8000	14468
Wet st.*	12332	–	6.7	8000	14468
Wet prep.**	11997	2.9	9.6	8000	14468
With hard-separable imp. st.	12438	–	5.7	8000	14468
With hard-separable imp. prep.	12312	3.7	6.8	8000	14468
With filmness up to 19 % st.	11997	–	9.6	8000	14468
With filmness 19 % prep.	11285	6.3	16.5	8000	14468
Containing germinated grain st.	11997	–	9.6	8000	14468
Containing germinated grain prep.	11734	2.4	12.1	7800	14468

Note. * st. – standard technology; ** prep. – proposed technology.

The presented data suggest that a comprehensive approach to the modernization of Russian equipment allow processing off-grade grain with obtaining end products in accordance with the requirements, reducing the cost to 6.3%. Thus, changing drying rate mode and fractionation methods make it possible to increase the efficiency of processing off-grade buckwheat grain.

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Please cite this article in press as: Mar'in V.A. and Vereshchagin A.L. Physical principles of processing off-grade buckwheat. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 51–60. doi: 10.21179/2308-4057-2016-1-51-60.

TECHNOLOGY OF AFTERPURIFICATION OF DRINKING WATER FROM ORGANIC CONTAMINANTS IN PRODUCTION OF FOODSTUFF

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Received April 28, 2016; Accepted in revised form May 30, 2016; Published June 27, 2016

Abstract: The technology of afterpurification of drinking water is developed for upgrading of foodstuff from organic contaminants periodically present at natural water or formed on a stage of disinfecting by ozonization. The adsorption research of phenol, formaldehyde and acetic aldehyde from individual water solutions and their mixes on active charcoals (AC) marks AG-3, ABG, KsAU, AG-OV-1, SKD-515 and BAU differing in contents, in the way of reception, structure and chemical state of a surface is carried out. The basic laws, features and the mechanism of adsorption organic contaminants on AC are established. The mechanism of mass carry is shown at adsorption of mixes of phenol and formaldehyde, formaldehyde and acetic aldehyde on AC of different marks. A method to optimise the parametres and modes of continuous absorption cleaning, based on the fundamental equation of external diffusion dynamics of absorption, using Dubinin-Radushkevich constants and kinetic dependences, is offered. The basic parametres of adsorption dynamics which allowed determining the operating period of the column, the quantity of refined water depending on the rate of transmission, height of fixed bed and sizes of a column are stated. According to the results of experimental researches and derivatographic analysis the technology of regeneration of active charcoals after adsorption of mixes by washing AC by water warmed up to 50°C with the subsequent warmup by a stream of air with temperature 200°C within 2 hours that allows to reduce sorptive capacity of sorbents on 95–98% is developed. The technology solution for afterpurification of drinking water from phenol, formaldehyde and acetaldehyde, occasionally presenting in natural water or arising in the stage of ozonation in water processing, was recommended on the basis of the complex analysis of absorption (balance, kinetics and dynamics) of organic substances, optimization of purification modes and parametres of absorption column, using mathematical modelling.

Keywords: adsorption, active charcoals, drinking water, phenol, formaldehyde, acetic aldehyde

DOI 10.21179/2308-4057-2016-1-61-69 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 61–69.

INTRODUCTION

Drinking water playing the major role in maintenance of people's health is one of irreplaceable components in production of much foodstuff: reduced dairy production, meat and bakery goods, alcoholic and soft drinks. Water from system of economic-drinking water supply or prepared of underground water sources is basically used on food industry enterprises.

Kemerovo region – is a large territorial and production complex of the Russian Federation, where the considerable quantity of the metallurgical, chemical enterprises is placed, the mines which are a source of intensive pollution of the main waterway of area, the river Tom'. Therefore in Kuzbas underground waters are also used for water treatment. In the structure of water supply of Kemerovo region underground waters make 37.2%. Practically in all underground waters of Kuzbas there are phenols which are washed away from coal layers and humic connections. So, in Kuzbas in 2015 33.1% of hallmarks of water from sources of the centralized water supply of population mismatched hygienic specifications on sanitarian-chemical indexes, including from open reservoirs of 13.6%, underground – 33.6% [1, 2].

Underground water is extracted from a source and is exposed to purification directly in a place of extraction with application of modern technologies, including the ozonization, excluding influence of external medium and contact to the person. Ozone is one of the strongest natural oxidants and a good disinfectant agent. Ozone is applied as disinfectant and oxidations of organic contaminants [3]. However as a result of water disinfecting by ozone, oxygen-containing organic compounds mainly aldehydes (formaldehyde, acetic aldehyde) can be formed. Being in water in the concentration exceeding maximum concentration limit, these contaminants damage central nervous system, liver, kidneys, possess toxic and carcinogenic properties [4–9].

So, there is a necessity of working out a technology of water afterpurification from organic contaminants, including phenol, formaldehyde and acetic aldehyde. For water purification from microadmixtures of organic compounds it is more expedient to use the

adsorptive methods. The efficiency of water adsorption cleaning from organic compounds is defined by active charcoals on set of researches of balance, kinetics and dynamics of the adsorptive process.

The purpose of the present work is producing the adsorptive technology of afterpurification of drinking water for foodstuff production from phenol, formaldehyde and acetic aldehyde, occasionally presenting in natural water or arising in the stage of ozonation in water processing.

OBJECTS AND METHODS OF STUDY

Research objects were: active charcoals of marks AG-OV-1, SKD-515, AG-3, BAU (manufacturer "Sorbent", JSC, Perm'), coconut active charcoal KsAU ("Eurocarb", England), ABG (lignite semi-coke, "Karbonika-F", CJSC, Krasnoyarsk), differing in raw materials, in the way of reception, chemical state of a surface, technical characteristics; water solutions of acetic aldehyde, formaldehyde, phenol with variable organic content (0.0001–50 mmol/dm^3) and their mixes. Balance of mixes adsorption of phenol and formaldehyde is studied in the ratio components 1 : 50 (mmol/dm^3), formaldehyde and acetic aldehyde – 9 : 1. The chosen parities of organic matters correspond to the periodic maintenance of phenol, formaldehyde, acetic aldehyde in water prepared with utilization of ozone. Studying of kinetics and dynamics of adsorption of systems formaldehyde-phenol – water-AC and formaldehyde-acetic aldehyde – water-AC were made on modelling water solutions with the greatest possible real maintenance of components during spring period in water with concentration of formaldehyde of 0.2000 mg/dm^3, phenol – 0.0120 mg/dm^3, acetic aldehyde – 0.0500 mg/dm^3. Production tests were made in the shop of bottled water of "Talinka", LLC using ozonization as disinfecting.

The maintenance of phenol, formaldehyde, acetic aldehyde in samples was defined by method of molecular absorption spectroscopy.

Porous characteristics of active charcoals (total area of surface, area of micropores, volumes) were studied by method of low-temperature adsorption of nitrogen at 77°C on the device "Sorbtometr M" (production of IR Siberian Branch of the Russian Academy of Science, Novosibirsk).

IR-spectroscopic researches were made on samples AC preliminarily slashed to a powdery state and mixed with KBr in the ratio 1 : 10. IR DOFP-spectra were registered on the infra-red Fourier-spectrometer "Infralyum FT-801', production of IFP Siberian Branch of the Russian Academy of Science (Novosibirsk) in the range of 4000–500 cm^{-1}, number scans 50.

RESULTS AND DISCUSSION

One of the basic criteria of estimation of sorbents' adsorption properties are adsorption isotherms. Adsorption isotherms were constructed under the received experimental data of adsorption of formaldehyde, phenol, acetic aldehyde from solutions of individual components in a wide interval of concentration by carbon sorbents (Fig. 1). Various adsorptive capacity of active charcoals at adsorption of organic matters from solutions is caused by physical and chemical properties of an adsorbent and adsorbate nature.

In the field of low concentration (Fig. 1) for all adsorption isotherms is typical the linear site, testifying that the maximum adsorbability has not been reached yet. The isotherms presented in Fig. 1a, 1b have a classical appearance and according to Giles's classification adsorption isotherm of phenol and formaldehyde from water solutions of individual components are isotherms of class L, isotherms of acetic aldehyde adsorption are isotherms of class S. The character of sorption isotherms according to Giles's classification allows to make the conclusion that interaction between the adsorbed molecules is not enough, and critical increment of energy does not depend on degree of fullness of sorbent surface. In case of adsorption of acetic aldehyde the force of interaction between the adsorbed molecules is more than force of interaction between a solute and an adsorbent, and energy activation increases [10, 11].

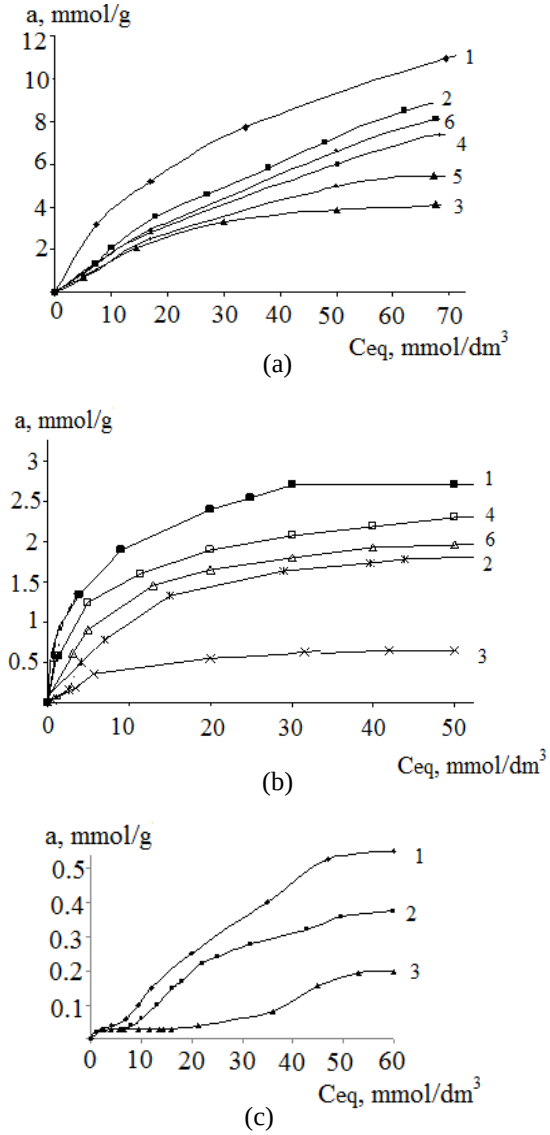

Fig. 1. Adsorption isotherms (a) formaldehyde, (b) phenol, (c) acetic aldehyde from individual water solutions on active charcoals: 1 – KsAU; 2 – AG-3; 3 – ABG; 4 – SKD-515; 5 – BAU; 6 – AG-OV-1.

The analysis of adsorption isotherms of organic contaminants also shows, that the maximum adsorbability of carbon sorbents depends on nature, contents, specific surface and porosity.

The influence of adsorbate properties is presented on Fig. 2.

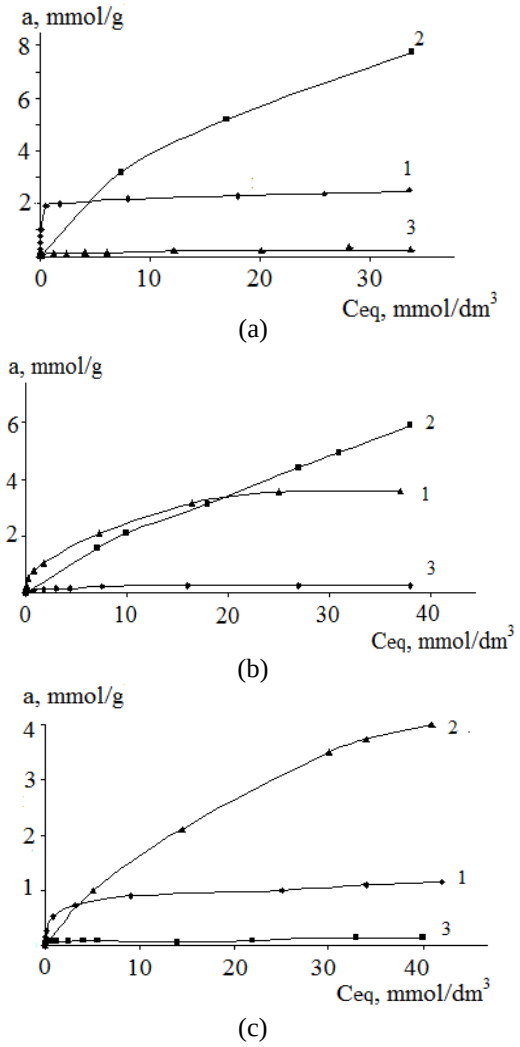

Fig. 2. Adsorption isotherms of organic matters by active charcoal (a) AG-3, (b) KsAU, and (c) ABG: 1 – phenol; 2 – formaldehyde; 3 – acetic aldehyde.

The dependence known from literary data [11] between solubility sorbate and its sorption activity proves to be true for all probed substances. The influence of solubility on the acetic aldehyde adsorption, which limit of solubility is infinite, is especially appreciable. Because of high water solubility it is more favourable for acetic aldehyde thermodynamicly to be in solution, than to be adsorbed on AC. Higher solubility of formaldehyde in water (12400 mmol/dm^3) in comparison with phenol in water (925 mmol/dm^3) and ability of formaldehyde to form clusters in the solution also reduces sorptive capacity because of dispersive interaction for the yielded component on all probed coals. In the yielded conditions it is more favourable for formaldehyde to be adsorbed on superficial active groups forming the secondary adsorptive centres. In spite of the fact that formaldehyde and acetic aldehyde have identical functional groups, in this case the influence of solubility prevails and acetic aldehyde practically is not adsorbed. In the field of high concentration formaldehyde adsorption exceeds phenol adsorption, i.e. the yielded dependence is not conserved, because solubility is not the unique factor defining adsorption from water solutions of individual substances. At concentration increasing formaldehyde is adsorbed on the secondary adsorptive centres, forming new active centres for formaldehyde adsorption. Formaldehyde adsorption exceeds phenol adsorption on AC AG-3 in the field of concentration more than 20 mmol/dm^3, on AS KsAU more than 5 mmol/dm^3, on AC ABG more than 3 mmol/dm^3, reflecting features of sorbents behaviour, caused in their various sizes of porosity and chemical surface state, and also adsorbate properties.

For substantiation of adsorption mechanism data of poremethry, potentiometric titration according to Byom and adsorptive curves in the field of low concentration were used. The results of research of a pore structure and titration are presented in Tables 1 and 2. Data of poremethry showed, that the greatest bulk volume of micro-and mesopores at AC KsAU in comparison with other coals (Table 1), assumes higher adsorptive capacity of the yielded sorbent in relation to the substances which adsorption has the physical nature and goes mainly in micropores (Fig. 1a, 1b).

The sorbent of mark ABG possesses the least bulk volume of micro-and mesopores, in comparison with others AC, that causes the least adsorptive capacity of the yielded sorbent in relation to contaminants (Fig. 1). Experimental data also show, that the important factor defining the adsorptive extraction of phenol, formaldehyde and acetic aldehyde is the chemical surface state meaning quantitative and qualitative contents of functional groups.

Potentiometric titration data according to Byom have allowed to explain higher attenuation range of phenol and formaldehyde, at AU KsAU in comparison with ABG and AG-3. A surface of sorbent KsAU (Table 2) contains acid (carboxylic and lacquer) KFG on surface area 3 times more, than ABG, and 2 times more than AG-3. It provides the additional adsorption of organic matters caused by specific interaction because of a hydrogen bridge with KFG of coals.

For the characteristic of carbon materials and calculation of the adsorptive parametres monolayer adsorption theories (Freindlikh and Lengmyur's equations), theory of volume filling of micropores (Dubinin-Radushkevich's equitation modified for a case of adsorption from water solution) and the generalized theory of polymolecular adsorption Brunauer, Emmet and Teller (BET) are used. Applicability of these equations for calculation of adsorption parametres was defined by pre-award experimental researches. The calculated values of adsorption parametres of phenol and formaldehyde from water solutions for all coals are resulted in Table 3. According to the received parametres theoretical adsorption isotherms of components are calculated.

Table 1. The main characteristics of pore structure of probed samples

Sample	S_{micro}, m^2g	*V_S, cm^3/g	V_{micro}, cm^3/g	**V_{meso},cm^3/g
SKD-515	404.0	0.561	0.359	0.202
AG-OV-1	369.0	0.459	0.218	0.241
BAC	586.0	0.455	0.352	0.103
AG-3	490.0	0.340	0.270	0.060
ABG	-	0.260	0.020	0.240
KsAU	1418.7	0.730	0.620	0.110

Note. * – general volume of pores with dimeter less than 150 nm; ** – volume of mesopores, got from the balance $V_S – V_{micro}$,

Table 2. Surface condition of active charcoals

AC	The maintenance of oxygen active in mmol/g of coal (n_{kfg}, mkmol/м^2)		
	-OH phenol	-$COOH_{strong}$ carboxylic	-COO- lacquer
SKD-515	0.181	-	0.157
AG-OV-1	0.213	0.032	0.078
AG-3	0.321	0.035	0.039
ABG	0.130	0.020	0.040
KsAU	0.194	0.090	0.060

Table 3. Parameters of adsorption of organic matters from water solutions, probed active charcoals in static conditions

Coal mark	Type of equitation							
	Freindlikh		Lengmyur	BET		Dubinin-Radushkevich		
	1/n	b, mmol/g	a_m, mmol/g	a_m, mmol/g	-Q, kJ/ mmol	a_{max}, mmol/g	E_0, kJ/mmol	W, dm^3/kg
formaldehyde								
AG-OV-I	1.34	0.04	13.5	25.0	13.4	21.0	15.9	0.56
SKD-515	0.80	3.80	11.7	20.0	12.5	19.1	13.6	0.51
AG-3	0.99	1.67	11.1	23.8	15.9	20.1	14.7	0.54
BAU	1.00	1.60	8.5	12.8	10.8	15.6	11.3	0.42
KsAU	1.23	2.93	20.8	33.3	17.3	33.1	10.1	0.334
ABG	1.07	0.89	3.92	17.54	16.7	9.2	10.4	0.09
phenol								
AG-3	0.43	10.53	0.75	0.894	13.45	2.14	15.9	0.25
KsAU	0.64	14.30	2.001	2.007	17.42	6.75	14.9	1.115
ABG	0.46	5.83	0.459	0.046	17.7	0.009	15.7	0.086
AG-OV-I	-	-	0.85	1.30	14.9	2.22	15.0	0.310
SKD-515	-	-	0.99	1.58	15.55	2.36	15.2	0.356

In connection with low initial concentration acetic aldehyde is practically completely adsorbed from water solutions on AC, therefore usage of monolayer adsorption theories, theory of volume filling of micropores and generalised theory of polymolecular adsorption BET for calculation of the basic adsorptive parametres of acetic aldehyde is not obviously possible.

Analyzing adsorptive characteristics, it is possible to draw a conclusion, that sizes of limiting adsorptive volume W for all carbon sorbents are in limits of 0.086–1.115 dm^3/kg (for phenol), 0.09–0.56 dm^3/kg (for formaldehyde) and allow to assume, that adsorption of phenol, formaldehyde from solutions of individual substances submits to the volume mechanism of micropores filling. The values of characteristic energy which are in limits 14.9–15.9 kJ/mole (for phenol), 10.1–15.9 kJ/mole (for formaldehyde), testify that adsorption occurs basically in micro-and mesopores of adsorbents, and for active charcoal ABG in mesopores. Formaldehyde and phenol AC adsorption isotherms assume physical nature of adsorption. The calculated sizes for heats of adsorbtion 13.42–17.70 кJ (for phenol), 10.8–17.6 кJ (for formaldehyde) confirm the conclusion about the physical nature of interaction of organic matters with a surface of the studied carbon sorbents.

The received experimental data and settlement parametres allow to draw a conclusion, that adsorption of phenol, formaldehyde, acetic aldehyde has physical

nature, and phenol mainly has dispersive (nonspecific) and partially specific interaction because of hydrogen connection, for formaldehyde is more typical specific interaction. The formaldehyde having the highest size of adsorption, besides nonspecific interaction in pores is capable to interact with functional acid groups of surface AC and even to serve for formaldehyde molecules as the secondary centres of adsorption.

The complex estimation of adsorptive characteristics of the probed carbon sorbents in static conditions allowed to arrange them by efficiency of extraction: phenol – KsAU> SKD-515> AG-OV-1> AG-3> ABG; formaldehyde – KsAU> AG-3> AG-OV-1> SKD> 515> BAU> ABG; acetic aldehyde – KsAU> AG-3> ABG.

As in natural water mixes of organic matters usually contain, the balance of adsorption in systems most often meeting in practice of water treatment with application of stage of ozonization is probed: formaldehyde – phenol – water – AC and formaldehyde-acetic aldehyde – water – AC [12].

According to the received experimental data of adsorption of formaldehyde and acetic aldehyde from water solution their mix various carbon sorbents are constructed by adsorption isotherms. Comparison of adsorption isotherms of acetic aldehyde and formaldehyde from water solution their mix with adsorption isotherms from water solutions of individual substances showed absence of components influence on adsorption of each other. At adsorption of formaldehyde and acetic aldehyde from a mix the form of isotherms does not vary, thus acetic aldehyde (Fig. 3) owing to its low initial concentration is adsorbed completely. On the basis of experimental data adsorption key parametres are received.

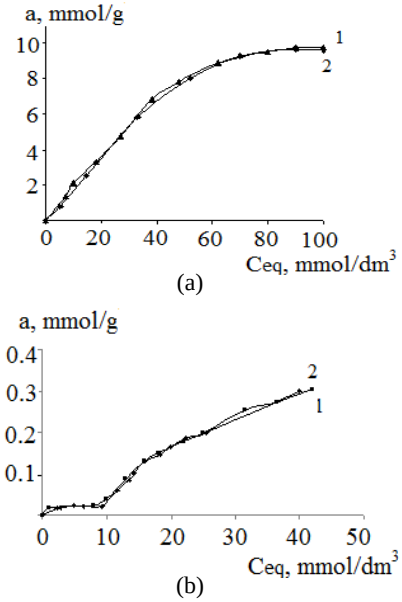

Fig. 3. Adsorption isotherms of formaldehyde (a) and acetic aldehyde (b) on AC AG-3: 1 – individual component; 2 – mix of components.

The obtained data allow to draw a conclusion, that adsorption of formaldehyde and weak adsorption of acetic aldehyde from a mix having physical nature, goes independently from each other, and for acetic aldehyde dispersive interaction in micropores prevails, for formaldehyde – both dispersive in micropores and specific on surface AC with hydrogen connection with oxygen-containing superficial groups of acid type; thus at formaldehyde adsorption some processes proceed: adsorption in accessible size of micropores and specific interaction with active centres (thus sizes of characteristic energies are averaged).

The changing of adsorptive activity of carbon sorbents during formaldehyde and acetic aldehyde extraction at joint presence decreases in KsAU> AG-3> ABG.

The adsorptive behaviour of mix phenol-formaldehyde-water is probed. Under the received experimental data of adsorption of formaldehyde and phenol from water solution their mixes adsorption isotherms are constructed by various carbon sorbents. Comparison of adsorption isotherms of phenol and formaldehyde from water solution of their mix with adsorption isotherms from water solutions of individual components showed, that in the field of low concentration (to 10 mmol/dm^3) there is no influence of components on adsorption of each other. In the field of high concentration at joint presence of organic matters phenol adsorption does not change, and formaldehyde is adsorbed a little bit poorer, than from its water solution of individual substance (Fig. 4). The form change of an isotherm (reduction of isotherm steepness) is thus observed that can testify about changes of interaction character of components in the solution and with AC surface. The form change of adsorption isotherm of formaldehyde from a mix on AC AG-3 with L on S_4, means growth of critical increment of energy and bigger force of interaction between adsorbed molecules than between a solute and an adsorbent, and also molecules aspiration of solute to settle down on a surface in the form of chains or clusters. The decrease in adsorption of formaldehyde from water solution of components mix in the field of high concentration (more than 10 mmol/dm^3) is caused by competitive adsorption on the adsorptive centres.

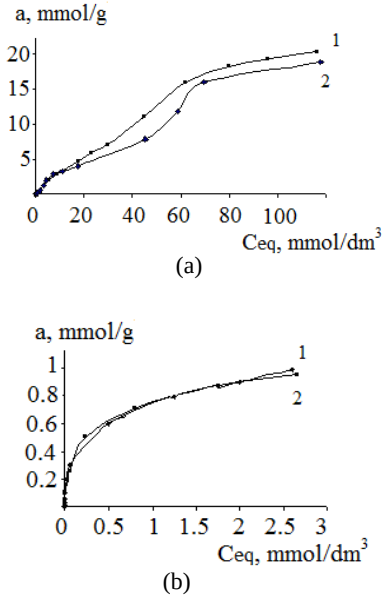

Fig. 4. Adsorption isotherms of formaldehyde (a) and phenol (b) on AC AG-3: 1 – individual component; 2 – components mix.

It is experimentally positioned, that Lengmyur's equation is not applicable for the adsorption equilibrium description in system water – phenol – formaldehyde – AC. The adsorption isotherms calculated on the equations of Dubinin-Radushkevich, Freindlikh and BET, testify a application possibility of the yielded equations for calculation of equilibrium parametres of adsorption in the system Au – water – phenol – formaldehyde.

The sizes of limiting adsorptive volume W for all carbon sorbents are in limits of 0.0862–1.0641 cm^3/g (for phenol), 0.33–1.231 cm^3/g (for formaldehyde) and allow to assume, that phenol and formaldehyde adsorption at joint presence submits to the volume mechanism of micropores filling. The values of characteristic energy which are in limits 14.932–15.902 kJ/mole (for phenol) and 13.576–14.668 kJ/mole (for formaldehyde), testify, that phenol and formaldehyde sorption at joint presence occurs basically in micro-and mesopores of adsorbents. The value of adsorbtion heats at a small filling the pores of active charcoals are close among themselves and are in limits 11.282–16.963 kJ/mole for phenol, assuming the adsorption caused as Van-der-Vaal'sovy forces (dispersive interaction), and because of formation of hydrogen bridges (specific interaction). The values of adsorbtion heats for formaldehyde 16.704–22.243 kJ/mole give the basis to consider, that at its adsorption specific interaction prevails because of formation of hydrogen bridges with superficial polar functional groups, that is absorption of phenol and formaldehyde by active charcoals is a total process of nonspecific adsorption (in the volume of accessible pores) and specific (on active centres).

For specification of adsorption mechanism of phenol and formaldehyde IR-spectroscopic researches are carried out. The IR-spectroscopy is used for identification of organic matters and their functional groups. The occurrence in a spectrum of characteristic frequency of a functional group is influenced by structure, power constant connections and a molecule environment. The fluctuations change of base units of a molecule because of interaction at formation of intermolecular communication (for example, hydrogen) or dispersive interaction at physical adsorption will also be reflected in a spectrum by change of frequencies and fluctuations intensities of corresponding functional groups that will allow to specify the mechanism of interaction an adsorbent - adsorbate [13]. IR-spectra of active charcoals are studied before and after adsorption of organic matters from water solution.

The analysis of the received results showed (Fig. 5), that for all spectra it is possible to evolve areas of valent and deformation fluctuations -OH of alcohol group, phenols and carboxylic acids (3700–3300 and 1300–1250 cm^{-1}, accordingly), free and connected hydrogen bridge; area of valent and deformation fluctuations-S-N of aliphatic group (2970–2850 and 1450–1300 cm^{-1}, accordingly); area of fluctuations> C=O groups in aldehydes, ketones and carboxylic acids (1780–1600 cm^{-1}); fluctuations from group of alcohol and aethers (1300–1000 cm^{-1}); the band corresponding to 2350 cm^{-1}, falls into to carbon dioxide absorption, this absorption band is visible on all infra-red spectra received on one-beam devices or two-beam device at not enough good indemnification of a working bunch and a bunch of comparison.

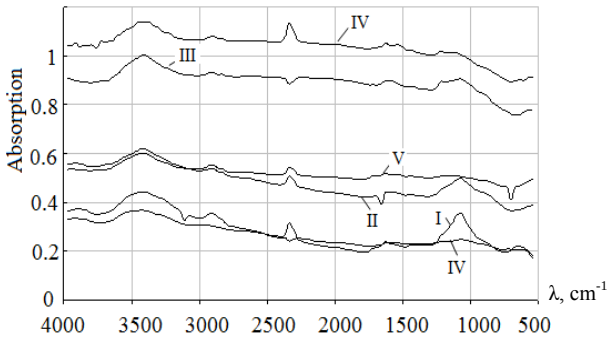

Fig. 5. IR-DOFP spectra of active charcoals AG-3 and KsAU and their samples after adsorption of organic matters: AG-3 – industrial sample (I), AG-3 after adsorption of phenol (II), AG-3 after adsorption of formaldehyde (III), AG-3 after adsorption of a mix of phenol and formaldehyde in the ratio 1 : 50 ($mmol/dm^3$) (IV), KsAU (V), KsAU after adsorption of a mix of phenol and formaldehyde (VI).

In Fig. 6 different IR – spectra are presented on which the studying of interaction of organic matters with superficial oxygen-containing groups AC is possible. Different spectra are received by subtraction of spectra AC after adsorption by organic matters from spectra AC [14].

On different spectra of samples II-IV and VI a set of bands of average intensity is seen, corresponding to fluctuation of -OH group with formation of an intermolecular hydrogen bridge (3600–3400 cm^{-1}). Hydroxyl group presence proves to be true by display of deformation fluctuations of -OH group and valent vibrations from -C-O- group in the field of 1300–1000 cm^{-1}.

At difference spectrum AG-3 after phenol adsorption there are sets of bands with fluctuations of group -C-O- (1300–1000 cm^{-1}), and also a benzene ring (1620 and 1450 cm^{-1}) of adsorbed phenol. The set of bands 1790–1640 cm^{-1}, probably, falls into to fluctuations of a carbonyl group of the carboxylic acids free and connected by a hydrogen bridge on an active charcoal surface.

In difference spectra AG-3 after formaldehyde adsorption there are bands of fluctuations of group -C-O- (1300–1000 cm^{-1}), also absorption bands for aliphatic aldehydes of 1230–1100 cm^{-1} caused by the valent and deformation fluctuations C-C-C in the group $\overset{O}{\overset{\|}{C}}\text{-}C\text{-}C$.

Different spectra AC AG-3 after adsorption of a mix of organic matters at joint presence differs presence of more intensive bands of groups -OH (3600–3400 cm^{-1}), -C-O- (1300–1000 cm^{-1}) and adsorbed phenol (3650–3520 and 1280–1010 cm^{-1}).

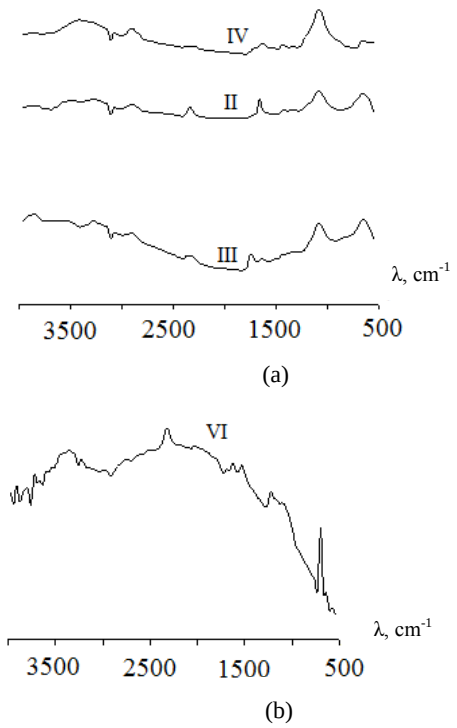

Fig. 6. Different spectra of active charcoals samples AG-3 (a), KsAU (b) after adsorption of organic matters.

Area expansion of fluctuations to 3600 cm^{-1}, probably, illustrates hydrogen connection between phenol and formaldehyde. In the field of 1790–1630 cm^{-1} fluctuations of a carbonyl group of the carboxylic acids free and connected by a hydrogen bridge, and also quinones are displayed.

In different spectra AC KsAU after adsorption of a mix of phenol and formaldehyde the set of bands 1870–1540 cm^{-1} is observed, connected with valence vibrations of group C=O. The field 1800–1630 cm^{-1} corresponds to fluctuations of a carbonyl group of carboxylic acids free and connected by a hydrogen bridge, and also quinones. The strengthening of absorption bands in area (3600–3400 cm^{-1}) in comparison with other samples is specified in occurrence of a hydrogen bridge -OH of groups of organic matters with oxygen-containing functional groups on AC surface. The absorption set of bands in area (3600–3400 cm^{-1}) can be correlated with fluctuations phenolic and carboxyl groups on the active charcoal surface, being hydrolysate in the course of interaction of solvent with a sorbent.

On the basis of experimental researches of the balance, the calculated adsorptive parametres and IR-spectroscopy data, it is possible to consider, that phenol and formaldehyde adsorption both from water solution of individual components, and from a mix has physical character and does not result in to strong linkage with an adsorbent. At joint adsorption of formaldehyde and phenol instead of adsorption of dimer structures on an active charcoal surface the increase aliphatic (-CH) and from -C-O- groups is observed. Hence, between phenol and formaldehyde there is an interaction both in water solution, and on an active charcoal surface where formaldehyde molecules are capable to represent themselves as the secondary adsorptive centres for phenol molecules. At joint adsorption between these connections, obviously, the effect of hydrogen bonding is displayed which is enough fragile that allows to assume possibility of effective regeneration of active charcoal after adsorption cleaning of water from a phenol and formaldehyde mix.

According to kinetic researches it is positioned, that a limiting stage of process of extraction of small concentration organic contaminants from water (phenol, formaldehyde, acetic aldehyde) is external mass carry. Factors of external mass carry, necessary for engineering calculations [15, 16] are calculated.

Experimental studying of adsorption dynamics assumes consecutive selection of parametres (sorbent type, length of fixed bed, rate of flow, etc.) and reception of experimental target curves depending on one varied variable (for example, rates of flow of a solution) at the fixed values of the others. Dynamics research of adsorption of mixes with phenol and formaldehyde, formaldehyde and acetic aldehyde from water on AC were made in columns in diameter of 1.7 cm with height of bed of loading 0.5 m, peripheral speed of a stream – 1m/h. Experimental researches of dynamics of adsorption showed, that at the continuous water purification containing mixes of organic contaminants, breakthrough of a dominating component is observed as the first from a column: for mixes phenol-formaldehyde and formaldehyde-acetic aldehyde – breakthrough of formaldehyde that allows to model adsorption process on a dominating component. Calculation is executed on the basis of the fundamental equation of external diffusion dynamics of adsorption in the field of low concentration with utilization of experimental data on balance and kinetics of sorption of organic matters on AC [17].

Coincidence of the experimental and theoretically calculated curves confirms legitimacy of the offered approach to modelling of adsorption and possibility of definition of dynamic characteristics of adsorption without additional carrying out of experimental researches (Fig. 7). According to the results of modelling of process of adsorption of the studied mixes dynamic characteristics are received: length of working bed, length of not used bed, protective effect factor, running time of a column and quantity of refined water depending on parametres of a column and a purification mode.

Experimental results confirm possibility of modelling of adsorption process. Engineering calculations for practical adsorptive installation can be made on a dominating component.

While working the efficiency of the adsorptive filters on extraction of pollutants drops, therefore their periodic regeneration is necessary for restoration of sorbents adsorbability. The possibility of utilization of the following methods of regeneration is experimentally studied: a steam, air stream, heated-up to 200°C and hot water.

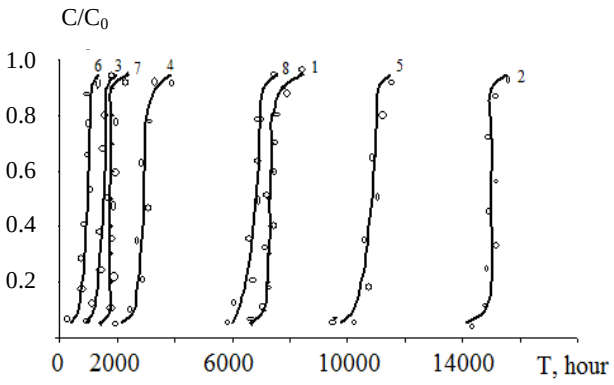

Fig. 7. Target curves of dynamics of adsorption of formaldehyde in the presence of phenol for dense bed AC AG-3 (on theoretically calculated curves points experimental data are put) at different rates of flow (ν) and height of bed of a sorbent (H): 1 – ν = 1m/h, H = 0.5 m; 2 – ν = 1 m/h, H = 1 m; 3 – ν = 5 m/h, H = 0,5 m; 4 – ν = 5 m/h, H = 1 m; 5 – ν = 5 m/h, H = 2 m; 6 – ν = 8 m/h, H = 0.5 m; 7 – ν = 8 m/h, H = 1 m; 8 – ν = 8 m/h, H = 2 m.

For restoration of adsorbability of sorbents the technology of regeneration AC after adsorption of probed mixes – application of washing AC by water warmed up to 50°C, with the subsequent warm-up by a stream of air with temperature 200°C within 2 hours is offered, allowing to reduce sorptive capacity of sorbents on 95–98%. Thermogravimetric researches have preliminarily been made for a choice of temperature of thermal regeneration. The estimation of restoration of adsorption properties of AC after adsorption of aqueous mixes in laboratory conditions showed, that after the fifth cycle regeneration-sorption adsorbability has dropped on 16–22%.

On the basis of theoretical and experimental researches of adsorption process the technology of afterpurification of drinking water from priority organic contaminants and their mixes on AC, providing drinking water upgrading is developed, the carbon sorbent and hardware decor of process is chosen, the way of regeneration of the fulfilled sorbents, allowing to carry out their repeated utilization without decrease in adsorption properties is offered. The production tests on "Talinka", LLC have shown efficiency of the offered technology.

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Please cite this article in press as: Timoshchuk I.V. Technology of afterpurification of drinking water from organic contaminants in production of foodstuff. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 61–69. doi: 10.21179/2308-4057-2016-1-61-69.

BIOTECHNOLOGY

THE STUDY OF RHEOLOGICAL BEHAVIOR AND SAFETY METRICS OF NATURAL BIOPOLYMERS

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Received April 10, 2016; Accepted in revised form June 01, 2016; Published June 27, 2016

Abstract: Traditionally, plastics are made from artificial synthetic polymers. These polymers have an unnatural structure, that's why they are not biodegradable. Based on the latest advances in polymers structure studies, the article sets forward new biodegradable materials highly competitive with base plastic. Biodegradable films were received by fill-and-drain method from agar-agar, carrageenan and hydroxypropyl methylcellulose with glycerol, used as a plasticizer. Various compositions of biodegradable films based on natural polysaccharides have been analyzed for their rheological behavior and stress-strain properties, as well as for their safety and ecotoxicity index. It is found that all compositions of received polymer films are biodegradable and relatively bio-safe (III-IV class of danger). The strength characteristics tests revealed that the compositions with carrageenan have higher strength (2.84 MPa) than polymers containing only agar-agar (1.64 MPa). Also biopolymers with the content of carrageenan have the elevated chemical resistance (prolonged time of dissolution in hydrochloric acid). The melting point of the samples narrowly varies from 35.3 to 35.9°C. The study of the received polymers showed no cracks and no serious heterogeneities of composition. According to the testing results the compounds have been selected, which have optimum characteristics for use of biodegradable polymers in various industries. The biopolymers obtained in the future will replace artificial polymers that can solve problems of non-biodegradable polymer systems waste.

Keywords: biodegradable polymers, rheology, diffusing, deformation, safety, ecotoxicity, ecology, bioconversion

DOI 10.21179/2308-4057-2016-1-70-78 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 70–78.

INTRODUCTION

At the present there have come a new approach to polymeric materials making, opposite to the traditional one [1, 2]. The purpose of this approach is the production of polymeric materials which retain producing characteristics only during the consumption period, and then go through physicochemical, chemical and biological transformations under the influence of environmental factors and easily join in processes of a metabolism of natural biosystems [3].

Biodegradable polymers is a class of the high-molecular compounds containing products of biological organisms (cellulose, protein, starch, nucleic acid, natural resin, etc.), and they are capable to decay on neutral for environment substances under corresponding conditions. In biologically active medium biodegradable polymers undergo significant changes in molecular weight and mechanical characteristics or give the nutrients providing growth of microorganisms. In such mediums there are processes of hydrolysis and photochemical destruction of biopolymers. Finally biopolymers decay on the components participating in a natural cycle – water, carbon dioxide, a biomass, etc. The basic advantage of biopolymers is their ability to biodegradation during rather short time, unlike the traditional analogues received from petrochemical raw materials [4, 5].

The market of biodegradable polymers is one of most emerging economic segments. Their production is already an integral part of national agrochemical complexes of Japan, the USA, European Union countries [6]. Biodegradable polymers possess more ample opportunities of utilization in comparison with traditional polymers, because they solve a problem of dependence on petrochemical raw materialsб as well as recycling problems. In Russia production of biodegradable polymers is in an incipient state, the overwhelming number of fabricators use basically the foreign workings out [7, 8].

In connection with the aforesaid the working out of Russian analogues of biodegradable films in packaging, food and pharmaceutical industry is considered to be actual.

Polymeric materials' ability to biological degradation is caused, mainly, by their chemical composition, structure and qualities of macromolecules [9, 10]. The great influence on polymers' stability to biodegradation is rendered by some macrostructural characteristics (pore volume, additives equality in polymeric mass, features of surfacing of products, etc.), and also by technological parameters [11, 12, 13].

The purpose of the this research was to investigate films samples made on the basis of natural polysaccharides, and also to choose of tailored composition of films, and conditions of polymers productions, by biodegradability and stress-strain properties.

OBJECTS AND METHODS OF STUDY

The objects of investigations were biodegradable polymers, produced from vegetable polysaccharides agar-agar, carrageenan and hydroxypropyl methylcellulose with glycerol by fill-and-drain method. 8% of glycerin, as a plasticizer, was added in each sample. Without addition of glycerin the films were rigid and curled at drying. The film-former solution was transferred to a dish with (1–2) mm high bead. The dish was covered with a glass cap. It was cooled at normal temperature. The film drying was carried out in various ways:

– at normal temperature;
– at air convection in a draught cupboard;
– in a vacuum drying chamber (with varied drying temperature);
– in a drying chamber (with varied drying temperature).

The best way of drying was chosen for each type of the sample, allowing to provide uniformity of drying and to exclude deformation of the sample (overdrying, formation of cracks).

Evaluation of thickness and density

Thickness of films was measured with an electronic micrometer "MK gladkii N 26591" by direct measurement method. At least 10 parallel meterages were carried out on 5 various sites of a film, and the midrange value was counted.

Water absorption measurement

Water absorption measurement was held in accordance with GOST 4650-80. Samples in the form of a square with the side of 50 mm were taken for tests. Before test samples were exsiccated at 50°C in a drying chamber within 24 h, and then were cooled in a desiccator over phosphorus pentoxide at 23°C. After cooling samples were weighed. Further samples were dipped in distilled water and maintained at temperature 50°C within 24 h. After that, samples were taken out from water, wiped with filter paper and were being weighed during 1 minute.

Chemical durability evaluation

For evaluation of chemical durability squares in the size of 10/10 mm were cut out for each sample. The received squares were dipped in the reactive mediums preliminarily prepared in glassware, and by means of a stop watch the time of sample degradation was defined.

Research of strength characteristics of the films

The load at tearing up was defined with desktop electromechanical testing machine Instron 3343 in accordance with GOST 14236-81. The parameters of measurements: a gross capacity is 50 kN, speed of movement of cross-arm is 100 mm/mines, the sizes of the sample, a clamping length of 150 mm. The size of samples was 170/10/0.2 mm/mm/mm with equal edges without defects. The test was made at temperature 23°C and relative humidity of 50%.

Evaluation of melting point of films

Evaluation of melting point of films was made by method of differential scanning calorimetry on DSC 204 F1 Phonix device at heat rate of 10 °C/minutes in air atmosphere by method STO TGU 074-2010 "Thermal analysis of film composite polymeric materials on the basis of polyolefins".

Estimation of biodegradability

Biodegradability degree was defined by method ISO 846:1997. Agar-agar was prepared without nutrients and was poured to Petri dish. Samples were sterilized before test by submergence in 70% ethanol during 1 minute, and then exsiccated within 72 h at a constant air адщц. Then samples were put in Petri dishes, and the suspension of viable fungus spores was put on a surface in number of 100 mkl. The suspension of fungus microorganisms was represented by *Aspergillus niger, Penicillium funiculosum, Paecilomyces variotii, Aspergillus terreus, Aureobasidium pullulans, Penicillium ochrochloron* in number of 10^6 spores in ml for each kind. The selected microorganisms are specific for growth on biodegradable plastics and inhabit in natural biocenosis. The samples were incubated in a thermostat within 28 days at temperature 29°C and relative humidity of ≥90% and were weighed on reference points.

Evaluations of ecotoxicity

For estimation of influence of films samples ecotoxicity we used the influence on phyto-and zooplankton. The first variant used an algological pure diurnal growth of seaweed *Chlorella vulgaris Beijer*, with optical density 0.625 according to FR 1.39.2011.10993 "Technique of evaluation of an index of ecological toxicity of concentrated aqueous disperse systems of nanoparticles on growth inhibition of test culture seaweed chlorella". 2 ml of the diurnal growth of seaweed was placed in each of 6 prepared glassfuls containing 48 ml of blank and test samples. The test samples were spilt for 5 ml in sample vial, in triplicate. Escalating of growth of seaweed chlorella was defined after 22 hours of cultivation. Toxicity estimation was carried out by measurement of optical density of test sample and by comparison of a diurnal gain of seaweed cells in blank and test samples (if the optical density in test vials were not lower than 0.120).

In the second variant the lower crustaceans were used, by technique FR 1.39.2010.09102 "Technique of evaluation of an index of toxicity nanopowders, products from nanomaterials, nanocoverings, waste and sludge containing nanoparticles, according to the mortality the test-organism *Daphniamagna Straus*" with modification STO TGU 137-2015 "Nanomaterials and superfine materials, waste and sludge containing nanoparticles. The evaluation index of toxicity on mortality the test-organism *Daphniamagna Straus*". The experiment took 72 h. The criterion of sharp toxicity was the death at least of 50%

daphnids in test sample for 48 hours, while in a blank sample all the bions keep the germinating power. The daphnias mortality account in blank and test samples was made by each 24 hours. The experiment was stopped, if the death of more than 50% bions was observed in all test tubes within 24 hours. Motionless bions considered to be dead if they did not start to move within 15 seconds after light rocking of a test tube. Besides, the behavior of daphnias (activity and character of movement), filling of bowels, the dumped ephippiums number were considered.

Electronic microscopy

The system with electronic and focused bunches Quanta 200 3D was used for electronic microscopy. The samples were analyzed according to TGU 041-2009 "Technique of carrying out researches of surface structure of a solid body by method of raster electronic microscopy". Researches were made on the samples extended before deformations, corresponding to a proportional limit, to a lower limit of fluidity and to ultimate strength.

Research light transmittance

The research is made on equipment CARY 100 SCAN. Light transmittance of polysaccharide films is measured in a range 180–780 nm. The samples have been preliminarily nonfattened with 70% ethanol and exsiccated.

RESULTS AND DISCUSSION

The work was made to two stages. At the first stage samples of films are made from natural polysaccharides of different composition (Table 1). Agar-agar, carrageenan and hydroxypropyl methyl-cellulose were used as the substances possessing a high potential to formation of hydrogels with stable chemical and a physical structure.

Table 1. Composition of probed films

No. п/п	Quantity of ingredient. mass, %			
	agar-agar	carrageenan	hydroxypropyl methylcellulose (HPMC)	water
1	5.0	-	-	95.0
2	20.0	-	-	80.0
3	-	2.5	-	97.5
4	-	5.0	-	95.0
5	-	-	2.5	97.5
6	-	-	10.0	90.0
7	5.0	2.5	-	92.5
8	20.0	2.5	-	77.5
9	5.0	5.0	-	90.0
10	5.0	10.0	-	85.0
11	5.0	-	2.5	92.5
12	20.0	-	2.5	77.5
13	5.0	-	5.0	90.0
14	5.0	-	10.0	85.0
15	-	2.5	2.5	95.0
16	-	10.0	2.5	87.5
17	-	2.5	5.0	92.5
18	-	2.5	10.0	87.5
19	5.0	2.5	2.5	90.0
20	10.0	5.0	5.0	80.0

The sample No. 1: small enough concentration of agar-agar in suspension allows to pulpify it on a magnetic stirrer with function of maintenance of temperature.

Stirrer temperature – 150°C.

Speed of rotation – 800 rpm.

After the solution became transparent, it was poured out on a substrate with 2 mm bead height.

Drying was made in a drying chamber at temperature 85°C.

The sample No. 2: Because of the big concentration of agar-agar the dry-mix which cannot be pulpified on a magnetic stirrer is formed. This sample was pulpified in an autoclave on a blowdown mode within 15 minutes.

The received solution poured out on a substrate with 1 mm bead height.

Drying was made in a drying chamber at temperature 90°C.

The sample No. 3: too small concentration of carrageenan. It was not possible to receive a film.

The sample No. 4: too small concentration of carrageenan. It was not possible to receive a film.

The sample No. 5, the sample No. 6: It was not possible for HPMC to dissolve completely. It was not possible to receive a film.

The sample No. 7: the mixture at dissolution in water forms moderately dense suspension that allows to weld it on heating mantle.

Agitator temperature – 150°C.

Speed of rotation – 800 rpm.

The received solution was poured out on a substrate with 2 mm bead height.

Drying was made in a drying chamber at temperature 85°C.

The sample No. 8: the big concentration of agar-agar with carrageenan. The sample was pulpified in an autoclave on a blowdown mode within 15 minutes.

The received solution poured out on a substrate with 1 mm bead height.

Drying was made in a drying chamber at temperature 90°C.

The sample No. 9: the yielded mixture was pulpified on heating mantle.

Agitator temperature – 150°C.

Speed of rotation – 800 rpm.

The received solution was poured out on a substrate with height of shoulders by 2 mm.

Drying was made in a drying chamber at temperature 85°C.

Sample No. 10 was pulpified in an autoclave on a blowdown mode within 15 minutes.

The received solution was poured out on a substrate with height of shoulders 1 mm.

Drying was made in a drying chamber at temperature 90°C.

The sample No. 11, Sample No. 12, Sample No. 13, Sample No. 14, Sample No. 15, Sample No. 16, Sample No. 17, Sample No. 18, Sample No. 19, Sample No. 20: it was not possible to receive a quality film, there are undissolved particles of HPMC.

According to appearance and structure from the received samples the greatest interest for the further research was introduced by samples:

– The film sample No. 1 consisting of 5.0 mass. Agar-agar %; 95.0 mass. water %;

– The film sample No. 2 consisting of 20.0 mass. Agar-agar %; 80.0 mass. water %;
– The film sample No. 7 consisting of 5.0 mass. Agar-agar %; 2.5 mass. % carrageenan; 92.5 mass. water %;
– The film sample No. 8 which composition includes 20.0 mass. % of agar-agar and 2.5 mass. % carrageenan, 77.5 mass. water %;
– The film sample No. 9 which composition includes 5.0 mass. % of agar-agar and 5.0 mass. % carrageenan; 90.0 mass. water %;
– The film sample No. 10 which composition includes 5.0 mass. % of agar-agar and 10.0 mass. % carrageenan; 85.0 mass. water %.

At the second stage various compositions of biodegradable films made of natural polysaccharides were probed on rheological behavior, stress-strain properties, and also on safety and ecotoxicity.

The received results on biodegradability are shown in Table 2.

From Table 2 it follows, that the samples of the film No.1 had the maximum degree of biodegradability (78.0% for 3 days, 86.0% for 28 days) consisting of 5.0 mass. agar-agar %; and the sample of the film No. 8 which composition includes 20.0 mass. % of agar-agar and 2.5 mass. % carrageenan had the minimum degree (54.0% for 3 days, 68.0% for 28 days). All probed samples correspond to the research problems on creation of biodegradable films, possessing high speed of biodegradation. According to the results of experiment the speed of biodegradability is in inversely proportional dependences on concentration of raw materials. Agar-agar possesses higher firmness to action of micro-organisms, than carrageenan, that is explained by the feature of a molecular structure of carrageenan which under the influence of microbial enzymes breaks up to low molecular weight complexes easier and assimilates living organisms.

Data of thickness and density of biodegradable films are shown in Table 3.

From Table 3 it follows, that the maximum thickness (1.518 mm) is typical for a film consisting of 20.0 mass. % of agar-agar and 2.5 mass. % carrageenan, minimum (0.593 mm) – for a film consisting of 5.0 mass. agar-agar %. The maximum density (1.4284 g/cm^3) characterizes a film consisting of 5.0 mass. % of agar-agar and 5.0 mass. % carrageenan, the minimum density (1.2344 g/cm^3) – a film consisting of 5.0 mass. % of agar-agar and 10.0 mass. % carrageenan. As a result of researches interrelation between thickness and density has not been registered, and also between these characteristics and composition of films. However the regularity was found, according to which is revealed at the maintenance of ingredients more than 10% mass. it is impossible to receive a sample with a thickness less than 1 mm in the bulk way (a demanded thickness 0.6 ± 0.1).

The results of evaluation for strengthening characteristics of biodegradable films on the basis of natural polysaccharides are introduced in Table 4. The extension strength is measured in MPa, it is the force enclosed to the area, i.e. kg/cm^2. The higher this value is, the more stable against efforts to a stretching the material is.

From Table 4 it is seen, that the maximum magnitude of tensile stress (2.84 MPa) is typical for a film which composition includes 20.0 mass. % of agar-agar and 2.5 mass. % carrageenan, minimum (1.64 MPa) – for a film consisting of 5.0 mass. % of agar-agar and 2.5 mass. % carrageenan. There is an implicit correlation between thickness of films and tensile stress, besides carrageenan attaches thickness to a finished product, and so also the tensile stress.

Table 2. Degree of films biodegradability on the basis of natural polysaccharides

Sample number	Average proportion of a weight loss of degraded samples, %				
	3 days	7 days	14 days	21 days	28 days
1	78.0 ± 0.3	79.0 ± 0.2	80.0 ± 0.3	82.0 ± 0.7	86.0 ± 0.1
2	63.0 ± 0.4	64.0 ± 0.3	66.0 ± 0.3	70.0 ± 0.6	74.0 ± 0.5
7	71.0 ± 0.5	72.0 ± 0.9	74.0 ± 0.9	79.0 ± 0.3	82.0 ± 0.3
8	54.0 ± 0.4	56.0 ± 0.8	58.0 ± 0.4	62.0 ± 0.5	68.0 ± 0.8
9	64.0 ± 0.5	65.0 ± 0.7	66.0 ± 0.4	69.0 ± 0.5	73.0 ± 0.5
10	52.0 ± 0.3	57.0 ± 0.7	58.0 ± 0.3	62.0 ± 0.5	69.0 ± 0.3

Table 3. Results of evaluation of thickness and density of biodegradable polymers on the basis of natural polysaccharides

Sample number	Thickness, mm	Density, g/cm^3
1	0.593 ± 0.030	1.2879 ± 0.0644
2	1.270 ± 0.064	1.3455 ± 0.0673
7	0.711 ± 0.036	1.3857 ± 0.0693
8	1.518 ± 0.076	1.3086 ± 0.0654
9	0.849 ± 0.042	1.4284 ± 0.0714
10	1.220 ± 0.061	1.2344 ± 0.0617

Table 4. Results of evaluation for strengthening characteristics of biodegradable films on the basis of natural polysaccharides

Sample number	Tensile stress at a gross capacity, MPa
1	2.09 ± 0.09
2	2.19 ± 0.45
7	1.64 ± 0.24
8	2.84 ± 0.26
9	2.72 ± 0.12
10	2.66 ± 0.08

As the developed biodegradable films are planned to use further for creation of packing materials, it is interesting to find out the measurement of water absorption of considered films. The influence of moisture on polymeric materials there can make essential changes. Moisture diffusion in polymer is accompanied by reduction in it of intermolecular interaction which can appear useful from the point of view of strengthening properties, but the further increase of specific humidity renders deleterious effect. Thus, it is necessary to estimate possibility of influence of external liquid medium on plastic.

The results of experiments on measurement of water absorption of films on the basis of natural polysaccharides are shown in Table 5.

Table 5. Results of water absorption evaluation of biodegradable polymers on the basis of natural polysaccharides

Sample number	Weight fraction of absorption of water, %	Water mass, absorbed by the sample, mg
1	3.0 ± 0.6	0.0071 ± 0.0015
2	19.0 ± 0.8	0.0913 ± 0.0036
7	81.0 ± 0.7	0.2039 ± 0.0084
8	72.0 ± 0.3	0.3844 ± 0.0052
9	165.0 ± 0.3	0.4949 ± 0.0090
10	168.0 ± 0.9	0.5369 ± 0.0120

The analysis of data of Table 5 testifies that samples of films No.9 and No.10 (165.0% and 168.0%) are characterized by the maximum water absorption, consisting, accordingly, from 5.0 mass. agar-agar %, 5.0 mass. % carrageenan and 5.0 mass. agar-agar %, 10.0 mass. % carrageenan. The minimum water absorption (3.0%) is observed for the sample of a film No.1 (5.0 mass. agar-agar %).

Along with susceptibility to biodegradability the polymers should possess chemical durability. This concept also falls into to one of the main protective criteria of the films characterizing ability to stand up to the influence of chemical agents of environment among which mineral and organic acids evolve, and also their solutions in water, solutions of alkalis; solutions of salts both other chemicals and their connections possessing strong redox potential. Parametres of chemical durability of polymer allow to receive representation about possibility of its utilization in various industries.

Chemical durability of biodegradable films was probed in relation to acids (sulfuric, saline) and to alkalis (sodium hydroxide). The received results are shown in Table 6.

From Table 6 it follows, that the sample No. 1 dissolves in HCl_{conc} during 3 mines, the sample No. 2 – during 17 mines, samples No. 7–10 – within 55 minutes.

Table 6. Research results of chemical durability of biodegradable polymers on the basis of natural polysaccharides

Sample \ Solvent	Duration of dissolution of the sample				
	H_2SO_4, concentration	HCl, concentration	HCl, 0.1 M	NaOH, 2 M	NaOH, 0.1 M
1	22 hours	3 min	Fims didn't break down	Films swelled, but didn't break down	Fims didn't break down
2		17 min			
7		55 min			
8					
9					
10					

The best dissolution of films samples occurs in a solution of concentrated muriatic acid that is explained by extreme aggressiveness of medium in which vegetable polysaccharides are exposed to hydrolysis.

The firmness of polymers to = various chemical reagents and solvents changes over a wide range not only from polymer to polymer, but in some cases and within various grade numbers of the same polymer. Generalizations concerning chemical durability of this or that polymer should be held with big care because of exceptions. Nevertheless, certain structural and chemical properties of polymer can be used for the approached estimation of firmness to various chemical reagents.

Films with carrageenan are marked by better firmness in concentrated muriatic acid, than the films including only agar-agar. This results from the fact that in sour mediums agar-agar is less stable, than the majority of polysaccharides, becouse 3,6-angidro-α-L-glycosidic bonds containing in agar-agar are decomposed by acids low fidelity in 100 times lighter than β-glycosidic bonds with carrageenan.

It is important to have the optimum indexes of melting temperature providing stability of a material to environmental conditions and thus not causing big expenses by production. Melting is a process of polymer transferring from the ordered state to the liquid one. Melting process has diffuse and relaxation character; its passing and result are defined by a parity of melting and heating speeds.

In Table 7 the evaluation results of melting point of biodegradable films on the basis of natural polysaccharides are resulted.

Table 7. Evaluation results of melting point of biodegradable films on the basis of natural polysaccharides

Sample number	Fusion temperature, °C
1	35.7 ± 0.2
2	35.5 ± 0.2
7	35.9 ± 0.4
8	35.3 ± 0.5
9	35.7 ± 0.5
10	35.4 ± 0.5

The data from Table 7 proves that melting temperature of all probed samples varies in a narrow range from 35.3 to 35.9°C.

A special value of biodegradable plastics is in ecological compatibility of production and utilization, in comparison with traditional polymers. Safety parameters are the important index for all types of biodegradable polymers. One of such indexes is ecotoxicity which shows hazard of studied substance to corresponding biocenosis. The ecotoxicity parameters of biodegradable films samples are shown in Table 8.

From Table 8 it follows, that the influence on environment (toxicity for natural biocenosis), according to a principle of an estimation of toxicity on the most expressed reaction, is the following: the sample No. 2 falls into to V class of hazard, samples No. 7 and No. 10 – to IV class of hazard, samples No. 1, No. 8 and No. 9 – to III class of hazard.

The micromorphology of films on the basis of natural polysaccharides was probed by method of scanning electronic microscopy as working with materials is important to define size and form of their particles, degrees of uniformity of particles distribution in a solution, interrelation of the form and particle size with other characteristics of a material. The received results are introduced in Fig. 1.

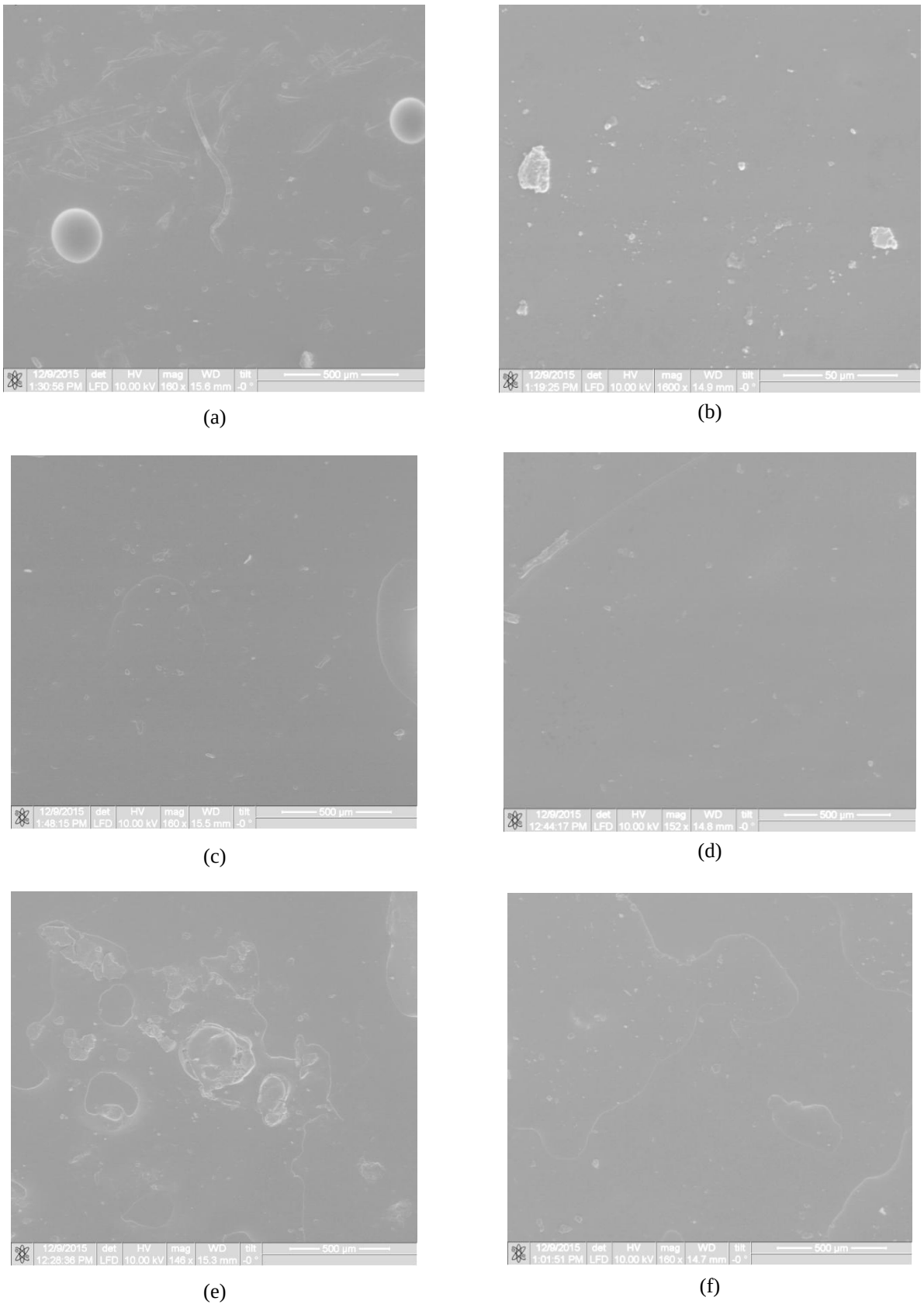
The research of films has not revealed cracks and serious inhomogeneities of composition, however samples No. 7, No. 8, No. 10 have more uniform structure in comparison with the others.

One of the major protective attributes of packing is property to detain a luminous flux, as ultraviolet and visible light are the reason of photodegradation and other changes in a product.

The received results on light transmittance of films are shown in Fig. 2.

Table 8. Ecotoxical indexes of biodegradable films samples on the basis of natural polysaccharides

Sample number	Test-organism	Test-reaction	Frequency rate of deluting to safe level	Class of hazard	General class of hazard
1	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	60.00	III	III
	Daphnia magna	Mortality	2.21	IV	
2	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	1.00	V	V
	Daphnia magna	Mortality	0.88	V	
7	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	5.55	IV	IV
	Daphnia magna	Mortality	3.13	IV	
8	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	535.70	III	III
	Daphnia magna	Mortality	2.69	IV	
9	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	352.20	III	III
	Daphnia magna	Mortality	5.20	IV	
10	*Chlorella vulgaris Beijer*	Inhibition of test culture growth	25.40	IV	IV
	Daphnia magna	Mortality	28.90	IV	



(a) (b) (c) (d) (e) (f)

Fig. 1. Photomicrography of films on the basis of natural polysaccharides: (a) sample No. 1; (b) sample No. 2; (c) sample No. 7; (d) sample No. 8; (e) sample No. 9; (f) sample No. 10.

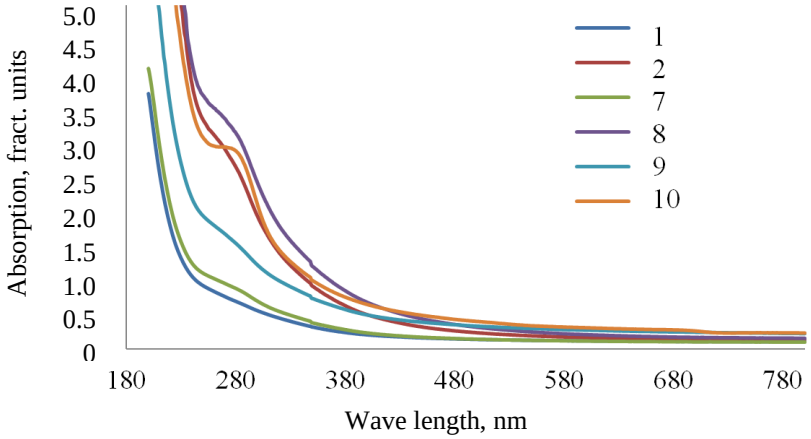


Fig. 2. Absorption spectra in UV, VID-areas for films samples.

It follows from Fig. 2, that all samples displayed similar properties in the analysis of light transmittance. Maximum light absorption was registered at wave length of 180 nanometers. In the process of wave length increase light holding ability of films falls and reaches less than 0.5 fract. units at 480 nanometers.

On a result of experiment the sample 8 possessed the best light barrier properties, and the sample 1 had maximum light transmittance. Also the regularity was revealed that the less thickness of films and concentration of substances in the sample corresponds to bigger light transmittance.

Thus, as a result of the made researches the following results are received:

(1) Six samples of the received films on the basis of the natural polysaccharides are chosen for the further researches, for their elasticity and form constancy. In the samples 5–20 it was not possible to receive qualitative film, as at solutions there were undissolved particles of HPMC and concentration of carrageenan was too low for a stable form of a total film in the samples 3–4.

(2) It is positioned, that all considered samples of films on the basis of natural polysaccharides are biodegradable. According to the results of experiments speed of biodegradability is inversely proportional dependences on concentration of raw materials. Agar-agar possesses higher firmness to action of microorganisms, than carrageenan.

(3) The thickness and density of biodegradable polymers on the basis of natural polysaccharides is defined. The interrelation between thickness and density has not been registered, and also between these characteristics and composition of films on the basis of polysaccharides. However the regularity is revealed according to which at the maintenance of ingredients more 10% mass. It is not possible to receive a sample in the thickness less than 1 mm in the bulk way.

(4) As a result of evaluation of strengthening characteristics of biodegradable films on the basis of natural polysaccharides it is positioned, that maximum magnitude of tensile stress (2.84 MPa) is characteristic for a film which composition includes 20.0 mass. % of agar-agar and 2.5 mass. % carrageenan, minimum (1.64 MPa) – for a film consisting of 5.0 mass. % of agar-agar and 2.5 mass. % carrageenan. There is an implicit correlation between thickness of films and tensile stress, besides carrageenan attaches to a finished product more thickness, and so also the tensile stress.

(5) Analysis of water absorption of biodegradable polymers on the basis of natural polysaccharides showed, that films samples No. 9 and No. 10 are characterized by maximum water absorption, and the sample of a film No. 1 – by the minimum one.

(6) All received samples of biodegradable polymers are stable against action of the diluted with sulfuric, saline acids and sodium hydroxide. In concentrated sulfuric acid all analyzed samples have dissolved within 22 hours. The probed films display the greatest sensitivity in relation to concentrated muriatic acid. Films with addition of carrageenan differ bigger firmness in concentrated muriatic acid, than the films including only agar-agar.

(7) Evaluation results of melting temperature of biodegradable films on the basis of natural polysaccharides testify that melting temperature of all probed samples varies in a narrow range from 35.3 to 35.9°C.

(8) Analysis of ecotoxicity samples of biodegradable films has shown, that the sample No.2 falls into to V class of hazard (the ecological system practically is not broken), samples No. 7 and No. 10 – to IV class of hazard (low-hazard substances), samples No. 1, No. 8 and No. 9 – to III class of hazard (moderately hazardous).

(9) Research of films has not revealed cracks and serious inhomogeneities of composition, however samples No. 7, No. 8, No. 10 have more uniform structure in comparison with the others.

(10) All samples displayed similar properties in the analysis of light transmittance. Maximum light absorption was registered at wave length of 180 nanometers. In the process of wave length increasing the light holding ability of films falls and reaches less than 0.5 fract. unit at 480 nanometers. On a result of experiment the sample No. 8 possessed the best light barrier properties, and the sample No.1had maximum light transmittance. Also regularity has been revealed: the smaller thickness of films and concentration of substances in the sample corresponds to the bigger light transmittance.

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Please cite this article in press as: Asyakina L.K., Dolganyuk V.F., Belova D.D., Peral M.M., and Dyshlyuk L.S. The study of rheological behavior and safety metrics of natural biopolymers. *Foods and Raw Materials*, 2015, vol. 4, no. 1, pp. 70–78. doi: 10.21179/2308-4057-2016-1-70-78.

AN EPISTEMOLOGICAL BACKGROUND ON PARADIGM FORMATION OF LIPIDOMICS OF DAIRY INDUSTRY

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Received May 19, 2015; Accepted in revised form November 20, 2015; Published June 27, 2016

Abstract: The article considers post-genomic view on formation of scientific ideas about one of the major components of raw milk and products produced from it (for example, cheese). It is a lipid complex (milk fat) of the brand Lipidomics. The cluster structure of milk fat, its components and derivatives are described. The author shows the dynamics of milk fat transformation in the process of cheese production in logistic link to lipolytic activity of bacterial starters and enzyme preparations. The characteristic of lipid complex of milk whey is given. Information allows us to formulate Lipidomics positions of dairy products. The aim of the article is to attract researchers' attention to the object, and practitioners to a rational and careful use of milk fat in food products.

Keywords: raw milk, dairy products, cheese, lipids, milk fat, Lipidomics

DOI 10.21179/2308-4057-2016-1-79-89 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 79–89.

INTRODUCTION

Formulation of the problem. According to the principles of Lactoomics [1] (science about milk) and logistics of dairy industry [2], at the present level of knowledge [3, 4] Lipidomics of milk and dairy products is reduced to milk fat – monitoring of composition and properties, cooking and use. The perfect model is the production of butter [5] and spreads [6]. This subject is fully and systematically covered by works of Franz Adamovich Vyshemirsky, Doctor of Technical Sciences, Professor, Laureate of the State Prize of the Russian Federation, by his students and followers. There are more than 1.000 publications on this topic which give all the necessary information about the subject – milk fat, butter-making, dairies and a wonderful indispensable product – butter [7] – which is the indicator of dignity and well-being of human civilization.

Based on post-genomic biotechnology view on concepts of objects and phenomena knowledge, taking into account the experience of Glikoomics formation [8], it is useful to formulate epistemologically and as a paradigm our vision of one of the major components of milk – the fat phase – Lipidomics. The starting material and the base model of the article are numerous researches of Professor M.S. Umansky [9]. This article is dedicated to his blessed memory.

OBJECTS AND METHODS OF STUDY

The object under review is the unified scheme of milk lipids classification by M.S. Umansky, which is shown in Fig. 1.

The basic problem-target structure of research of raw milk lipids by M.S. Umansky with the interpretation of the whole range of milk and milk products, at the example of cheese-making is shown in Fig. 2.

Taking into account the lack or absence of identification procedures for simulation of milk lipids and its products, as well as bacterial cultures and enzymes, M.S. Umansky developed and modified 17 original methods of studying the objects of knowledge. A list of some of the information is provided:

– preparative TLC analysis of N-lipid;
– micro TLC analysis of N-lipid;
– preparative TLC analysis of P-lipid;
– Micro-TLC analysis of P-lipid;
– GLC analysis of fatty acid composition;
– GLC analysis of free fatty acids;
– GLC analysis of volatile fatty acids;
– esterase activity of mesophilic and thermophilic lactic acid bacteria;
– esterase activity of propionic acid bacteria.

Fig. 3 shows the logistic scheme of the system of complex analysis of milk lipids and milk products according to M.S. Umansky.

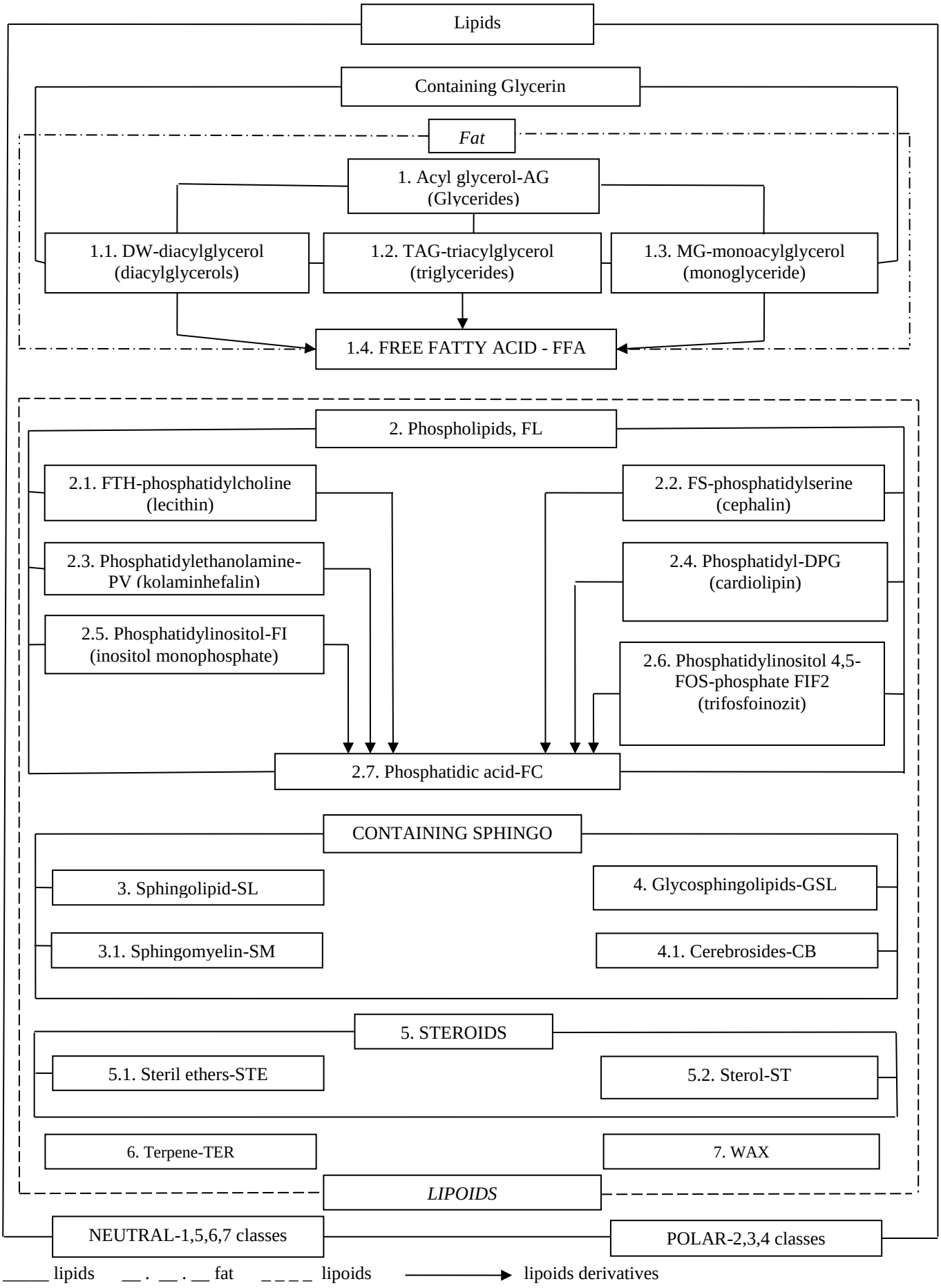


Fig. 1. A unified scheme of classification of milk lipids.

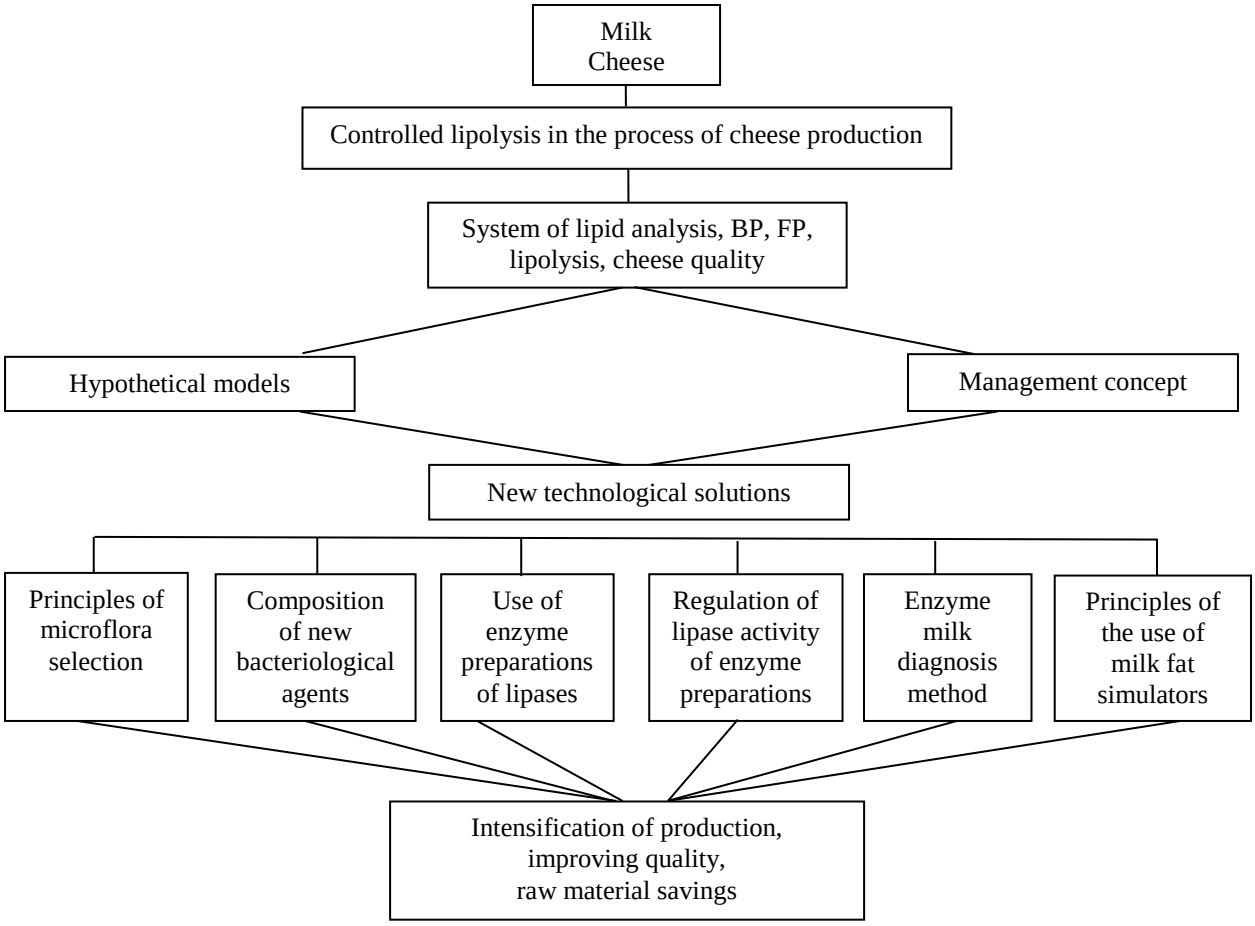


Fig. 2. Logistics of Lipidomics scheme research of raw milk and products.

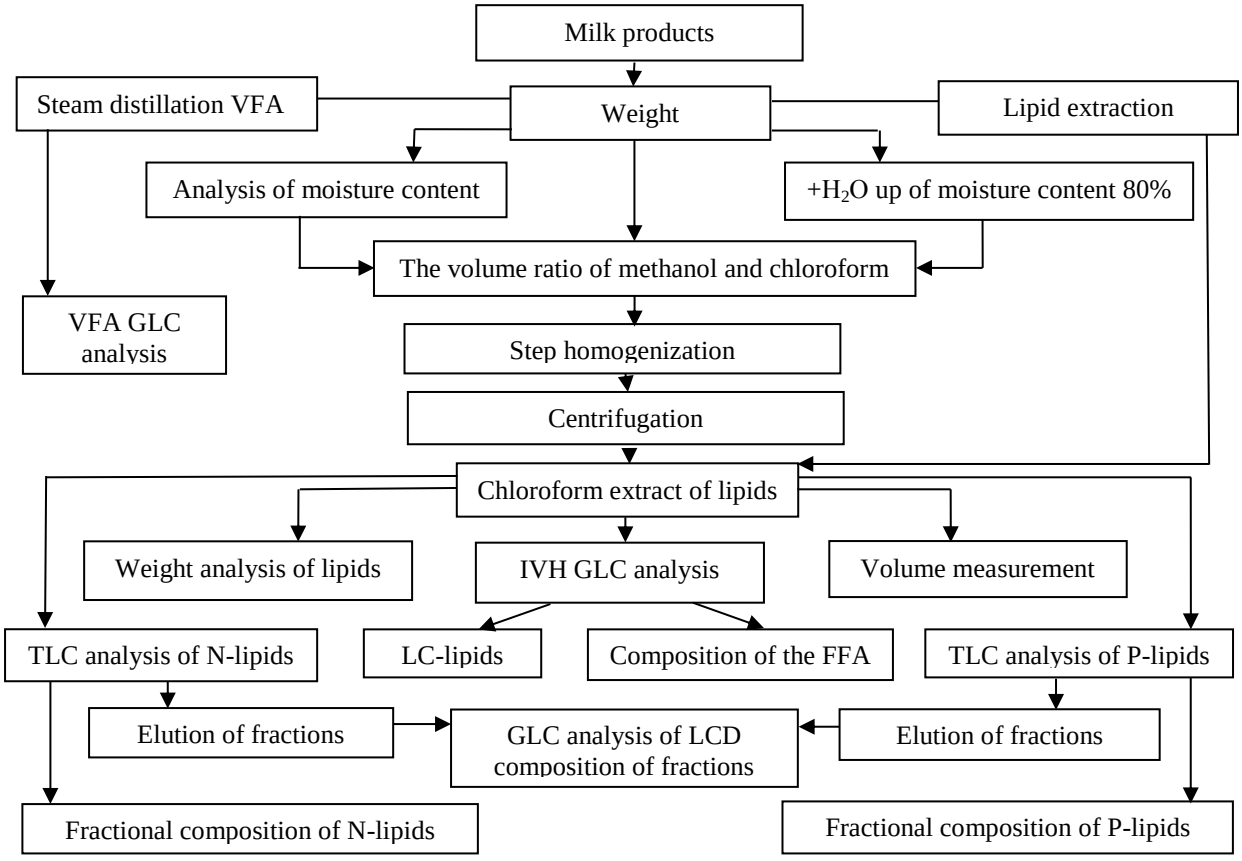


Fig. 3. Scheme implementation of the system of lipid complex analysis of milk and dairy products.

As a result of the above system of cognition research object, at the example of an idealized model of the most sophisticated biotechnological object – cheesemaking, we can formulate principles of Lipidomics of dairy industry: lipid complex and lipase of raw milk, analytics of lipids and lipase, enzymes-builders, biogenic enzymes of bacterial cultures, lipolysis in the cheese during its production, storage and maturation. A single technique aimed to establish laws governing the presence of somatic cells in raw milk and active lipoprotein (which is not considered in industry) needs a separate independent study and practical implementation. Then the problem is in a logical sequence.

RESULTS AND DISCUSSION

Lipids (*Greek* Lipos – fat) are hydrophobic (insoluble in water but soluble in organic solvents) chemical compounds with different composition and structure [10]. In everyday practice we know animal fats, such as milk fat, vegetable oil and microbial oil (there are six product groups with more than 20 items). Among lipids (fats) of animal origin, and in particular in vegetable oils, a unique place is taken by the subject of this article – milk fat, the main indicator of the value of raw milk in Russia. The uniqueness of this bioenergy power given by nature at the disposal of "homo-sapies" can not be overestimated, and to understand it is very simple. Just have a portion of the natural (preferably fresh) and skim milk. Everyone will understand the difference! Milk fat generates a dairy product – its taste, aftertaste, color and even "childhood memories" about your nurse-mother, "fairy tales and songs" (Alexander Pushkin).

This systematic file represents identified and isolated milk lipids according to A. Tepel [3] as neutral fat (acyl glycerol), lipoids (fat-like compounds), isoprenoid lipids and substances related to lipids (Fig. 4).

The main component of the lipid complex of milk-milk fat (glycerides) – occupy more than 98%. The remaining 2% of the lipids (related substances) are phospholipids, glycolipids, sterols, vitamins, pigments; they are usually a part of the lipoprotein membranes of the fat globules – formalized milk fat. Milk fat is a complex hierarchical system which according to Mikhail Umansky includes all partially lipid compounds. It is shown in Fig. 5.

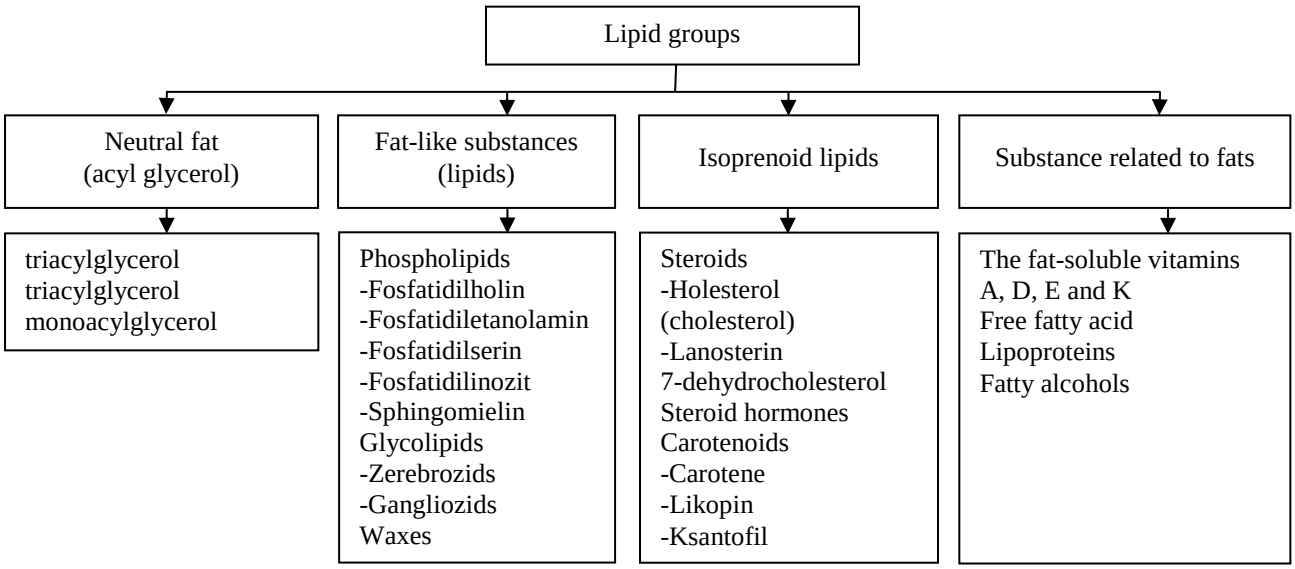


Fig. 4. Milk lipids groups.

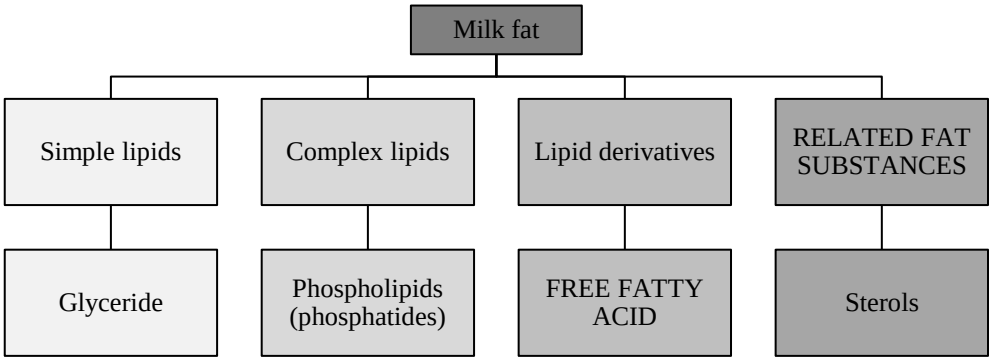
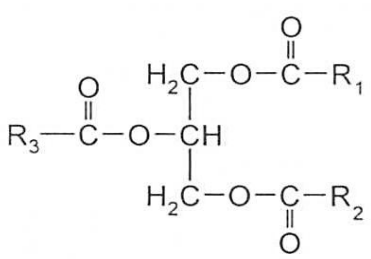


Fig. 5. Systemology of milk fat by M.S. Umansky.

Thus, milk lipid complex shown in Fig. 1 and 2 is unique and is reduced in practice to milk fat cluster structures – globules. Biotechnology fat-containing milk products (cream, sour cream, and of course, butter) is connected with the fat globules. They also play a significant role in the formation of the entire range of protein-fat products – drinks, canned milk, condensed and dried milk, cottage cheese, all kinds of cheeses, etc. A common scientific base of the whole range of milk fat modification and possible derivatives is Lipidomics (science about lipid complex – milk fat). Its principles are formed for all assortment groups and individual milk and milk products. Lipidomics of cheese in complete technological cycle – cheese and whey is considered as a basic example (analog).

The general structural formula of milk fat triglycerides is shown below (R, R1 and R2 are hydrocarbon radicals of higher carboxylic acids).



Schematically fat globules of cow's milk at the present level according to A.Tepel [3] are shown in Fig. 6.

The starting components of the milk fat-structural elements of acyl glycerol – are fatty acids, characteristic of which is shown in Table 1.

The complexity of the structure of one of the groups of milk lipids is shown in Fig. 7.

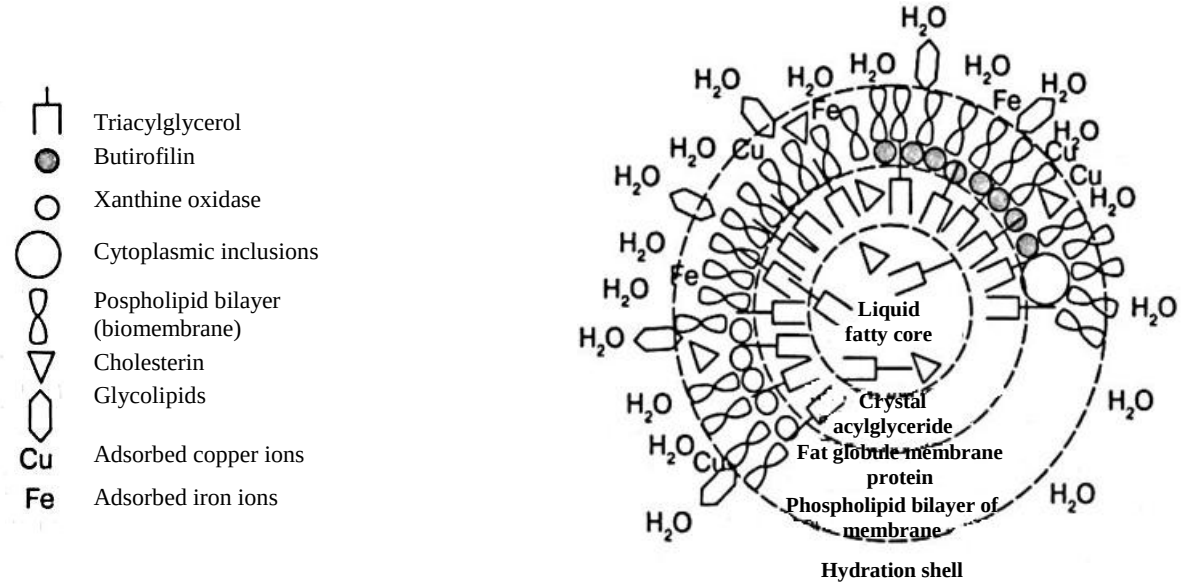


Fig. 6. Schematic representation of fat globules and the membrane.

Table 1. Fatty acid composition of milk fat

Index	Acid		Amount, %
	Systematic	Every day	
Saturated acids			
4:00	butane	oil	3.60
iso-4:0	2-methylpropanoic	isobutyric	0.20
5:00	pentane	valeric	0.20
6:00	hexane	kapron	1.80
7:00	heptane	heptyl	0.01
8:00	octane	caprylic	1.00
9:00	nonanoic	pelargonic	0.30
10:00	decanoic	capric	2.10
11:00	Gendekanoic	undecyl	0.30
12:00	dodecanoic	lauric	2.70
13:00	tridecanoic	tridecylic	0.05
iso-13:0	11-metildodekanoic	izotridcylic	0.01
14:00	tetradecanoic	myristic	11.00
iso-14:0	I2-metiltridekanoic	izomyristic	0.20
15:00	pentadecanoic	pentadecylic	1.10
iso-15:0	12-metiltetradekanoic	izopentadecylic	0.08

Table 1. Ending. Fatty acid composition of milk fat

Index	Acid		Amount, %
	Systematic	Every day	
16:00	hexadecanoic	palmitic	27.50
iso-16:0	13-methyl pentadecanoic	izopalmitic	0.20
17:00	heptadecanoic	margarine	1.00
iso-17:0	14-metilgeksadekanoic	izomargarine	0.01
18:00	octadecanoic	stearic	10.20
19:00	nonadecanoic	nonadedecylic	0.10
20:00	eicosanoic	arachidic	1.80
21:00	Geneycosanoic	-	0.01
22:00	docosanoic	behenic	0.08
23:00	Tricosanoic	-	0.01
24:00:00	Tetracosanoic	lignoceric	0.05
26:00:00	Geksacosanoic	tseritonic	0.07
28:00:00	Oktacosanoic	montanic	0.01
Unsaturated acids			
Δ9-10:1	9-decene	kaprolenoic	0.20
Δ10-11:1	10-undecyl	-	0.06
Δ9-12:1	9-dodecenoic	lauroleinoic	0.30
Δ9-14:1	9-tetracenoic	myristoleic	1.00
Δ9-16:1	9-geksadecenoic	palmitoleic	4.00
Δ9-18:1	9-octadecenoic	oleic	29.80
trans-Δ11-18:1	trans-11-octadecenoic	vaccenic	0.80
Δ9-20:1	9-eykozsnooc	gadoleic	0.10
Δ13-22:1	13-dokochsnoic	erucic	0.05
trans-Δ13-22:1	trans-13-docosenoic	brassidic	0.02
Δ9,12-18:2	9,12-okgadekadienoic	linoleic	3.20
Δ9,12,15-18:3	9,12,15-ktadekatrnenoic	linolenic	1.40

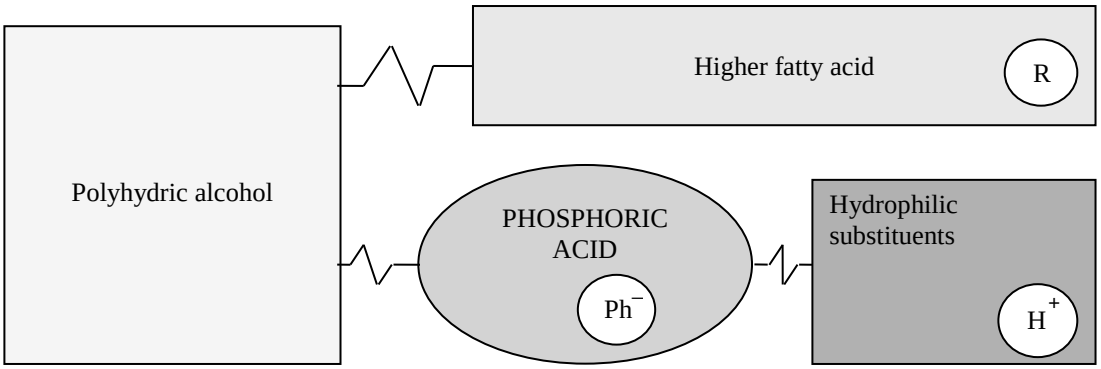


Fig. 7. Block diagram of the structure of the phospholipids.

Systemology of raw milk lipids can be illustrated in details by the example of fat fraction of milk whey in my monograph "Milk whey phenomenon" [11]. This combination of objects of knowledge seems logical in terms of the formation of a Lipidomics paradigm in dairy industry according to the scheme: milk = cheese + whey.

Lipid complex of milk whey is associated with the milk fat in the form of globules in the form of emulsion or suspension, depending on the temperature. The native (fresh) milk whey is emulsion that should be taken into account when organizing processing such as separation.

Study of lipid complex of milk whey in classical science Lipidomics has not only informative, but also practical importance to replenish food and software technology, for example, in the preparation of milk sugar.

The cluster structure of milk fat as an object of nanotenology is quite clearly demonstrated in the works of T. Smykova [12, 13]. Figure 8 shows the cluster structure of the system of lipid complex of milk whey.

The formation basis of lipid complex clusters of milk whey is volatile fatty acids (VFA) in the form of chromatograms, which is shown in Fig. 9.

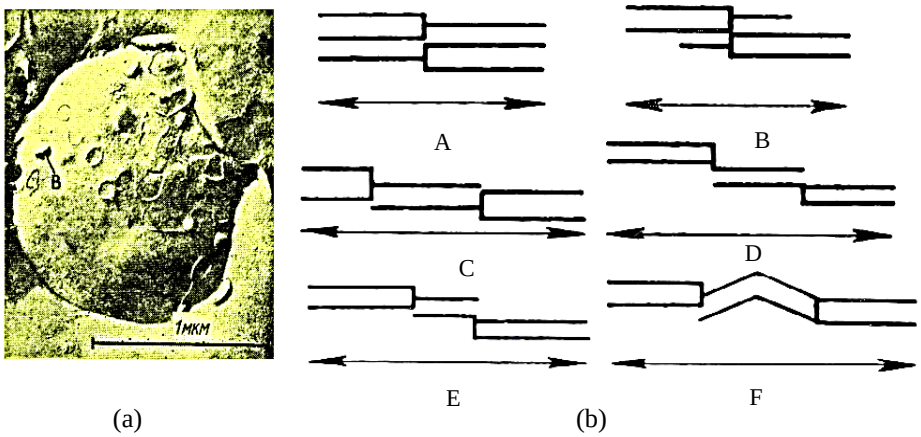


Fig. 8. Lipid complex of milk whey: (a) fat globules, and (b) structure glycerides of milk fat: saturated monoacids (A), saturated diacid with a difference in the length of the acid chain by two carbon atoms (B), saturated diacid with a difference in length of the acid chains of more than two carbon atoms (C, D), saturated different acids (E), oleodisaturated acids (F).

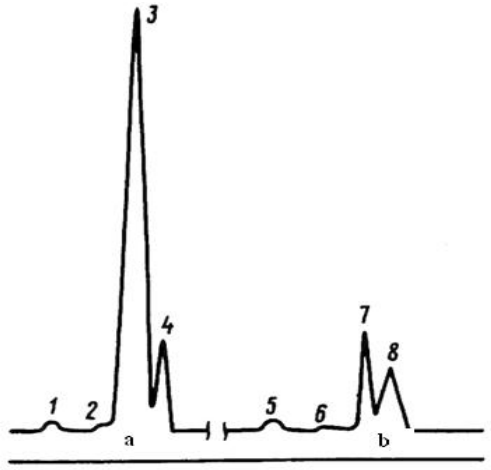


Fig. 9. The chromatogram of volatile fatty acids of curd (a) and cheese (b) whey: butyric acid (1, 5); propionic acid (2, 6); acetic acid (3, 7); formic acid (4, 8).

It should be noted that based on the VFA of E.I. Melnikova, Professor of VGUIT [14] a new perspective is formed on the original component of milk whey – osmophoric compounds that deserve special consideration.

Milk fat has only about 5% in dry milk whey, but it plays an important role in industrial processes, even indirectly – phospholipids, lipoproteins and even fat sugar. Lipid complex is certainly separated from milk whey in the identified complex, separate fractions or together with other components. It should be noted that the component of milk fat globular membrane whey cream (MFGM), shown in Fig. 8 (on the left), attracted the attention of physicians. It is proved by the special report on the Sixth International Conference on milk whey (US, 2011) [15].

A special place in M.S. Umansky's researches [9] is given to lipolytic enzymes of raw milk, which classification is shown in Table 2.

Table 2. Classification of lipolytic enzymes

Systematic number	Systematic title	Everyday title
3.1.1.3	Triacylglycerol-atsilgidrolase (glycerol ester hydrolase)	Lipase
3.1.1.34	Triacylglycerol-atsilgidrolase (glycerol ester hydrolase)	Lipoprotedlipase
3.1.1.13	Sterol ester hydrolase	Cholesterol esterase
3.1.1.4	Phosphatide-2-atsilgidrolase	2 phospholipase (PLA2)
3.1.1.4.-	Phosphatide-1-atsilgidrolase	Phospholipase 1 (phospholipase A1)
3.1.1.5	Lysolecithin-atsilgidrolase	lysophospholipase
3.1.3.4	Phosphatidate-phosphogidrolase	Phosfatidatfosfatase
3.1.4.3	Fosfatidilholin- choline phosphogidrolase	Phospholipase 3 (phospholipase C)
3.1.4.-	Sphingomyelin-N-atsilsfingozingidrola za	sphingomyelinase

In [9] it is shown that lipolysis and milk quality are interrelated, especially in mechanically activated raw materials (mixing) (Fig. 10) and abnormal (not cheesable) milk.

As it was mentioned above even more important is M.S. Umansky's dependence of the content pattern of somatic cells in raw milk-enzyme on lipoprotein lipase activity (Fig. 11).

This fundamentally new (Patent, AS USSR № 1010946) method for the determination of cheesable milk by the number of somatic cells is unique and requires implementation.

A separate topic of M.S. Umansky research regarding Lipidomics is devoted to the study of the lipolytic activity of lactic and propionic acid bacteria. It fully solves a complex problem – the emergence of biogenic elistors in cheesemaking in vitro and in vivo, which requires separate consideration. Further, according to logistics principles lipolysis of milk fat in cheese is discussed. For example, the identification sheet may be composed of phospholipids content in cheese (Table 3).

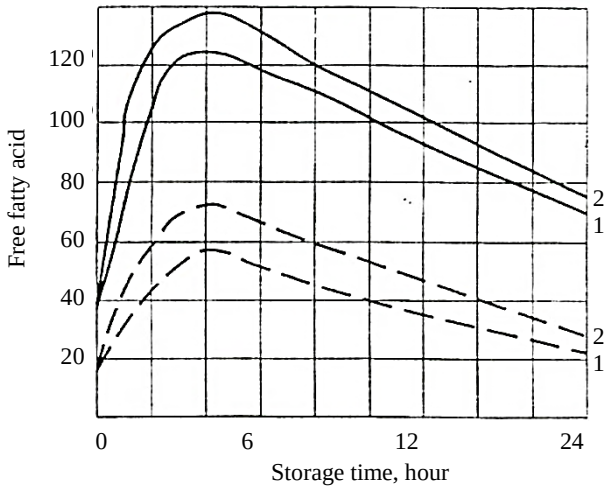


Fig. 10. Changes in the amount of free fatty acids in mechanically active milk (- - -) and cream (————) depending on the duration of storage temperature: 1 – at 4°C, 2 – at 18°C.

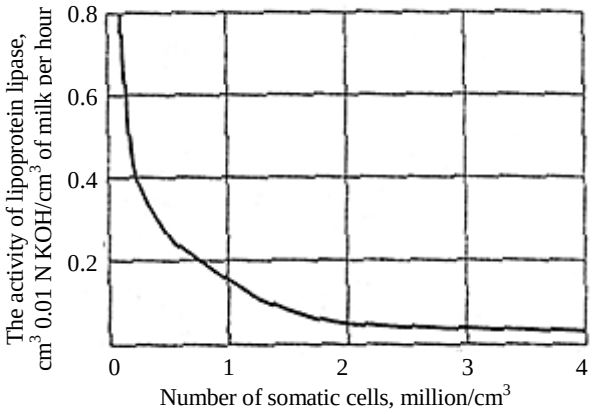


Fig. 11. Dependence of lipoprotein lipase activity on somatic cells in milk.

Table 3. The content of phospholipids in the cheese

Type of cheese	Phospholipids, % 10^{-3}	Type of cheese	Phospholipids, % 10^{-3}
Roquefort	2.0	Krestsentsa	82.6
Parmigiano	60.4	Russian	83.0
Emmental	76.8	Dutch	115.0
Lithuanian	77.0	Kostroma	124.0
Fontana	80.7	Cottage	376.0

Seven guidelines of lipolysis cheese formulated by M.S. Umansky have no analogues and are waiting for research. Below (Fig. 12) you can see a hypothetical scheme of bioconversion of lipid complex in cheese milk.

On the basis of the postulates and revealed laws, using original research methods, we obtained unique results (criteria optimization and neural network modeling) on the regulation of lipolysis in cheeses with low (Table 4) and high (Table 5) temperatures of the second heat.

A simple analysis of Tables 4 and 5 shows the depth and practical value of cheese lipidology as a "launching pad" for Lipidomics of dairy industry.

The most valuable research made by M.S. Umansky [9] are studies of activity of lipase of milk coagulated enzymes (Table 6) and original research methods of lipolysis. They are unique and should be (as innovative) the intellectual property of the Siberian Institute of Cheesemaking (Barnaul, Altai Territory), where M.S. Umansky worked for many years.

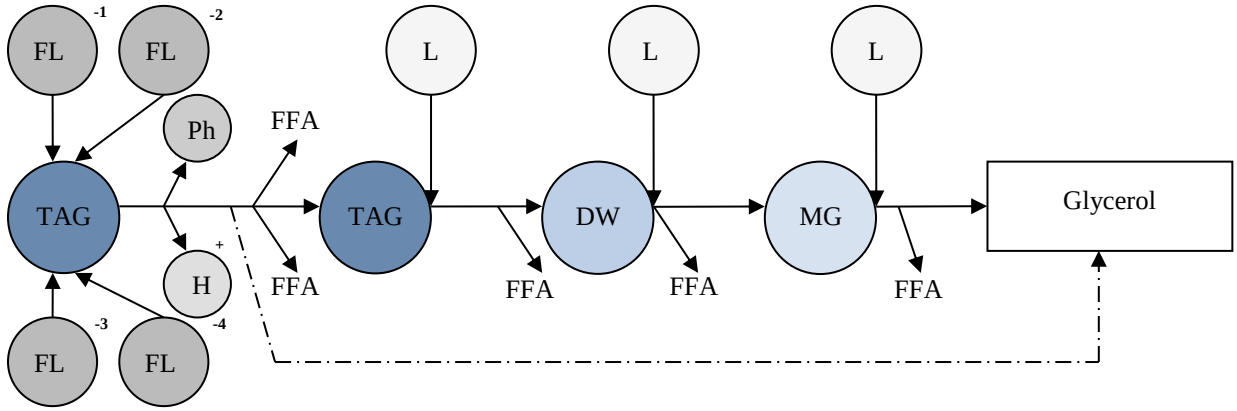


Fig. 12. Schematic diagram of a hypothetical sequential enzymatic hydrolysis of lipids in cheese (Fig. 1).

Table 4. The content of the lipid fraction in cheese

Lipid fractions	The content of the fractions in cheeses with different starter cultures (% of total)	
	Strong lipolytic activity	Weak lipolytic activity
Phospholipids	1.44	1.86
Mono + 1,2-diacylglycerols	8.47	6.65
Sterols + unidentified fraction	8.06	6.60
Free fatty acid 1,3-diacylglycerols +	9.01	7.34
triacylglycerol	67.84	72.72
Steridy + hydrocarbons	5.18	6.03
lipolysis ratio (Cl)	0.34	0.26

Table 5. The mass proportion of phospholipid component in the mature cheese, 10^{3} %

Phospholipid component	Variants of test cheese		
	I	II	III
hospho sphingosine	16.2 ± 1.8	15.0 ± 2.6	18.4 ± 3.1
hosphatidylcholine	18.6 ± 1.4	18.2 + 1.5	18.9 ± 1.3
phosphatidylethanolamine	18.8 ± 1.2	19.9 ± 1.7	19.6 ± 1.2
phosphatidylserine	3.3 ± 2.6	3.6 ± 1.9	4.4 ± 1.4
phosphatidylinositol	1.7 ± 1.5	2.0 ± 2.1	2.6 ± 1.8

Table 6. Lipolytic activity of milk coagulated enzymes on different substrates (10^{-9} mol. sec^{-1})

Enzyme preparations	Olive oil		Synthetic milk fat emulsion		Cream	
	A_1	A_1^{rel}	A_2	A_2^{rel}	A_3	A_3^{rel}
Rennet calves	0.12 ± 0.00	1.0	0.12 ± 0.00	1.0	0.27 ± 0.01	1.0
Pepsin beef	0.09 ± 0.00	0.8	0.10 + 0.00	0.9	0.29 ± 0.01	1.1
Pepsin porcine	0.13 ± 0.01	1.1	0.08 ± 0.01	0.7	0.41 ± 0.01	1.5
Pepsin chicken	0.15 ± 0.01	1.3	0.11 ± 0.00	0.9	0.79 ± 0.02	2.9
Pepsin duck	0.02 ± 0.00	0.2	0.23 ± 0.01	1.9	0.29 ± 0.01	1.1
Fromaza	0.05 ± 0.00	0.4	0.08 ± 0.01	0.7	0.24 ± 0.01	0.9

Table 6. Ending. Lipolytic activity of milk coagulated enzymes on different substrates (10^{-9} mol. sec^{-1})

Enzyme preparations	Olive oil		Synthetic milk fat emulsion		Cream	
	A_1	A_1^{rel}	A_2	A_2^{rel}	A_3	A_3^{rel}
Rennilaza	0.04 ± 0.00	0.3	0.23 ± 0.01	1.9	0.41 ± 0.02	1.5
Rennie-nomiin	1.28 ± 0.04	10.7	2.02 ± 0.06	16.8	1.76 ± 0.04	6.5
Meyto- rennet	0.12 ± 0.00	1.0	0.16 ± 0.01	1.3	0.27 ± 0.01	1.0
Mucor	0.14 ± 0.00	1.2	0.17 ± 0.02	1.4	0.29 ± 0.01	1.1
Mezen-terrine	0.20 + 0.01	1.7	0.16 ± 0.01	1.3	0.60 ± 0.02	2.2
Kazorus-Sulin	0.27 ± 0.01	2.3	0.43 ± 0.03	3.6	0.35 ± 0.01	1.3

CONCLUSION

In general, a brief analysis of M.S. Umansky's contribution to the development of selective lipolysis in cheese in the author's edition "lipidology of cheese" underlines the greatness of his work and the possibility of forming Lipodimics of dairy industry. Moreover, it allows us to formulate the following conclusions.

(1) An independent scientific direction – lipidology of cheeses (initiators and leaders are Professor H.H. Dilanyan and L.A. Ostroumov) began to form in biotechnological research in milk production and dairy products in 70 years of the last century. Its development was promoted by the appearance of such research methods as gas-liquid and thin-layer chromatography, spectrophotometry, alkalimetry and others.

(2) Methodological framework for the implementation of the management ideas of lipolysis in cheese is created. It includes the development and modification of 17 original analytical methods of milk research, culture media, bacterial whey and starter cultures at different stages of cheese production. The main processes of lipolytic in milk are studied. It is shown that lipolysis scale depends on the presence of lipoprotein lipase in the milk and other lipolytic enzyme, which is the main producer of psychrophilic microorganisms. Lipid and fatty acid composition of different kinds of cheese is investigated. It is found that each of them is characterized by a certain content of ester compounds and their hydrolysis products. To assess the level of enzymatic catalysis of lipid fractions an integral indicator – ratio of fatty acid specificity – is proposed.

(3) We proved experimentally the ability of lactobacilli to produce enzymes of lipolytic orientation that hydrolyze triacylglycerols of milk fat forming diacylglycerols, monoacylglycerol and fatty acids. The degree of lipase and phospholipase activity of Lactococcus and Lactobacillus has interspecific and intraspecific differences.

(4) We prove the existence of lipase and phospholipase activity in the majority of the representatives of propionic acid bacteria involved in the maturation of cheeses with a high temperature of second heating. The intensity of these symptoms at propionic acid bacteria is higher than at lactic acid bacteria.

(5) The hypothesis is put forward that there is a connection between specificity and degree of lipolytic activity of lactic and propionic acid bacteria used as starter cultures, and the intensity and direction of enzymatic hydrolysis of lipids in ripening cheeses. Selection of microflora in such a way is an effective quality management tool and the length of cheese ripening.

(6) The dependence of the formation processes of cheeses with a low temperature of second heating on the level of lipolytic activity of the lactic microflora starter cultures is set.

(7) We determined lipolytic activity of 12 different kinds of milk-products and proposed the criterion system of evaluation on this indicator of their suitability in cheese making.

(8) Original solution for the management process of cheese lipolysis is created, the novelty of them is protected by 15 copyright certificates for inventions (patents).

All above mentioned allows us to start the formation of Lipidomics postulates system form within Lactomics of dairy industry, starting with raw milk and its products in dairy products with a mandatory full and rational use of byproduct of raw milk-skim milk, milk whey and buttermilk. The legality of the implementation of systematic research is convincingly proved by Professor M.S. Umansky. The field of activity here is huge – all product groups and separate types of dairy products.

To the blessed memory of Professor Mark Solomonovich Umanskiy (04.07.1941 – 13.02 2011)

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Please cite this article in press as: Khramtsov A.G. An epistemological background on paradigm formation of lipidomics of dairy industry. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 79–89. doi: 10.21179/2308-4057-2016-1-79-89.

TECHNOLOGICAL OPTIONS FOR THE PRODUCTION OF LACTOFERRIN

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Received January 28, 2016; Accepted in revised form March 10, 2016; Published June 27, 2016

Abstract: Lactoferrin (LTF) is a multifunctional protein of the transferrin family present in human and other mammals' milk. Global demand for originated protein is currently much higher than the supply, and lactoferrin remains the one of the most expensive proteins. Therefore, the development of cost efficient methods to produce lactofferin is extremely important. In this study, human lactoferrin is obtained in bacterial system. *ltf* gene, coding sequence of protein - human lactoferrin (hLTF), was cloned into the expression vector pET28a+, modified signal sequence IMKKTAIAIAVALAGFATVAQA AS. *E.coli* BL21DE3 strain was selected for expression. Productivity of the producing strain in regard to recombinant human lactoferrin was 30–35% of the cellular protein. The strain produced the recombinant lactoferrin in the form of inclusion bodies. The maximum yield of the target protein (including in soluble form) was achieved by culturing of the recombinant strain at 25°C, induction by 1.1 mM concentration of IPTG and 8-h period after induction. Optimization of solubilization and renaturation conditions allowed once to recover lactoferrin in a concentration of 2.9 mg/ml by the pulsed system. The protein of high purity at least 90% was obtained by means of affinity chromatography on Ni+-agarose.

Keywords: lactoferrin, milk protein, gene, *E.coli*

DOI 10.21179/2308-4057-2016-1-90-101 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 90–101.

INTRODUCTION

Milk can be considered the first functional food in life as it contains, not only nutrients for the newborn, but also essential components for organ development and normal physiology. An overarching goal is to identify and produce bene ficial components of milk for improving nutrition and for specific therapeutic interventions. While some components are already being isolated from bovine milk to meet this goal, others, especially components of human milk cannot be easily obtained from the natural source. This is illustrated by lactoferrin [1, 2].

Lactoferrin is a polyfunctional protein of the transferring family, which has anti-bacterial, anti-viral, anti-cancer, antifungal, antiparasitic, antioxidant and regenerative properties. Lactoferrin can be found in the human and other mammal's milk [3]. On the basis of the many biological activities of hLF, researchers have considered a wide variety of possible applications in human health care, such as prophylaxis and treatment of infectious and inflammatory diseases [4].

Until recently, the therapeutic use of lactoferrin has been limited by the lack of an efficient and cost-effective method for the production of the human rotein in large uantities. The limited availability of human milk and purified hLF has been a major hurdle for (clinical) studies on potential nutraceutical and pharmaceutical applications of hLF. To overcome this limitation, the feasibility of large-scale production of functional recombinant human lactoferrin (rhLF) was studied in a variety of expression systems [5]. The small quantities of lactoferrin have been expressed in eukaryotic systems, including baby hamster cells and human 293 cells. While the recombinant lactoferrin produced was biologically active, these system are not readily amenable for scale production. The lactoferrin has been expressed in yeast expression system: *Saccharomyces cerevisiae*, *Pichia pastors* [6]. The secretion of recombinant lactoferrin in these systems was inefficient, with less than 10% of the lactoferrin produced being secreted into the growth medium and this host cell itself is found to be highly susceptible for

the lactoferrin produced. The ability to get lactoferrin by means of fungal expression system (*Aspergillus* [7]) was demonstrated. The bioavailability of the lactoferrin, obtained by means of this system, was less than 0.5%. Also, the using of *Aspergillus* for the lactoferrin production was associated with a high risk of contamination with aflatoxins, which are potential carcinogens. *Ltf* has been expressed in plant culture: potato plants [8], tobacco plants [9], ginseng cell culture [10], rice cell culture [11]. However, such problems as low level of lactoferrin expression, even when using a strong promoters; congestion with carbohydrate component; risk of the transgene leakage into the environment (out of control) make these approaches not suitable for large scale production.

Ltf has been expressed in the mammalian expression system: mice, rabbits [12], transgenic cows [13], transgenic goats [14]. However, the expression level was unstable and not suitable for fast expression of the proteins. Also, the success of this approach, including the ability to purify the recombinant protein in a viral- and toxin- free form and the cost-effectiveness of large transgenic animal program, remains to be established.

The advantages making microbal synthesis of the lactoferrin promising are metabolic flexibility and high ability of the microorganisms to adapt, high growth rate, ease of cultivation, the study of genetics and othe [15].

Expression of recombinant proteins in *E.coli* cytoplasm is widely used. However, improper folding of many target proteins may occur during this process. Improper folding often results in the formation of inclusion bodies despite attempts to optimize growth conditions. One possible approach to obtaining a correctly folded recombinant protein is to export the protein into the *E. coli* periplasm [16, 17].

The purpose of this study is to develop a technology of obtaining human lactoferrin from *E. coli* cells.

OBJECTS AND METHODS OF STUDY

Reagents. The following reagents were used in the work: isopropyl-β-thiogalactopyranoside (IPTG) (”ALMABION”, Russia), lactose (“ProfiPark”, Russia), T4 DNA ligase, *Taq*- and *pfu* DNA polymerase, buffer for *taq*-polymerase, buffer for *pfu*-polymerase, DNA markers, deoxyribonucleases, mineral oil, the restriction enzyme XhoI and HindIII, buffer XhoI, buffer HindIII (“Sibensim”, Russia), ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulphate (SDS), magnesium chloride, boric acid, sodium hydroxide, acrylamide, N,N,N’,N’-tetramethylethylenediamine (TIMED), persulfate ammonium, glycine, Coomassie R-250, sodium perchlorate, bromophenol blue, Tris, hydrochloric acid, 0,1% DS-Na, glucose, PEG, the solution of TE, phenol and chloroform, potassium acetate, sodium chloride (“Invitrogen”, USA), suspension of silica, carbod (“Helicon”, Russia), LB-medium (“Gibco BRL”, US), ribonuclease, kit “DNA isolation from agarose gels”, (“Sileks”, Russia), ampicillin, agarose, ethidium bromide, 2-mercaptoethanol, lactoferrin (“Sigma”, USA), kit “Lactoferrin-IFA-best” (“Vector-best”, Russia).

Plasmids.

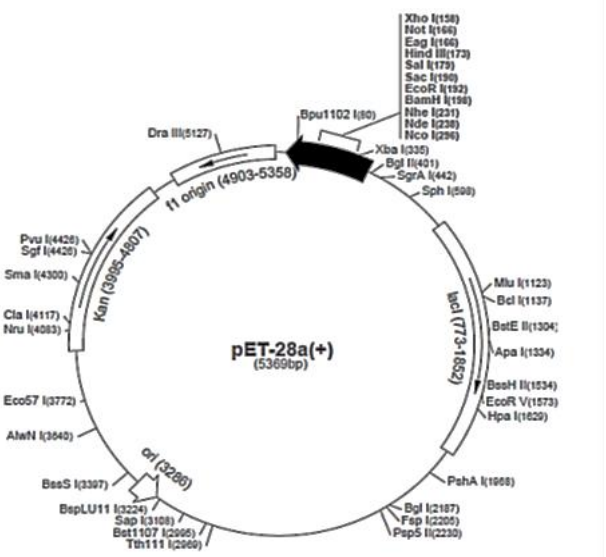


Fig. 1. pET28a+ plasmid which was used in the work

Construction (Fig. 1) was modified by signal sequence of OmpA, providing transport of proteins across the inner membrane into the periplasm - IMKKTAIAIAVALAGFATVAQA AS on sites (BspH I)OmpA (HindIII):

atcatgaagaaaaccgccatcgccatcgccgtggcgctggcaggtttcgccaccgtggca

I M K K T A I A I A V A L A G F A T V A

caggccgctag

Q A A S

Strain – *E.coli* BL21 DE3

mRNA synthesis. mRNA of human lactoferrin (LTF laciotransferrin [Homo sapiens]) based on the sequence of GeneBank database (GenBak: 23268458), in which were held insignificant nucleotide substitutions, was synthesized by the Evrogen (Russia).

mRNA of lactoferrin gene was synthesized so that directly behind the coding sequence was located the site corresponding to hexahistidine sequence. As a result the induced expression of the cloned gene will be synthesized lactoferrin, containing additional hexahistidine sequence in the C-terminal region of the polypeptide chain, allowing to carry out subsequent affine cleaning of the protein on Ni-NTA-agarose.

Hydrolysis of the DNA fragments of XhoI u HindIII by means of restriction enzymes was performed in 100 µl of special buffers at optimal temperature to each enzyme – 37°C. Completeness of the hydrolysis was monitored by mans of gel – electrophoresis on agarose gel.

Agarose gel electrophoresis is carried out according to the standard procedure described in [18].

Isolation of DNA fragments from the gel. The DNA samples were separated by means of electrophoresis in TBE buffer in 0.8–1% of a gel block containing 0.5 µg/ml ethidium bromide, and were analyzed in the UV. Pieces of the gel containing the fragment of interest was excised and transferred to microcentrifuge tubes. Then elution of fragments was performed using the kit "Isolation of DNA fragments from agarose gels".

Ligation. Electrophoretically purified HindIII-ltf-His-Tag-XhoI fragment and fragment of the mpET28a+ vector were ligated in ligase buffer with T4 DNA ligase for 12 hours at 10°C. Thereafter ligase was inactivated by heating of the mixture for 5 min at 70°C. Purification from unreacted PCR fragments was performed by excising from the gel.

Preparation and transformation of competent cells with the obtained genetic structure were performed by mens of heat shock. To this, 1 ml of overnight culture was grown at 30°C with intensive aeration in medium, supplemented with 0.9% glycine, 0.02 M $MgCl_2$ and ampicillin, in a shaker. The cells were pelleted by centrifugation after cooling on ice for 10 minutes. The pellet was resuspended in 1 ml of cooled TB1 buffer, further tubes were incubated on ice for 10 minutes and placed for 30 sec. in a water bath preheated to 42°C.

2M glucose was added in the tube after the thermal shock and the mix was incubated for 1 hour at 37ºC (or 1.5 h at 30ºC). Then mix was plated on Petri dishes with LB medium, containing 30 ug/ml ampicillin. The dishes were incubated in a thermostat at 37°C for 16 hours.

Isolation of plasmid DNA was carried out according to the method of Maniatis.

Lactoferrin heterologous expression. The plasmid was transformed into BL21(DE3)/mpET28a competent cells, then the cells were spread on LB-agar plates, followed by overnight culture at 37°C. The colonies from LB-agar plates were selected and cultured in 5 ml of LB liquid medium, plus 50 µl of 50% glucose, overnight at 37°C with shaking. The next morning, 1 ml of overnight culture was inoculated in 100 ml of fresh LB liquid medium, plus 1 ml of 50% glucose, and cell culture was continued at 37°C with shaking while monitoring growth of the culture by measuring the optical density at 600 nm (OD600). At OD 600 of 0.6–0.8, 100 ml of culture was inoculated into 4 L LB, plus 30 ml of 50% glucose. The cell culture was continued again at 37°C with shaking while monitoring growth of the culture. Once OD600 reached 0.6 again, the temperature was decreased to 23°C, the inducer was added (1 mM IPTG). All plates and LB liquid media used here contained 25 µg/ml of kanamycin.

Screening of cultivation conditions. The experiments were performed in 250 ml of LB medium containing 25 µg/ml kanamycin. 1 ml of culture was diluted 50 times in fresh LB nutrient medium and then cultivated (at 37°C, 250 rpm) until OD 600 nm of 0.4–0.8 of the cell density.

Response surface methodology (RSM) of statistic. RSM was used for optimize the induction conditions selected at univariate experiments, and for increase the production of recombinant lactoferrin. Composite design was used for optimization of three significant factors: temperature after induction, concentration of inducer and period after induction. The following polynomial second order equation explains the relationship between dependent and independent variables:

$$Y= \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+ +\beta_{23}X_2X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2 , \quad (1)$$

where Y is the dependent variable; X_1, X_2 and X_3 are the independent variables (temperature after induction, concentration of inducer and timeafter induction); β_0 is the time of intersection; β_1, β_2, β_3 the linear correction factors; β_{12}, β_{13} and β_{23} the interaction coefficients and β_{11}, β_{22} and β_{33} the quadratic coefficients. The approximating polynomial equation in the form of contour and surface response plots was used for image the relationship between responses and experimental levels of each of the variables.

Determination of the lactoferrin concentration in accordance with the protocol to "Lactoferrin-ELISA-BEST" ("Vector-best", Russia).

Protein analysis. SDS-PAGE was carried out using a 10% gradient gel according to Laemmli [19]. TotalLAb program was used for the processing of electrophoregram results.

Isolation, solubilization and renaturation. After fermentation the culture medium was separately by centrifugation. The cells were harvested by centrifugation and suspended in 50 mM phosphate buffered saline (PBS, pH 8.0) buffer containing 200 mM NaCl. Protease inhibitors were added to the suspension. The cells were destroyed by ultrasound homogenizer Scienta-IID (China) by 4 times in the mode: total time – 30 seconds (2 sec – voicing, 2 sec – break), cooled in an ice bath for 1 minute. After sonification, the suspension was centrifuged at 5800g for 30 min at 4°C. The pellet was suspended several times in 50 mM PBS (pH 8.0) buffer containing 10% Triton X-100 and 200 mM NaCl to remove

nonspecifically adsorbed proteins, and the solution was centrifuged again at 5800 g for 30 min at 4°C. The pellet was used for optimization of solubilization with different concentration of Gn-HCl at pH 12 in 2 M Tris-HCl buffer. Optimization of renaturation was carried out in standart buffer: 0.5 M/L Gn-HCl, 50 mM/L Tris-HCl (pH 8.5), 0.75 M/L arginine, 1 M/L NaCl, 5 mM/L EDTA and 3 mM of glutathione in a ratio of 10 : 1 GSH : GSSG.

Purification on a Ni^{+2}-NTA agarose. The sample was loaded onto a column of Ni^{+2}-NTA agarose equilibrated with 150 mM PBS (pH 8.0) comprising 10 mM imidazole. The column was washed of unbound material with the source buffer, then with the 150 mM phosphate buffer (pH 8.0), comprising 20 mM imidazole. Then it was eluted with150 mM phosphate buffer (pH 8.0), comprising 250 mM imidazole.

RESULTS AND DISCUSSION

The *ltf* gene expression in *E.coli* BL21DE3/mpET28a+-l*tf*

The cells received after the transformation were cultivated at 37°C for 24 h until optical density 0.6–0.8 in conditions when transcription of lactoferrin cloned gene was repressed. Then effective transcription was induced by the addition of the inductor. This approach allows to grow cells containing the mRNA of human lactoferrin gene to a high titer and only after to trigger the production of protein.

The target protein with a molecular mass of 78–80 kDa was accumulated in conditions of induction in *E. coli* BL21DE3/mpET28a+-*ltf* (Fig. 2).

The level of target protein expression (~78–80 kDa) was from 30 to 33% of the total cellular protein, as shown by electrophoresis in 10% polyacrylamide gel in the presence of sodium dodecyl sulfate (Fig. 2).

The level of the lactoferrin in the samples washed precipitations (inclusion bodies) was 1–3% less than the total expressed protein. Thus, the strain producing the recombinant lactoferrin mainly in the form of inclusion bodies (IB) (Fig. 2) in standart condition was obtained.

Selection of cultivation conditions

The expression of heterologous genes leads to metabolic depletion of cells, which is expressed in the inhibition of cell growth, decrease of the biomass yield and the target protein. Several one-factor experiments for study the impact of various factors on the production of target protein were carried out.

The following parameters presented in Table 1 were selected for testing. The experiments were conducted in three replicates.

Cell concentration

The cell density before induction is important factor for the expression of recombinant proteins.

Testing was performed at different cell concentrations from 0.4 to 1.0 at OD 540 nm. The results are presented in Fig. 3.

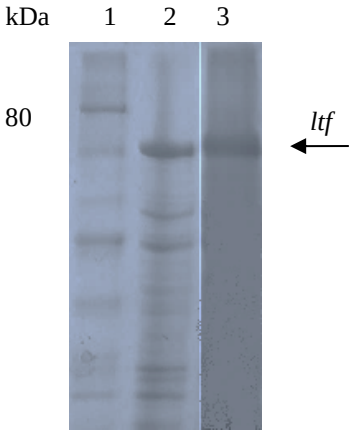


Fig. 2. The *ltf* gene expression in *E. coli* BL21DE3/mpET28a+-*ltf*. Lane: 1 – markers; 2 – lysate of the strain, transformed with the genetic construct containing *ltf* gene; 3 – after solubilisation of IB.

Table 1. Induction parameters selected for the study of lactoferrin production

Induction condition	Range
Cell concentration	OD 540 nm 0.4–1.0
Type of inductor	IPTG / lactose
Concentration of inductor	0.2–2.0 mM
Temperature cultivation after induction	15–37°C
Time after induction	2–24 h

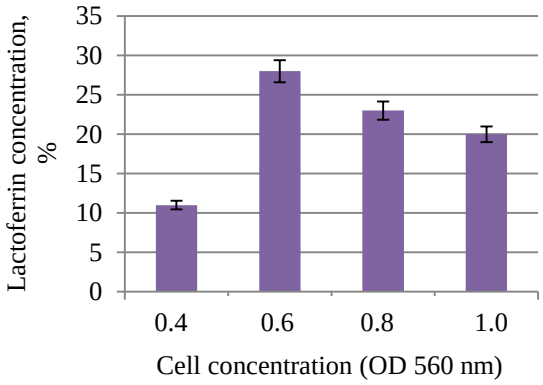


Fig. 3. The effect of cell concentration on the lactoferrin synthesis by cultivation with 1 mM IPTG, 37°C and 24 h after induction of strain.

The concentration of lactoferrin varies over wide range. The most amount of lactoferrin (28% of total cellular protein) was obtained during early induction (the cell concentration 0.6)

The effect of concentration and type of inducer

The used expression system of lactoferrin requires the addition of inducer (IPTG) for ensure of the cloned gene transcription in the plasmid and initiation of lactoferrin translation. This leads to some changes in the metabolism of the host cell. The concentration of the inductor depends on how expressed product is toxic to the strain and used vector. In order to reduce the cost of induction, the effect of lactose concentration on the efficiency of expression and the ability to effectively replace IPTG were studied. This strain was grown at 37°C. The inductor was added in concentrations from 0.2 to 2 mM. The results are presented in Fig. 4.

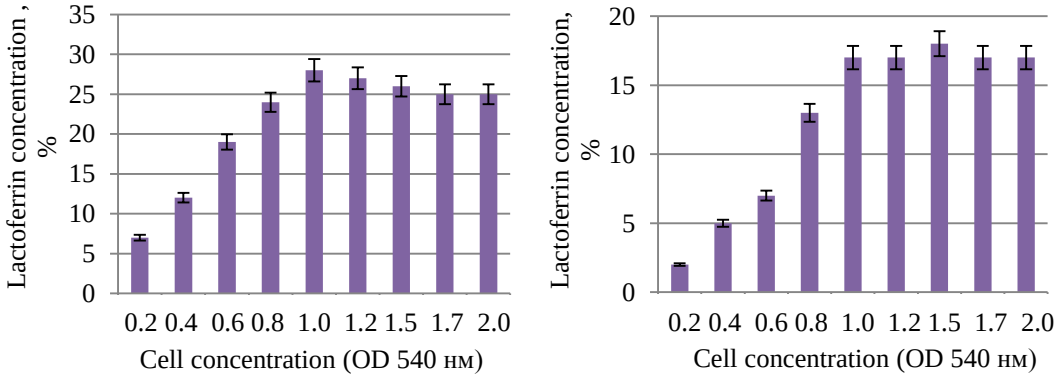


Fig. 4. The effect of IPTG (a) and lactose (b) concentrations on the lactoferrin synthesis (percentage of the total cellular protein) by cultivation at 37°C and 24 h after induction.

As Fig. 4a shows, the concentration of inducer (IPTG) affects on the lactoferrin synthesis. The increase of the lactoferrin concentration was observed by increasing of the IPTG concentration from 0.2 to 1.0 mM. The maximum percentage (28%) of lactoferrin was reached at a concentration of 1.0 mM. From 1.2 mM of IPTG the decrease of lactoferrin synthesis was observed due to the slower growth of bacteria at high concentrations of IPTG. High concentrations of IPTG in this case can lead to ribosomal degradation and the production of heat shock proteins and eventually to cell death.

As Fig. 4b shows, the concentration of the inducer (lactose) also affects on the lactoferrin synthesis. The increase of the lactoferrin concentration was observed by increasing of IPTG concentration from 0.2 to 1.5 mM. The maximum percentage was reached at a concentration of 1.5 mM.

Temperature of cultivation after induction

Lactoferrin expression in cells, transformed with pET28a+ plasmid, was studied at different temperatures. That is how the selection of temperature for cultivation of bacteria aimed at the maximum yield of the protein in a soluble form, was carried out. After induction, the recombinant strains were cultivated at different temperatures (37°C, 23°C, 15°C). The concentration of lactoferrin was measured in the culture medium after the destruction and dissolution of the inclusion bodies (IB).

As SDS-analysis shows (Fig 5), the expression of lactoferrin was observed at all investigated temperatures. Maximum protein synthesis was observed at 23°C. The maximum yields (62%) of target protein in the soluble form were achieved by culturing the recombinant strain also at 23°C. This is because

slower rates of production give sufficient time to the system to fold a protein that decreases the rate of aggregation and the possibility of random formation of intermolecular hydrophobic interactions. Almost all lactoferrin is produced in the form of inclusion bodies at 37°C. Lowering of temperature to 15°C also was not lead to greater accumulation of lactoferrin than at 23°C. Thus, cultivation of the strain at 23°C is the most appropriate.

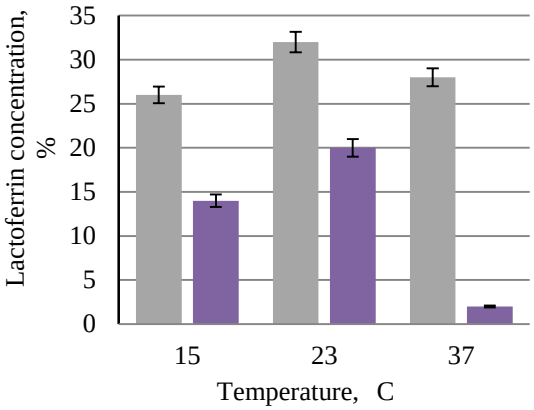


Fig. 5. The effect of temperature after induction on the lactoferrin synthesis (percentage of the total cellular protein) by cultivation at 37°C and 24 h after induction. The first column is total lactoferrin, second column – soluble lactoferrin.

Period after induction

The optimal time of expression was determined by analysis of samples taken after 2, 4, 6, 8, 10, 12 and 24 hours after induction. The total product (soluble and insoluble forms) of the total cellular protein was analyzed.

The effect of the duration of period after induction on the lactoferrin production is shown in Fig. 6.

As Fig. 6 shows, the maximum percentage of product to regard to total protein was reached in 8 h after induction.

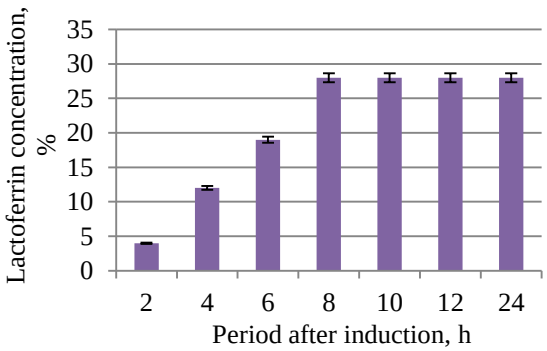


Fig. 6. The effect of duration of period after induction on the lactoferrin synthesis (percentage of total cellular protein) at cultivation at 37°C and 24 h period after induction.

Analysis of the effect of variable parameters on the lactoferrin synthesis by RSM

RSM (Response surface methodology) method of statistics was used for further optimization of the conditions. The use of statistically based experimental design is an important tool in optimizing of induction conditions. The analysis gives several important advantages, such as the effect of the studied factors, the determination of the optimal values, etc. Multifactorial experiment with 3 variable parameters selected in previous research: temperature after induction – X_1, the concentration of inducer – X_2, and period after induction – X_3 was conducted for optimization of induction conditions. Each parameter was varied on three levels (Table 3): low (-1) middle (0) and high (+1) (Table 2).

Composite matrix of multi-factorial experiment and obtained results of protein yield are presented in Table 3.

The mathematical processing of data presented in Table 3 was carried out for impact assess of variable induction parameters on the lactoferrin concentration. The results of the statistical analysis of effects of variable factors are showed in Fig 7.

Table 2. Levels of variation of independent parameters

Parameter	Variable	Range		
		-1	0	1
Temperature after induction, C	X_1	15	23	37
Concentration of inducer, mM	X_2	0.6	1	1.7
Period after induction, h	X_3	6	8	12

Table 3. Matrix multi-factor experiment for optimize the conditions (a key parameter is the concentration of lactoferrin)

№	Levels of factors			Lactoferrin concentration, mg/L
	X_1	X_2	X_3	Y
1	1	1	1	20,2
2	+1	-1	-1	22
3	-1	+1	-1	46,4
4	-1	-1	+1	36,8
5	+1	+1	-1	27,8
6	+1	-1	+1	42,1
7	-1	+1	+1	65,7
8	+1	+1	+1	80,9
9	1	-1	+1	63,8

Table 3. Ending. Matrix multi-factor experiment for optimize the conditions (a key parameter is the concentration of lactoferrin)

№	Levels of factors			Lactoferrin concentration, mg/L
	X_1	X_2	X_3	Y
10	-1	1	-1	59.1
11	-1	-1	1	29.6
12	1	1	-1	70.4
13	1	-1	1	57.4
14	-1	1	1	75.2
15	1	1	1	96.9
16	+1	+1	1	84.6
17	+1	1	+1	93.1
18	1	+1	+1	87.5
19	1	1	+1	102.0
20	1	+1	1	76.7
21	+1	1	1	93.5
22	-1	1	+1	83.5
23	-1	+1	1	64.5
24	+1	1	-1	50.7
25	1	-1	-1	39.9
26	1	+1	-1	56.5
27	+1	-1	1	32.1

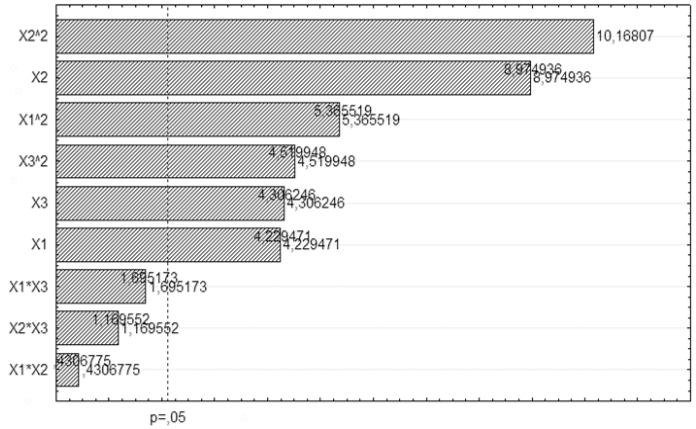


Fig. 7. Pareto diagram for assess of effects significance of variable factors on the lactoferrin concentration.

As the data shows, the concentration of lactoferrin, producted by *E. coli* BL21DE3/mpET28a+-*ltf* strain, is determined by all three considered parameters (X_1, X_2, X_3). Linear and quadratic factors of inductor concentration have most pronounced effect on protein yield. The results of the analysis show no significant interaction effect between variable parameters.

Multiple regression analysis of the experimental data provided parameters of equation. The polynomial equation of the second order (equation 1) was used for express the empirical relationship between the response and significant variables:

$$Y = -324.6 + 7.02X_1 - 0.15X_1^2 + 273.242X_2 - 115.4X_2^2 + 32.9X_3 - 1.81X_3^2, \quad (2)$$

where Y is the concentration of lactoferrin, mg/L; X_1, X_2, X_3 – temperature after induction, concentration of inducer and period after induction respectively.

The three surface are showed (Fig. 8), considering all possible combinations. The quadratic dependence of the lactoferrin concentration on the temperature after induction, inducer concentration and period after induction was showed.

The profiles of desirability are presented in Fig. 9. Profiles of desirability function also show a quadratic dependence between variable parameters and the lactoferrin concentration. Optimal levels for variable parameters according to the profile of the desirability are following: induction by 1.1 mM of IPTG, followed by 8.6 hour period after induction at 25°C. The change of desirability function for variation of the relevant independent variables was presented in the lower series of graphs.

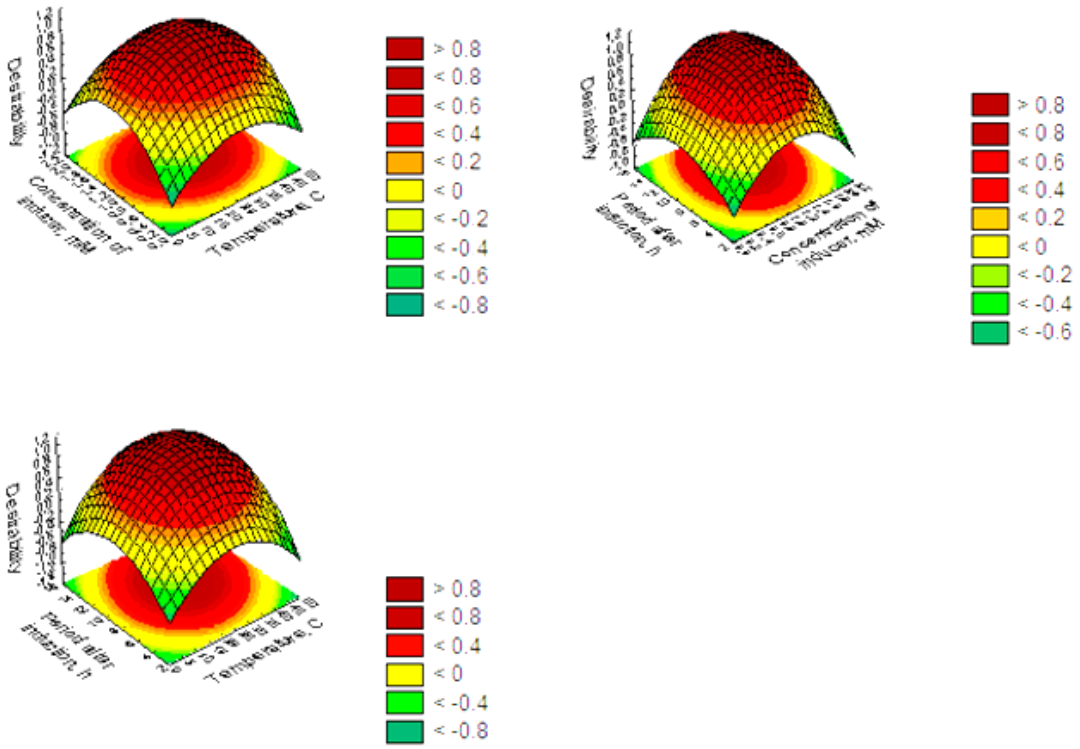


Fig. 8. Response surface showing the dependence of lactoferrin yield on variable parameters of induction.

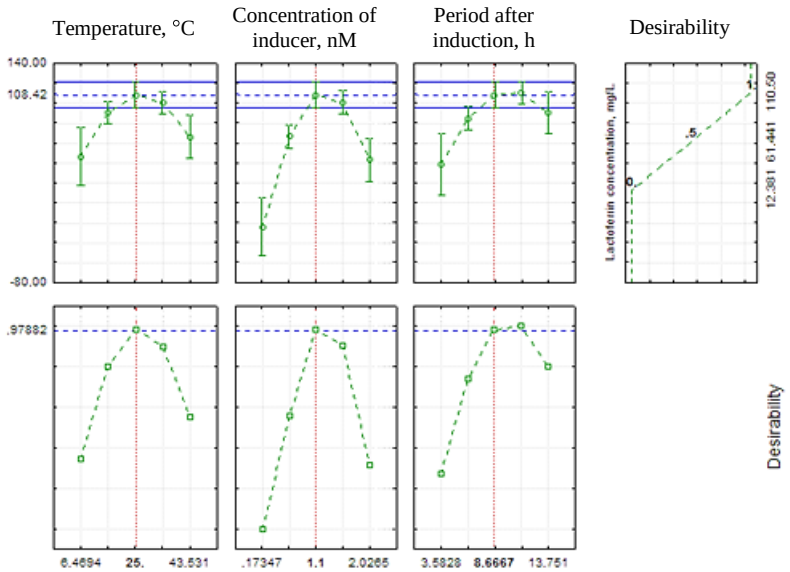


Fig. 9. Desirability profiles of lactoferrin release by culturing BL21DE3/mpET28a+-*ltf* strain.

Optimization of solubilization, renaturation and purification of lactoferrin

Optimization of solubilization

Portion of lactoferrin was synthesized in the form of inclusion bodies by cultivation of strain under optimal conditions. Additional stages of isolation, solubilization and renaturation were conducted for translate the protein in a soluble form.

Before solubilization inclusion bodies were washed with 20 mM/L Tris-HCl (pH 8.5), 0.5 mM/L EDTA and 2% Triton X-100.

For solubilization of protein from inclusion bodies, the experiments were carried out with different concentrations of IB both in the presence and in the absence of Gn-HCl. Fig. 10 shows the results obtained by dissolving various concentrations of inclusion bodies at pH 12 in 2 M Tris-HCl buffer with various Gn-HCl concentration.

With increasing of protein concentration from 0.05 to 5 mg/ml the solubility of inclusion bodies was decreased from 68% to 20% due to the alkaline pH (without adding of Gn-HCl). The addition of Gn-HCl in the buffer improve the solubility of protein inclusion bodies in higher concentrations.

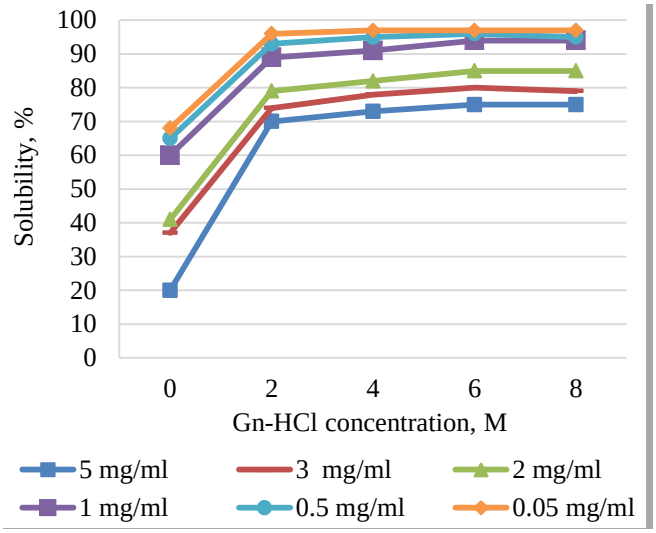


Fig. 10. The effect of Gn-HCl concentration on the solubility of inclusion bodies at different concentrations of inclusion bodies. Protein concentration varied from 0.05 to 5 mg/ml.

Solubilization more than 89% of the total protein was achieved by addition of 2M Gn-HCl to 1 mg/ml of IB. Approximately 70% of the total protein was received in soluble form at solubilization 5 mg/ml of inclusion bodies. Further adding of large amounts of Gn-HCl increased 1–4% more the yield of soluble protein. The maximum soluble protein was achieved at 6M Gn-HCl (from 75% at solubilization of 5 mg/ml protein to 97% at 0.05 mg/ml).

The increasing of Gn-HCl concentration to 8 M or more did not lead to greater yield of soluble lactoferrin. At low concentrations of protein 2 M Gn-HCl was sufficient for maximum efficiency of solubilization. Subsequent increase of Gn-HCl concentration did not lead to a significant increase of protein yield. The optimal mode is solubilization 1 mg/ml of protein by 6 M Gn-HCl, allowing to solubilisate 96% of protein.

Optimization of refolding

Refolding of solubilized protein is initiated by the removal of the denaturing agent.

The following factors: composition of the buffer, protein concentration, temperature, reagents suppressing aggregation and redox conditions were considered for optimizing of refolding.

Effect of pH

Experiments were performed at different pH to create the optimal conditions for refolding (Fig. 11). 0.5 M/L Gn-HCl, 50 mM/L Tris-HCl (pH 8.5), 0.75 M/L arginine, 1 M/L NaCl, 5 mM/L EDTA and 3 mM of glutathione in a ratio of 10 : 1 GSH : GSSG solution was used as standart buffer.

As Fig. 11 shows, at pH below 8 the renaturation was not observed, the pH prevents the formation of disulfide bonds. In more alkaline conditions, the renaturation of lactoferrin is also unfavorable, probably due to the instability of the protein.

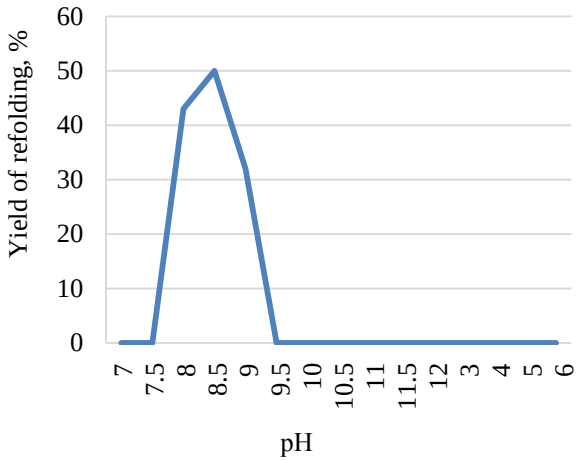


Fig. 11. Influence of pH conditions on lactoferrin renaturation was carried out at 20°C and a total protein concentration of 0.1 mg/ml in standard renaturation buffer with 3 mM/L of glutathione in a ratio of 10 : 1 (GSH : GSSG).

The influence of redox conditions

Since lactoferrin is a protein containing disulfide bonds, the refolding was carried out using redox systems. Adding in a mixture of oxidized and reduced forms of thiol reagent of low molecular weight usually provides the appropriate redox potential, which promotes the formation and rearrangement of disulfide bonds. In our research both oxidized and reduced glutathione (GSH : GSSG) were used.

In contrast to the relatively narrow range of renaturation pH, the constant of lactoferrin renaturation yield was obtained in a wide range of redox conditions (Fig. 12a).

Only strong oxidizing conditions did not lead to the lactoferrin renaturation. The maximum yield of renaturation was received in excess of reduced glutathione (2 : 1), in which case the yield of renaturation was 51%. Only 2 and 6% less the yield of renaturation was obtained at a ratio of 10 : 1 and 1 : 1.

Thus, in the case of lactoferrin, pH and the ratio of GSH : GSSG is critical variable for optimization of renaturation.

The influence of protein concentration and temperature on the lactoferrin renaturation

Protein aggregation is one of the major side reactions at the renaturation high concentrations of protein.

Several strategies, including renaturation at very low protein concentrations, low temperature, and/or addition of aggregation suppressors prevents and reduces aggregation. However, low protein concentrations slow down the process of renaturation, which lead to a decrease of the renaturation yield.

For identify the best conditions for the renaturation, the experiments were carried out in standard solutions of renaturation at temperature in the range of 10–30°C and the lactoferrin concentration 1.0 mg/ml. Severe aggregation was observed at all temperatures with the renaturation 1.0 mg/ml of protein.

At lower concentration of lactoferrin and temperature aggregation was decreased (Fig. 12b). For example, by reducing the temperature from 30 to 10°C the refolding yield was increased from 36 to 57% (on 21%). The best conditions for the renaturation are 10°C and 0.2 mg/ml of lactoferrin.

As the results show, the concentrations used in this refolding were not approached for application. In this regard, more research was investigated for increase of the protein concentration. Denatured protein can be added in pulses mode, avoiding aggregation.

In the experiments used 10 hour pulse system. With each impulse (total 10 pulses) the protein concentration was increased on 0.3 mg/ml to a final concentration of 2.9 mg/ml protein, followed by 48 hour incubation. This approach allowed to increase the concentration of protein to 2.4 mg/ml and to achieve a yield of 55%.

Optimization of solubilization and renaturation conditions to regard to pH, redox conditions, temperature, protein concentration, aggregation suppressors allowed to recover lactoferrin in a concentration of 0.2 mg/ml, resulting the renaturation yield was 58%. The increase of the lactoferrin concentration to 2.9 mg/ml was achieved by a pulsed system. Yield of renaturation was 55%.

The replacement of buffer at 4°C by dialysis of received solution against 5 volumes of buffer (with 0.2 mM of Gn-HCl, 10 mM Na2HPO4/NaH2PO4, 1 mM EDTA) was carried out for further purification.

Purification of lactoferrin

For purification of lactoferrin, mRNA of lactoferrin gene was synthesized so that immediately after the coding sequence was located the plot corresponding hexahistidine sequence (Xho I - gene - His-Tag - Eco I). As a result of induced expression of the cloned gene lactoferrin containing additional hexahistidine sequence in the C-terminal region of the polypeptide chain, allowing subsequent affine cleaning protein on Ni-NTA-agarose, is synthesized.

Purification of lactoferrin was based on affinity chromatography.

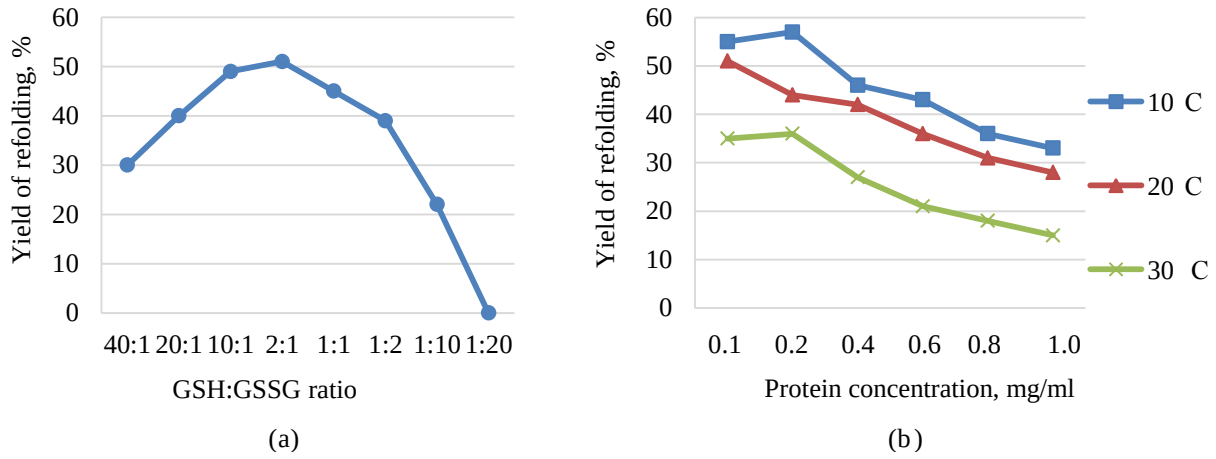


Fig. 12. The effect of redox conditions (a) and temperature and protein concentration (b) on lactoferrin renaturation.

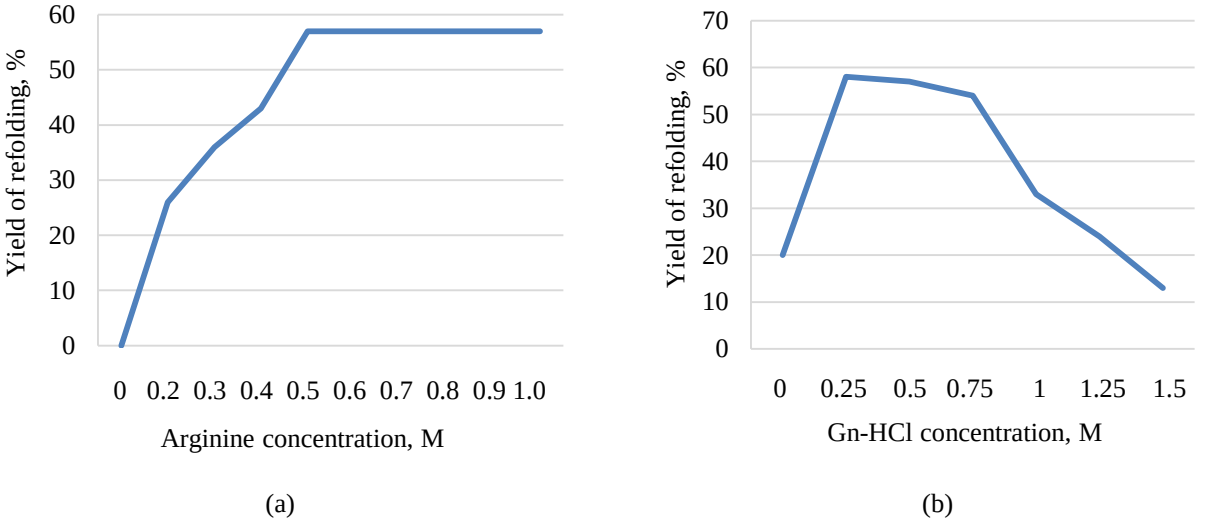


Fig. 13. Effect of arginine concentration (a) and Gn-HCl concentration (b) on the lactoferrin renaturation.

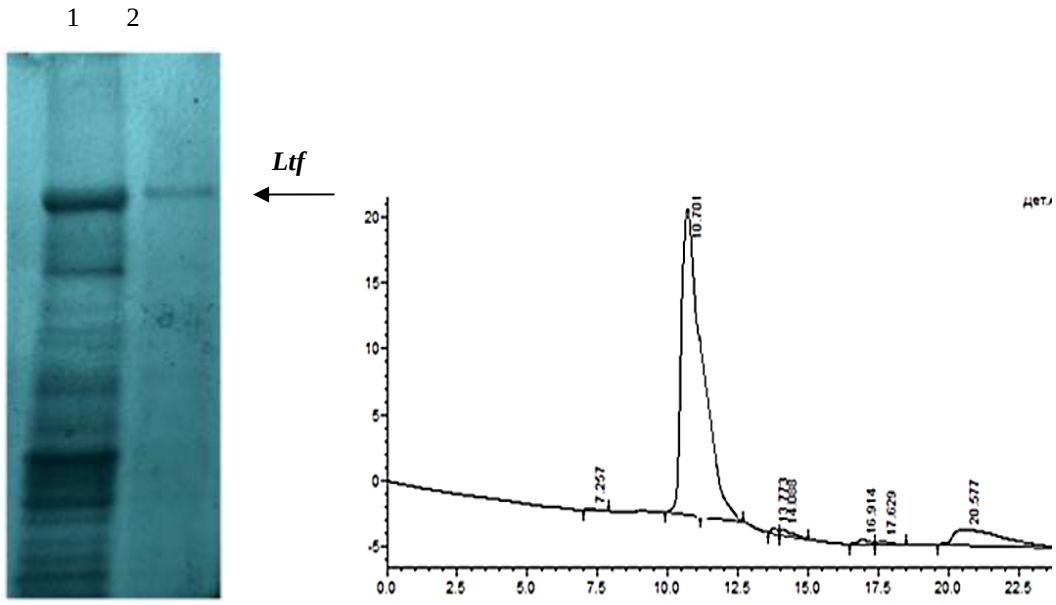
Data analysis of the protein electrophoresis, that is shown in Fig. 14a, presents that the fractions giving the highest efficiency bands near 76–80 kDa are fractions of lactoferrin.

The chromatogram in Fig. 14b shows the results obtained after the purification. The fraction of lactoferrin was from 10 to 12 minutes. The purity of the isolated protein was not less than 90%.

The yields of total protein and lactoferrin at different stages of isolation, solubilization, renaturation and purification were analyzed for determine the effectiveness of optimized methods. The results are presented in Table 4.

The maximum yield of lactoferrin (97.2 mg) was obtained by cultivation of *E. coli* BL21DE3/mpET28a+-*ltf* strain, 58 mg of which was in the form of a dissolved protein and 38.8 in the form of inclusion bodies. Such stages as: washing of inclusion bodies, renaturation and solubilization was required for 38.8 mg of protein. Total of 11 mg of lactoferrin from inclusion bodies, representing 11% of the primary was obtained after washing of inclusion bodies, renaturation, solubilization and purification. Most of the protein was lost at the stage of renaturation, which is mainly connected with the formation of insoluble aggregates.

Thus, 61 mg of lactoferrin, 11 mg of which was obtained during the renaturation of solubilised inclusion bodies, and 50 mg in the form of dissolved protein was obtained at full processing of biomass obtained from 1 L.



(a) (b)

Fig. 14. Purification of lactoferrin received from *E. coli* BL21DE3 / mpET28a + cells: (a) electrophoregram: 1 – the culture medium after sonication; 2 – purified lactoferrin obtained after optimization of purification conditions; (b) the chromatogram of the purified lactoferrin.

Table 4. The parameters of the lactoferrin production with optimised methods of solubilization, renaturation and purification

Stage	$M_{protein}$, mg	M_{lf}, mg		Yield of LF, %	
		S	IB	S	IB
Biomass after separation	270	102		100	
After sonication	230	97.2		96	
Separated fractions	116	58	39	56	38
Washing of inclusion bodies	116	–	38	–	36
Solubilization	113	–	35	–	34
Renaturation	72	–	19	–	18
Purification	63	50	11	49	11
Total yield of IB and S	63	61		60	

Discussion

Thus, the cloning of *ltf* gene encoding the human lactoferrin was described in this paper. Evidence of the created recombinant construct functionality was submitted. Ability to produce lactoferrin at level of lactoferrin synthesis – 30–35% of the total protein content was shown. The maximum yield of the target protein (including in soluble form) was achieved by culturing of the recombinant strain at 25°C, induction by 1.1 mM concentration of IPTG and 8-h period after induction. Optimization of solubilization and renaturation conditions to regard to pH, redox conditions, protein concentration, reagents suppressing aggregation and temperature allowed once to recover lactoferrin in a concentration of 2.9 mg/ml by the pulsed system, the income of which renaturation was 55%.The protein of high purity at least 90% was obtained by means of affinity chromatography. 61 mg of lactoferrin, 11 mg of which was obtained during the renaturation of solubilised inclusion bodies, and 50 mg in the form of dissolved protein was obtained at full processing of biomass obtained from 1 L.

ACKNOWLEDGMENT

The authors are grateful to the company "Evrogen" (Moscow, Russia) for providing mpET28a+ plasmid, containing *ltf* gene in its composition. We also gratefully acknowledge the Ministry of Science and Education of the Russian Federation for funding of this project within the Fellowship of the President of the Russian Federation program.

List of abbreviations

LTF – lactoferrin hltf- human lactoferrin; *rhlf* – recombinant human lactoferrin; IPTG – isopropyl-β-thiogalactopyranoside; SDS – sodium dodecyl sulphate; IB – inclusion bodies; SDS-PAGE – polyacrylamide gel electrophoresis; EDTA – ethylene-diaminetetraacetic acid; PBS – Phosphate buffered saline.

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Please cite this article in press as: Novoselova M.V. and Prosekov A.Yu. Technological options for the production of lactoferrin. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 90–101. doi: 10.21179/2308-4057-2016-1-90-101.

PROCESSES, EQUIPMENT, AND APPARATUS FOR FOOD PRODUCTION

THEORETICAL BACKGROUNDS FOR ENHANCEMENT OF DRY MILK DISSOLUTION PROCESS: MATHEMATICAL MODELING OF THE SYSTEM "SOLID PARTICLES – LIQUID"

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Received November 26, 2015; Accepted in revised form February 18, 2016; Published June 27, 2016

Abstract: The mathematical model of immersion of insoluble spherical particle with smooth surface under absolute statics (incl. assumption – its spontaneous formation on the surface) at the particle density ranging from 1.05 to 1.75 kg/m^3 and contact angle of moistening from 0º to 180º was created for development of theoretical and practical backgrounds of the reconstitution process. This model was used as the base of model of immersion in water and drowning of cubic grid of spherical insoluble particles under full static condition. Regularities of layers' drawning were established and an algorithm for calculating the missing force for full grid immersion was developed. It is possible to determine the coefficient of correlation between the calculated and actual data, taking into account the heat and mass transfer processes occurring during the dissolution of the dry products that will bring model to real systems and, in such a way, unify the process.

Keywords: reconstitution, mathematical model, dry milk products, a particle, the particle frame

DOI 10.21179/2308-4057-2016-1-102-109 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 102–109.

INTRODUCTION

It is known that basic volumes of dry milk products (DMP) processing in different branches of the industry suppose the availability of the tentative process of their solution in water. Practical results show that technical-technological presentation of the mentioned process stipulates to a great extent the qualitative characteristics and quantitative yield of the product, efficiency of the technological equipment operation and impacts indirectly the enterprise working routine [1].

A wide assortment and heterogenous nature of DMP raw material, disparity in operating principles of the industrial equipment as well as undeveloped methodological base of evaluative criteria of the process efficiency make it impossible to find dependence by generalization of production and experimental data. Accordingly the actual task is substantiation of the rational parameters of reconstitution technology from the point of the process simulation with the model complication from monoparticle immersion with variable form, density range and wetting contact angle in static conditions up to real polycomponent system in dynamics.

METHODOLOGY OF INVESTIGATION

The object of investigation – the mathematical model of immersion of insoluble spherical particle with smooth surface under absolute statics (incl. assumption – its spontaneous formation on the surface) at the particle density from 1050 to 1750 kg/m^3 and contact angle of moistening from 0°C to 180°C. The liquid with physical-chemical parameters of distilled water [2, 3] was used as solvent component of the system. The model should be able to determine the particle optimal minimal weight under the condition of its sphericity, required for overcoming surface tension. It is necessary as well to set up the rate of immersion after its sinking if: the particle is insoluble, the pass length is endless. Thus interaction of the particle with the liquid is considered in two variants: solid – liquid – gas (S/L/G) if the contact is running on the liquid surface and liquid – solid (L/S) when the particle is completely immersed. It is evident that force ratio influencing the particle is not always constant and either sets up the balance in the frame of the defined variant or determines its transition from one to another.

Methods of investigation – adaptive integration of hydrostatics and hydrodynamics classical results into applied fields of technology.

1. Critical Parameters of the statistic systems for immersion

The scheme of balanced interaction at S-L-G three-phase contact is presented at Fig. 1 (projection at Oxz). Fig. 2 shows visualization of wetting angle virtual configuration. The balance supposes balancing of all forces effecting the particle: Buyancy forces, surface tension forces and gravity forces.

We have the following system parameters: R – particle radius, m; ρ – particle density, kg/m^3 (values range 1100–1700); θ – angle of wetting, ^{o} (values range – 0–180); ϑ – angle of immersion, ^{o} (values range considering density of the studied particle 0–90), the particle embedding occurs at $\vartheta = 0$ (considering casual fluctuations near water surface the critical values for spontaneous embedding $\vartheta_c = 5^o$ are accepted); ρ_0 – liquid density (water), kg/m^3 (assumed equal to 1000; σ – surface tension, H/m (assumed equal to 72,86 · 10^{-3}); g – acceleration of gravity, m/s^2 (assumed equal to 9.8) [1, 4–8].

Introduce the symbol (difference between angle of wetting and angle of immersion is the basic parameter influencing the system balance, see then):

$$\alpha = \theta - \vartheta. \quad (1)$$

1.1. Surface tension force

Definition 1. Limiting wetting angle θ – the angle at the point of three-phase contact between tangents to particle surface and to liquid surface.

Surface tension force forms the angle at contact point with tangent to the particle equal to wetting angle. Modulus of vertical projection at surface tension axis Oz equals to:

$$\sigma_z = \sigma \cos\left(\frac{\pi}{2} - \theta + \vartheta\right) = \sigma \sin\alpha\,. \quad (2)$$

Calculation of surface tension force is carried out by the length of three-phase interface. In this case it is radius circumference:

$$r = R \sin\vartheta. \quad (3)$$

Accordingly circumference is:

$$l = 2\pi R \sin\vartheta. \quad (4)$$

The resultant value of Oz force component is:

$$F_\sigma = 2\pi R\sigma \cdot \sin\vartheta \cdot \sin\alpha. \quad (5)$$

1.2. Radius of liquid curvature

Imagine that uncurved liquid surface contacted the particle at L point (Fig. 3) and rose to point A over particle surface by R radius, ϑ_o – angle between radiuses scored to points L and A.

Draw perpendicular through point A to liquid surface up to point B and then the height capillary rise equals the length of AB segment.

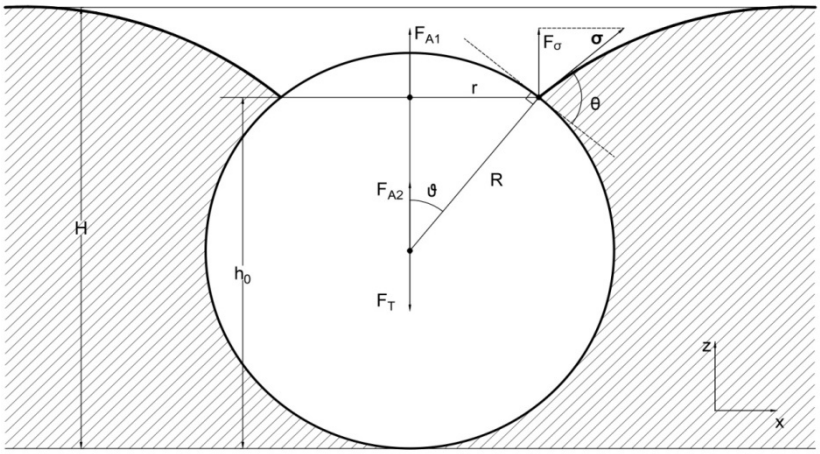
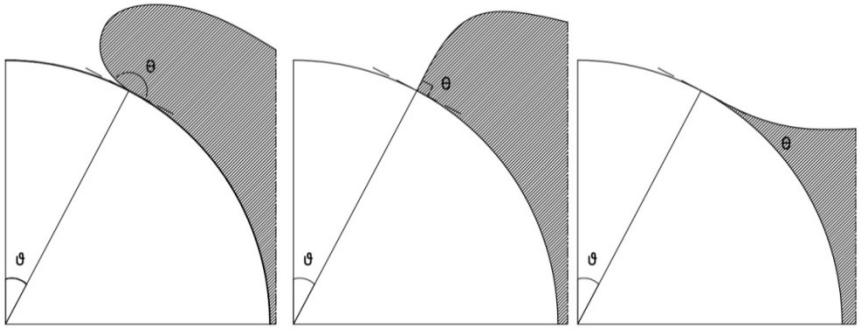


Fig. 1. Immersion of the particle into liquid.



(a) (b) (c)

Fig. 2. Visualization of wetting angle configuration: (a) $\theta = 180^o$, (b) $\theta = 90^o$, (c) $\theta = 0^o$.

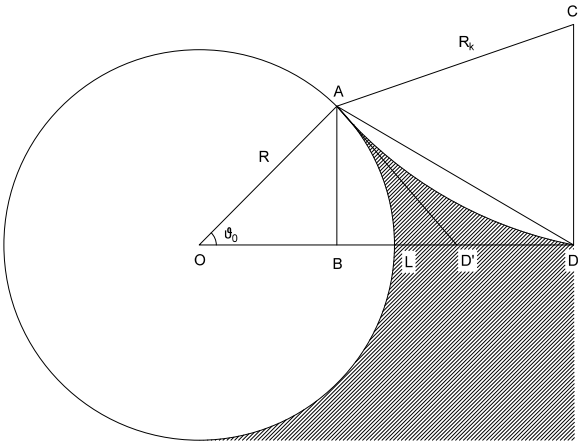


Fig. 3. The particle wetted by liquid.

The sizes of the studied particles are small then the amounts of clearances are relatively not big. The profile of curved liquid surface is spherical as the impact of gravity forces on it is practically low. Thus meniscus profile represents the circle touching the particle at contact point A and touching the surface of uncurved liquid at point D. So the circle lies at two tangents AD' and DD'. In triangle OAD' the angle OAD' equals the sum of the right angle and wetting angle.

$$OAD' = \frac{\pi}{2} + \theta, \quad (6)$$

$$AD'O = \frac{\pi}{2} - \alpha. \quad (7)$$

Thus the angle between tangents equals

$$AD'D = \alpha. \quad (8)$$

To calculate the radius of curvature examine triangle AOD'. According to theorem of sines:

$$\frac{R}{\cos\alpha} = \frac{AD}{\sin\vartheta_0}. \quad (9)$$

Finally, the radius of curvature can be found from right triangle AD'C where angle AD'C equals α/2

$$AD' = \frac{\sin\vartheta_0}{\cos\alpha}\tan\frac{\alpha}{2}R. \quad (10)$$

Assuming the liquid radius of curvature we can calculate h=|H-h_o| at which the liquid rises [9]:

$$h = |2\sqrt{\frac{\sigma}{\rho_0 g}}\sin\frac{\vartheta}{2}|. \quad (11)$$

1.3. Buoyancy force

Buoyance force is up-directed endwise O_z and is divided into two parts: F_{A1} – the force acting on the wetted but not immersed below the level of uncurved liquid surface and F_{A2} – the force acting on the immersed part of the particle.

The first component. If the immersed segment of the particle was replaced by liquid hydrostatic pressure would be equal to the weight of liquid column with by volume.

We have correlation for force:

$$F_{A1} = 2\pi r^2\sqrt{\rho_0 g\sigma}\sin\frac{\vartheta}{2}. \quad (12)$$

The second component of buoyancy force acts at the sphere segment with height

$$h_0 = R(1+\cos\vartheta). \quad (13)$$

Accordingly the segment volume equals to:

$$V = \int_0^{R\cos\vartheta}\pi\,(R^2 - x^2)\,dx + \\ + \int_0^R \pi\,(R^2 - x^2)\,dx = \frac{1}{3}\pi R^3(2-\cos\vartheta)(\cos\vartheta+1)^2. \quad (14)$$

As a result we have the equation for the second component of buoyancy force:

$$F_{A2} = \frac{1}{3}\pi R^3(2-\cos\vartheta)(\cos\vartheta+1)^2\cdot\rho_0\cdot g. \quad (15)$$

1.4. Gravity force

Gravity force is down-directed along axis O_z and equals to:

$$F_T = \frac{4}{3}\pi R^3\rho g. \quad (16)$$

1.5. Balance of forces

Buoyancy force and projection of surface tension force to axis O_z are directed upwards, gravity force is directed downwards. Considering that note the balance equation where the particle will be in equilibrium state in liquid:

$$F_\sigma + F_{A1} + F_{A2} - F_T = 0. \quad (17)$$

Fill incoming forces – equations (5), (11), (15), (16)

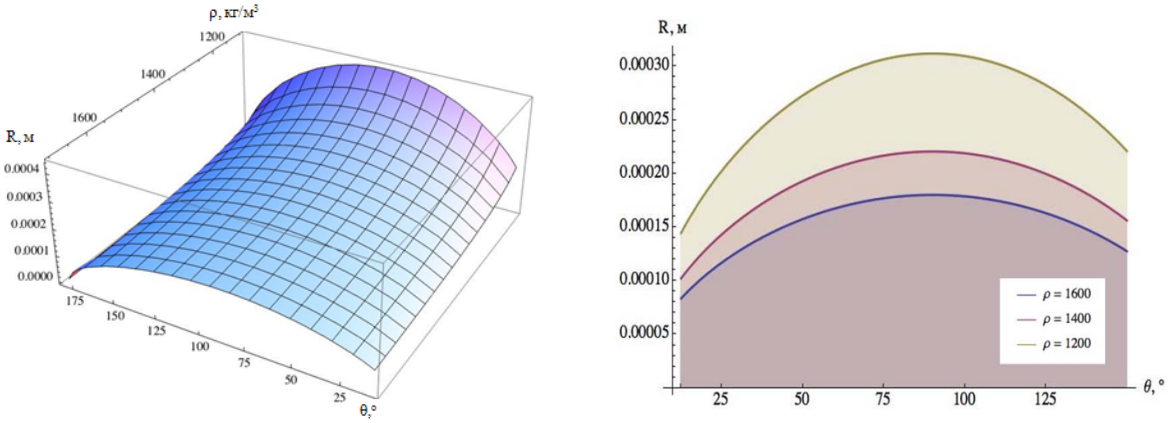
$$[(2-\cos\vartheta)(\cos\vartheta+1)^2\rho_0 g - 4\rho g]R^2 + \\ + 6\sqrt{\rho_0 g\sigma}\sin^2\vartheta\,\sin\frac{\vartheta}{2}R + 6\sigma\sin\vartheta\sin\alpha = 0. \quad (18)$$

It is possible to specify for this system the particle radius required for determination balance of forces with angle of immersion. In this case spontaneous particle immersion at low values of (0–5°) occurs.

Dependence of R(p) is presented at Fig. 4 and 5. Minimal size of the particle required for spontaneous immersion equals to 0.05 mm for ϑ =5 (wetting angle ϑ = 5°, density – 1700 kg/m^3) and 0.15 mm for ϑ = 0.1° (wetting angle ϑ = 5°, density – 1700 kg/m^3). At $\alpha \geq 0$ the equation has the solution as:

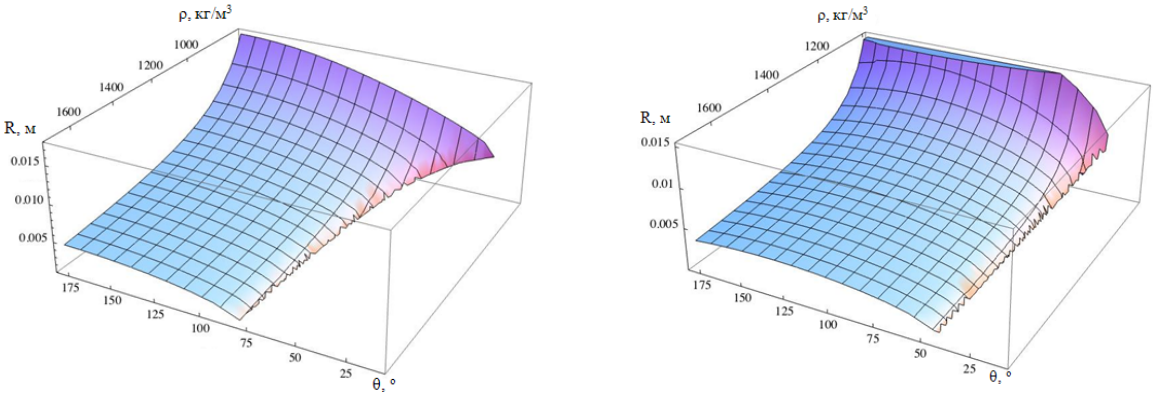
$$\rho_0 < \rho$$
$$(2 - \cos\vartheta)(1 + \cos\vartheta)^2 < 4$$

At $\alpha < 0$ two variants are possible: absence or presence of strictly one value of radius for each specific angle of immersion in the concerned range of the system parameters (ϑ and p). The example is shown at Fig. 6 – the particle location with p = 1400 kg/m^3 and value ϑ = 50° is not stabilized at the angle ϑ = 90° (a) under no radius, but is stabilized at ϑ = 45° (b).



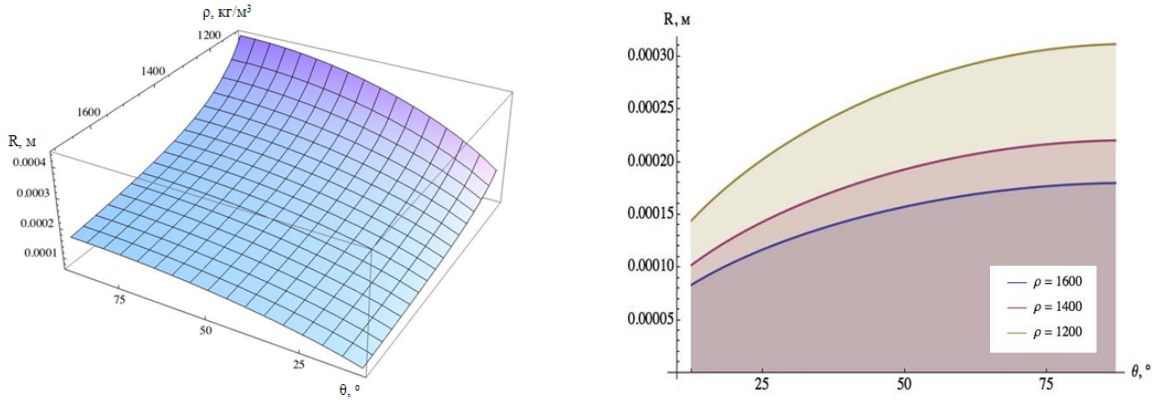
(a) (b)

Fig. 4. R (ϑ, p) – minimum radius at ϑ= 5° (a) with detailing at fixed values of density (b).



(a) (b)

Fig. 5. R (ϑ, p) – minimum radius at ϑ= 0.1° with detailing at fixed values of density.



(a) (b)

Fig. 6. R (ϑ,p) – minimum radius at ϑ= 90° (a) and ϑ = 45° (b).

2. Fluid motion

2.1. Behavior after sinking

After breakage of three-phase contact limit (at the excepted terms, with obtaining of critical angle of immersion ϑ) Buoyancy force working on the particle may be presented as the unified value. Therefore force F_α promoting the particle sinking (down-directed) is the difference of two forces:

$$F_a = \frac{4}{3}\pi g R^3 \cdot (\rho - \rho_0). \quad (19)$$

As the particle is assumed as spherical and has small size Stokes low is accepted. Force F_d of water head resistance according to this law equals to:

$$F_d = 6\pi\nu v R, \quad (20)$$

where ν is the liquid dynamic velocity, Па ·с, v is the particle velocity, m/s. Then the second Newton's law is acceptable for determination of embedding velocity:

$$F_a - F_d = \rho g a, \quad (21)$$

where α is the particle acceleration, m/s^2 .

We have Cauchy problem relatively to the particle velocity v (at zero time moment velocity is supposed to be zero):

$$\begin{cases} \rho g \cdot \dot{v} + 6\pi\nu R \cdot v - \frac{4}{3}\pi g R^3 \cdot (\rho - \rho_0) = 0 \\ v(0) = 0 \end{cases} \quad (22)$$

The following function will be the solution:

$$v(t) = \frac{2}{9}\frac{gR^2(\rho-\rho_0)}{\nu}\left(1 - \exp\left\{-\frac{6\pi\nu R}{\rho g}t\right\}\right). \quad (23)$$

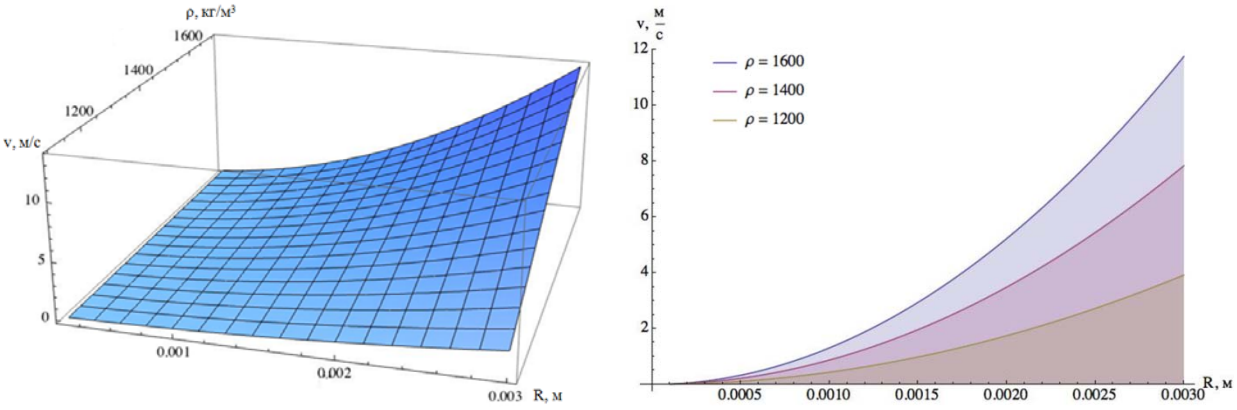
Accepting the value of viscosity dynamic coefficient equals to 0.001 Па · с (at 20°C) we obtain the dependence of the fixed velocity and the particle density specified at Fig. 7.

2.2. Liquid motion in clearance

In this paragraph we present the theoretical base of capillary impregnation influencing sinking of powder milk products located on the liquid surface. The process of capillary impregnation in the simplest form represents filling of clearance between the particles. The liquid starts motion in the formed clearance due to pressure of the twisted liquid surface. We shall examine the liquid motion between adjacent particles.

As pressure at the upper mark of the lattice from spheres is permanent we can suppose that pressure change on the lattice height will be only the function of the liquid fluid velocity along this lattice (Fig. 8).

$$\frac{dP}{dz} = -f(u). \quad (24)$$



(a) (b)

Fig. 7. v (R,p) – the particle fixed velocity after balance of forces subject to radius (a) with detailing at the fixed values of density (b).

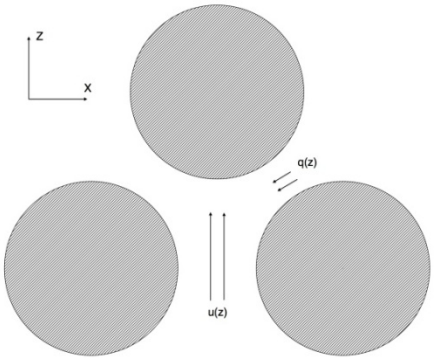


Fig. 8. Liquid motion in the clearance u(z) – motion velocity of the liquid along the lattice from spheres, m/s; dq(z)-liquid inflow from adjacent cells, m^3 /s.

With regard to geometrical shape it is advisable to calculate in co-ordinate cylindrical system.

For a start Gashen- Puazel formula can be used for viscous liquid flowing along the lattice body:

$$f(u) = \frac{8\mu u}{r_\varphi^{\,2}}, \qquad (25)$$

where μ is the dynamic coefficient of viscosity, Pa s; r_φ is the radius of lattice elementary tube, m.

The known Dupui formula can be used for determination of liquid amount entering at the dz height:

$$dq(z) = \frac{2\pi k_1 (P_z - P(z))dz}{\mu \ln\left(\frac{R}{r_\varphi}\right)}, \qquad (26)$$

where P_3 is the external pressure on the net, Pa; R is the radius of zone of liquid accumulation, m.

The liquid flowing from z mark will be:

$$dq = \pi r_\varphi^{\,2} u(z). \qquad (27)$$

Thus liquid velocity at z+dz mark will be:

$$u(z+dz) = \frac{\pi r_\varphi^2 u(z) + dq(z)}{\pi r_\varphi^2} = u(z) + \frac{2k_1 (P_z - P(z))dz}{\mu r_\varphi^2 \ln(\frac{R}{r_\varphi})}. \qquad (28)$$

Consequently, according to formula (26) we have the chance to forecast liquid velocity at every height

$$\frac{dP(z+dz)}{dz} = -f(u(z+dz)). \qquad (29)$$

Then taking into account the previous value change at section dz will be:

$$\frac{dP(z+dz)}{dz} - \frac{dP(z)}{dz} = -(f(u(z)) - f(u(z+dz)), \qquad (30)$$

$$\frac{d^2 P(z)}{dz^2} = -\frac{2k_1 (P_3 - P(z))dz}{\mu r_\varphi^2 \ln(\frac{R}{r_\varphi})} f'(u(z)). \qquad (31)$$

In equation (31) two functions P(z) and u(z) are unknown. According to (27) we can find u(z) using Lagrange theorems and values dz = 0:

$$u(z) = \frac{dq}{\pi r_\phi^2} = \frac{2k_1 (P_3 - P(z))dz}{r_\phi^2 \mu \ln\left(\frac{R}{r_\phi}\right)} = \frac{2k_1}{r_\phi^2 \mu \ln\left(\frac{R}{r_\phi}\right)} \int_0^z (P_3 - P(z))dz. \qquad (32)$$

Edge conditions for u(z) function are calculated by means of clearance curvature radius.

3. Build-in of the particle lattices

We shall examine the conditional cubic lattice broken to squares. Spherical particles of identical radius are located in lattice sites and at that the particle layers are located in staggered order (Fig. 9). The lattice is immersed into liquid with water characteristics transversely to the surface.

We shall examine the model in which M kg of dry milk product is filled into reservoir with water with surface area S m^2. It is required to calculate solution rate of the whole volume of dry milk product with the given impact force Fv and known parameters of particles R, p, θ, u.

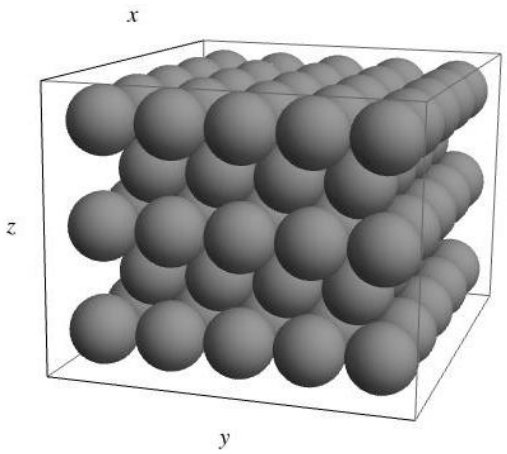
We shall modify balance of forces for immersion of one particle described subject to radius of the liquid curvature between particles and capillary impregnation process.

$$F_\sigma + F_{A1} + F_{A2} - F_T + F_v = ma,$$
$$F_\sigma = 2\pi R\sigma \cdot \sin\vartheta \cdot \sin\alpha,$$
$$F_{A1} = 2\pi r^2 \sqrt{\rho_0 g\sigma} \sin\frac{\vartheta}{2}, \qquad (33)$$
$$F_{A2} = \frac{1}{3}\pi R^3 (2 - \cos\vartheta)(\cos\vartheta + 1)^2 \cdot \rho_0 \cdot g,$$
$$F_T = \frac{4}{3}\pi R^3 \rho g N.$$

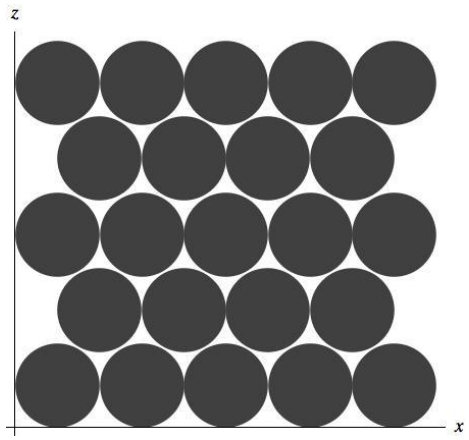
Solving the obtained differential equation (7)

$$F(\vartheta(t)) = m\ddot{\vartheta}(t). \qquad (34)$$

Relatively to angle of immersion one can calculate the time required for immersion of one layer – obtaining (t) = 180°C.



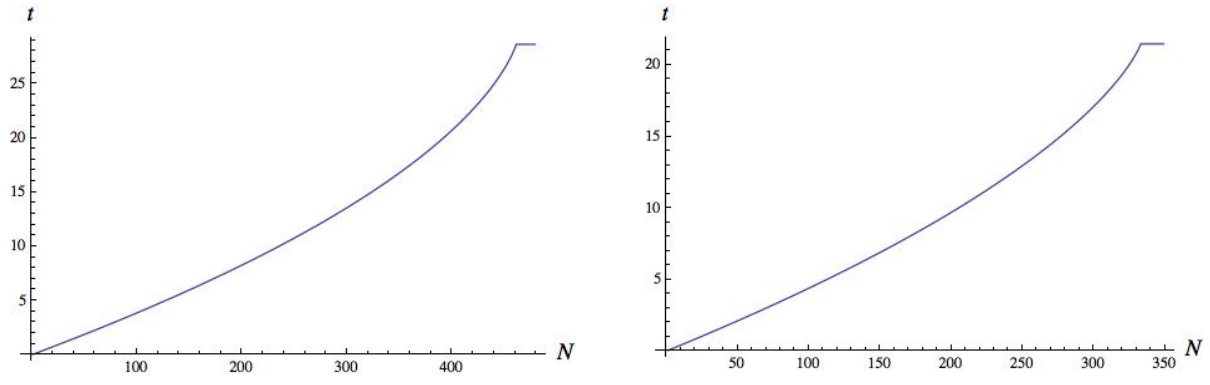
(a)



(b)

Fig. 9. Packing of the lattice from particles (a) and profile on surface Oxz (b).

Let us examine two DMP – dry whole milk (p = 1320 kg/m^3 and dry skim milk (p = 1510 kg/m^3). The sizes of these products are in the range of 0.05….0.25 mm; for manufacture of 1 t of the product 125 kg and 90 kg, respectively are required. Theoretical rates of solution in 1 m diameter tank is presented at Fig. 10. According to the drawing the layer portion will be remained on the liquid surface without application of force. Fig. 11 shows dry skimmed milk solution under secondary force action.



(a) (b)

Fig. 10. Period of dry milk product immersion (a) Dry whole milk (DWM), (b) Dry skim milk (DSM).

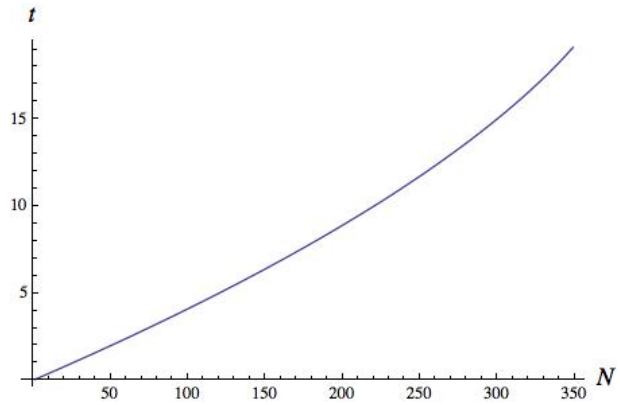


Fig. 11. Dry skim milk (DSM) solution under 4053 Pa pressure.

RESULTS

Simulated model of immersion in water and drowning of cubic grid of spherical insoluble particles under full static condition. Established regularities of layers' drawning and developed an algorithm for calculating the missing force for full grid immersion. In the future, it is possible through pilot studies to determine the coefficient of correlation between the calculated and actual data, taking into account the heat and mass transfer processes occurring during the dissolution of the dry products that will bring model to real systems and, in such a way, unify the process.

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Please cite this article in press as: Galstyan A.G., Petrov A.N., and Semipyatniy V.K. Theoretical backgrounds for enhancement of dry milk dissolution process: mathematical modeling of the system “Solid particles – liquid”. *Foods and Raw Materials,* 2016, vol. 4, no. 1, pp. 102–109. doi: 10.21179/2308-4057-2016-1-102-109.

ENERGY EFFICIENCY ANALYSIS OF THE SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*) FRUITS QUICK FREEZING

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Received March 28, 2016; Accepted in revised form May 18, 2016; Published June 27, 2016

Abstract: Low temperature preservation of perishable produce is a widespread technology of it's long-term conservation, at the same time freezing of foods being an extremely power-consuming process. When developing low temperature preservation technology it is important to aim both at retaining high quality of frozen food and improving the energy efficiency of the processes. This article presents the results of research that explores the energy efficiency when freezing the sea buckthorn (*Hippophae rhamnoides*) fruits in a fluidized bed quick freezer. The study offers a method to calculate an amount of energy consumed in the freezing process of the sea buckthorn cultivars in an air-blast quick freezer. It also outlines geometrical and mass parameters of the cultivars. The study simultaneously demonstrates an air flow rates calculation for the sea buckthorn fruits fluidization to occur; defines the air circulation energy cost at a velocity necessary for fluidization to be accomplished at different air temperatures, and calculates the energy consumption to produce an artificial cold to ensure the required temperatures of heat transferring air. Further, the article conducts an analysis of the overall interconnected factors that impact the berries freezing energy consumption. Based on data obtained through research the authors reveal the energy efficient regimes of the sea buckthorn fruits low temperature treatment in an air-blast quick freezer; types of refrigerating machines and refrigerant that would ensure the less power consuming quick freezing of the sea buckthorn. The research used the species grown in Kemerovo region.

Keywords: Sea buckthorn, quick freezing, freezing technologies energy efficiency

DOI 10.21179/2308-4057-2016-1-110-120 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 110–120.

INTRODUCTION

Nowadays, vitamin and mineral deficiency has become one of the most common features in the modern individual diet [1]. Fruits and berries are the natural source of biologically active substances. Entering the human body the substances reveal their physiologically active properties. They have a substantial effect on metabolism and organism vital activity [2]. The sea buckthorn is known to be one of the most valuable vitamin rich plants in the flora of Russia. The sea buckthorn fruit accumulates a significant amount (up to 350 mg%) of carotenoids, up to 450 mg% of ascorbic acid, up to 165 mg% of vitamin E, up to 0.8 mg% of folic acid, and also vitamins B, F, and P. It contains up to 3.5% of sugars and 3.2% of organic acids, oils, flavones, sterols, minerals of iron, boron, manganese, etc. [3].

Freezing is considered to be one of the most efficient food preservation methods. Frozen fruits and berries can be stored for many months as the moisture transfers into a solid state. Low temperatures and crystallization of moisture contained in fruits and berries create unfavorable conditions for the biochemical processes to occur, and speed of the process decreases [4].

Formation of ice crystals accompanies the freezing of product that contains moisture and destroys the object's structure. Both mechanical and osmotic factors lead to the destruction. In the process of freezing, crystals form outside cells, increase in size, and thus, deform and rupture cell membranes. Besides, growth of ice crystals found in intercellular space causes cell moisture to diffuse though membrane, and cells dehydrate.

The intensity and nature of changes occurring in the product treated by the low temperature depend on the conditions and parameters of the process, and the quality characteristics of the object being treated. The intensification of heat removal increases the amount of crystallization clusters, which encourage the formation of microcrystalline structure. Moreover, the higher the intensiveness of heat transfer is, the smaller crystals in the frozen product are [5]. The conditions provided, crystalline structure will be more homogenous and ice crystals will form both inside intercellular space and inside the cells.

An increase in the quantity of heat removed during fruit freezing can be achieved either through temperature decrease in heat transfer medium or acceleration of its

circulation. The first scenario is of an extensive nature and can be attained at the expense of temperature difference increase between object treated by low temperature and heat removal medium. In the second case, a change in the quantity of heat removed will be of an intensive nature and accomplished at the expense of heat transfer coefficient increase between refrigerated object and refrigerating medium [6].

Intensification of heat transfer during fruit and berry freezing causes growth in energy use in both scenarios. The real processes use the combined effect of the two above listed factors to intensify heat removal during fruits and berries freezing.

Energy component in the cost of final product prepared from fresh-frozen fruits and berries plays an important role in the development of low temperature processing technology. Hence, the relation between temperature and convection factors becomes an essential element in optimization of energy consumption during low temperature treatment of fruits and berries.

The objective of this paper is to determine the low temperature treatment regimes that would minimize the amount of energy used in production of the quick frozen sea buckthorn fruits.

OBJECTS AND METHODS OF STUDY

The sea buckthorn fruits were fast frozen in a quick freezer, which is a mechanism providing high air circulation velocity and temperatures required for the low temperature treatment processes.

Fruits mass and volumetric characteristics are necessary to determine parameters of air flow in the freezer. Characteristics of fruits used in research are shown in Table 1 for each cultivar.

Critical air flow velocity w'_{cr} (m/s) characterizes the onset of fluidization process [7].

$$w'_{cr} = \frac{\nu_{air}}{d_{eq}} \times \frac{Ar}{1400 + 5.22\sqrt{Ar}}, \quad (1)$$

where d_{eq} is the product's equivalent diameter (m); ν_v is the kinematic viscosity of air (m^2/s); Ar is the Arckimedes number.

$$Ar = \frac{g \cdot d_{eq} \cdot \rho_{pr}}{\nu_{air}^2 \cdot \rho_{air}}, \quad (2)$$

where g is the gravitational acceleration (m/s^2); ρ_{pr}, ρ_{air} are the product and air densities respectively (kg/m^3).

Critical air velocity w''_{cr} (m/s) characterizes the velocity of air medium when fruits can be blown away [7].

$$w''_{cr} = \frac{\nu_{air}}{d_{eq}} \times \frac{Ar}{18 + 0.6 \cdot \sqrt{Ar}}. \quad (3)$$

The sea buckthorn fruits freezing time was calculated from the equation of Plank [8]:

$$\tau_f = \frac{q_f \cdot \rho_{pr}}{t_{cryo} - t_{air}} \times \frac{d_{eq}}{6} \left(\frac{d_{eq}}{4\lambda_f} + \frac{1}{\alpha} \right), \quad (4)$$

where α is the heat transfer coefficient ($W/(m^2\ K)$); λ_f is the heat transfer value of the frozen fruits portion ($W/(m\ K)$); ρ_{pr} is the frozen sea buckthorn fruits density (kg/m^3); q_f is the heat of solidification (J/kg); t_{cryo} is the sea buckthorn fruits cryoscopic temperature (°C); t_{air} is the air temperature (°C).

Heat removal is determined by the air condition and air flow velocity depending on the product's geometrical parameters.

Heat transfer coefficient was calculated according to the formula [9]:

$$\alpha = Nu \cdot \lambda_{air} / d_{eq}, \quad (5)$$

where λ_{air} is the air thermal conductivity; Nu is the Nusselt number.

During the fluidization we use the following empirical equation to determine Nusselt number [10]:

$$Nu = 0.03 \cdot Pr^{1/3} \cdot Re, \quad (6)$$

where $Pr = c_{air} \cdot \mu_{air} / \lambda_{air}$ is the Prandtl number; μ_{air}, c_{air} are the air dynamic viscosity and specific heat respectively; $Re = \omega \cdot d_{eq} \cdot \rho_{air} / \mu_{air}$ is the Reynolds number; ω, ρ_{air} are the air velocity and air density respectively.

The air mass m_{air} (kg) and air volume V_{air} (m^3) required to freeze 1kg of the sea buckthorn fruits are determined from the formulas:

$$m_{air} = \frac{\Delta h}{c_{air} \cdot \Delta t_{air}}, \quad (7)$$

where Δh is the difference between the fruits enthalpies before and after freezing (J);

$$V_{air} = m_{air} / \rho_{air}. \quad (8)$$

Quantity of heat removed from fruits Δh equals the quantity of heat transferred to air Δh_a in refrigerated volume.

Air heating Δt_{air} during the sea buckthorn fruits freezing determined according to the formula:

$$\Delta t_{air} = \alpha \cdot F_{pr} \cdot \Delta t_t, \quad (9)$$

where F_{pr} is the sea buckthorn fruits surface area (m^2); Δt_t is the logarithmic mean difference between air temperature and the temperature of the sea buckthorn fruits being frozen (°C):

$$\Delta t_t = \frac{t_{air2} - t_{air1}}{\ln \frac{t_{cryo} - t_{air1}}{t_{cryo} - t_{air2}}}, \quad (10)$$

here t_{air1} is the initial air temperature; t_{air2} is the final air temperature, $\Delta t_{air} = t_{air2} - t_{air1}$.

The following formula determines consumption of energy (L_a, J) necessary to reach the required air circulation velocity:

$$L_a = V_{air} \cdot \Delta P / \eta_f, \quad (11)$$

where η_f is the fans performance factor; ΔP is the aerodynamic resistance of quick freezer (Pa) [7].

$$\Delta P_f = 1.67 \left(Re \frac{H_f}{d_{eq}} \right)^{0.2} \times \frac{G_{pr}}{F_{pr}}, \quad (12)$$

where H_f is the fluidized bed depth (m); G_{pr} is the

mass; F_{pr} is the fluidized bed depth flow area of the product (m^2).

$$H_f = H_0\left(\frac{1-\varepsilon_0}{1-\varepsilon}\right), \quad (13)$$

where H_0, ε_0 is the depth and porosity of the bed; $\varepsilon = \left(\frac{18\,\mathrm{Re} + 0.36\,\mathrm{Re}^2}{Ar}\right)^{0.21}$ is the porosity of the fluidized bed.

The aerodynamic resistance of the tray (ΔP_g):

$$\Delta P_g = 13.72 \cdot w^2 - 43.12 \cdot w + 119.36, \quad (14)$$

where w is the air flow velocity.

Aerodynamic resistance of air cooling agents (ΔP_{ac}) was calculated according to the formula:

$$\Delta P_{ac} = 1.35 \cdot A \cdot \mathrm{Re}^{-0.24} \rho_{air} \cdot w^2, \quad (15)$$

where A is the coefficient to account for the form factors.

Aerodynamic resistance to air flow in the circuit of quick freezer:

$$\Delta P = (\Delta P_f + \Delta P_g + \Delta P_{ac})\alpha, \quad (16)$$

where $\alpha = 1.1$ is the air friction coefficient.

Working substance of the refrigerating machine removes heat from the low temperature treated object and transfers it towards the surrounding environment. This substance largely determines refrigerating system efficiency [11].

The research studied the efficiency of artificial cold production in one-, two-stage, and cascade refrigerating machines, their operation based on Freons R-134a, R-22, R-404a, R-23, R-717. Energy consumption was measured in accordance with the work [12].

RESULTS AND DISCUSSION

Based on the experimental data shown in Table 1 and formulas 1÷3 we obtained values of critical velocities of the studied fruits. Fig. 1 displays the results of the calculations.

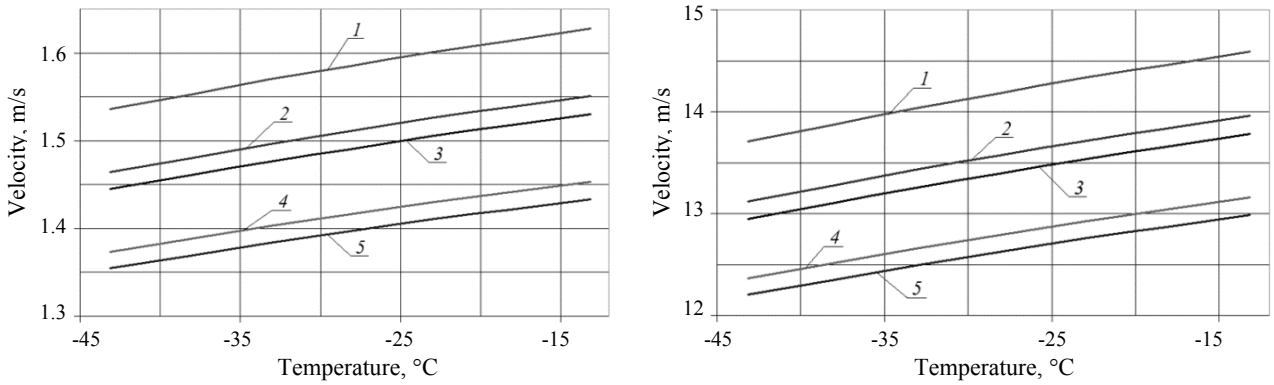
Drawing from the calculations, velocities that allow stable fluidization process to occur, range from 1.8 to 12 m/s. The velocity regime holds for the air temperature -43÷-13°C.

In accordance with formulas 4÷6 the sea buckthorn fruits freezing time was determined in relation to the air velocity and temperature. Fig. 2 displays the results of the calculations.

From formulas 7÷16 we found energy inputs required to circulate the air in quick freezer at variable velocities and temperatures. Energy consumption during the sea buckthorn fruits freezing determined from initial temperature t_i = 10°C to t_f = -18°C. The results are represented in Fig. 3.

Table 1. Mass and geometrical characteristics of the fruits by cultivars used in the research

Sea buckthorn cultivar	Diameter of a single product, mm	Mass of a single product	Product density, kg/m^3	Bulk density, kg/m^3	Bed porosity
Chuyskaya	10/12	0.6	943	662	0.301
Panteleevskaya	10/15	0.8	968	679	0.315
Dar Katuni	9/12	0.6	957	672	0.309
Maslyanichnaya	9/11	0.4	932	654	0.311
Zolotoy pochatok	11/11	0.7	960	674	0.310



(a) (b)

Fig. 1. Critical fluidization velocities dependence for the sea buckthorn fruits:
1 – "Zolotoy pochatok"; *2* – "Panteleevskaya"; *3* – "Chuyskaya"; *4* – "Dar Katuni; *5* – "Maslyanichnaya"
(a) initial fluidization velocity; (b) final fluidization velocity.

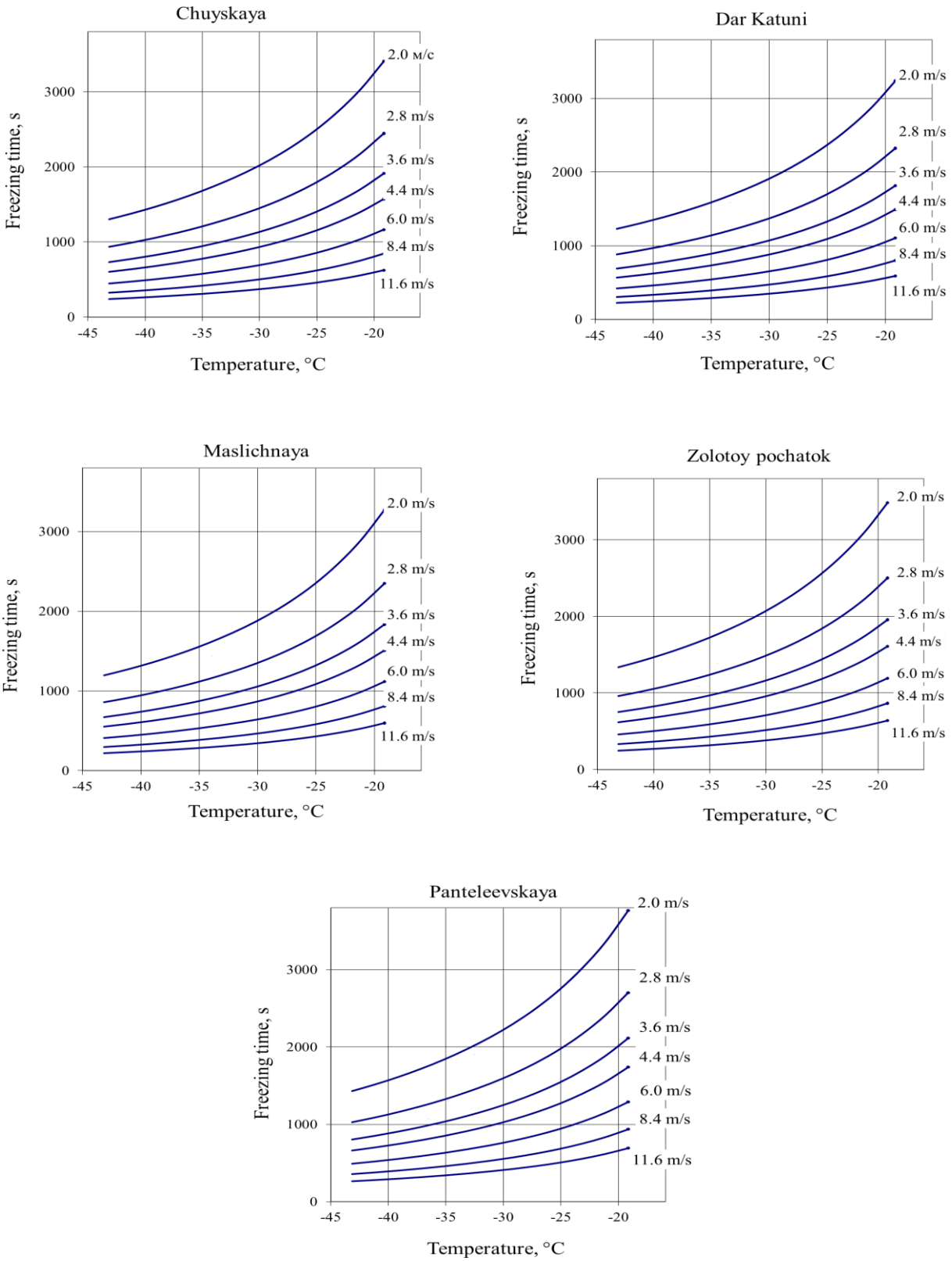


Fig. 2. The sea buckthorn fruits freezing time in relation to the temperature of air medium and fluidization velocity.

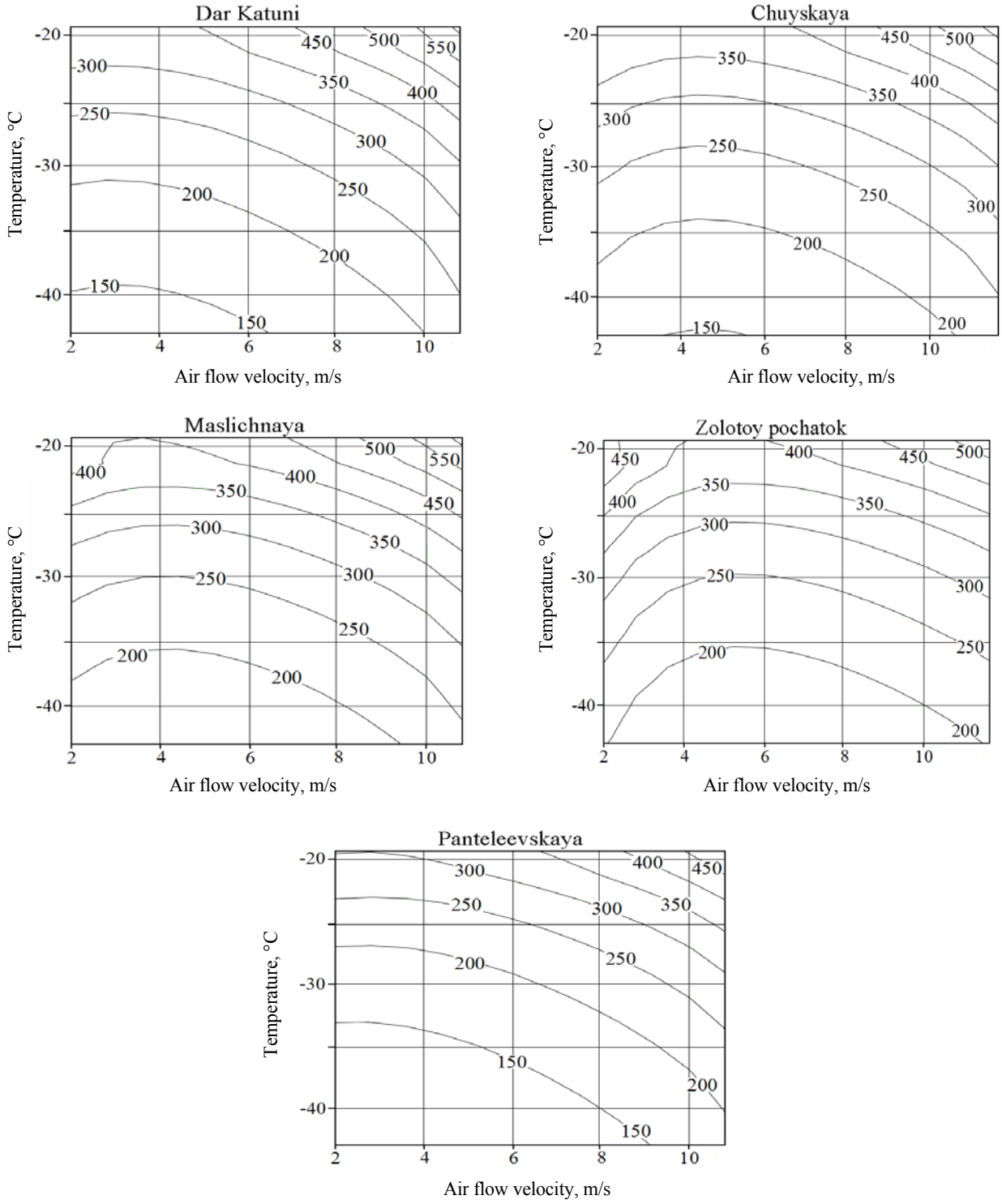


Fig. 3. Energy consumption (kJ/kg) to provide air flow in quick freezing machine to freeze 1kg of the sea buckthorn fruits in relation to air velocity and temperature.

The sea buckthorn fruits have quite a significant variability of air flow optimal velocity where energy consumption for its circulation is minimal. The optimal air velocity in the sea buckthorn freezing depends on the fruit cultivars. The fruit has oblong shape. During the freezing of the sea buckthorn cultivar "Zolotoy pochatok", its fruits having the least oblong shape, the optimal velocity regime for fluidization range from 5 to 6 m/s. And to freeze the sea buckthorn cultivar "Panteleevskaya", the fruits having the most oblong shape, the optimal fluidization regime is a velocity of approximately 2 m/s.

An increase in fluidization speed improves heat transfer from the fruits being frozen and hence, reduces the time of low temperature treatment and lowers consumption of air needed to remove the heat from freezing process. At the same time as the air flow velocity increases in quick freezer, the aerodynamic

losses rise proportionally to the square of velocity. Thus, in the initial stage, an increase in air flow velocity leads to the reduction of energy consumed during product freezing. It occurs because energy used for reduction related to the decrease in amount of air consumed for freezing is more intensive than energy input growth, necessary to overcome aerodynamic resistances. Provided air velocity in quick freezer increases further, energy consumption growth necessary to overcome dynamic resistance takes the lead over the reduced amount of energy related to the decrease in the required amount of circulating air; therefore, total energy consumption for providing for air circulation grows.

Refrigeration capacity required to freeze 1 kg of the sea buckthorn fruits is calculated in relation to the air temperature in quick freezer and the temperature of surrounding air and is shown in Fig. 4.

The results outlined in Fig. 4 show that the Refrigeration capacity required to freeze the fruits of different cultivars varies insignificantly – not more than 1.9%.

For comparative energy efficiency analysis of the different refrigerating circuits we studied the cultivar "Zolotoy pochatok".

Fig. 5 shows the results obtained through the comparative analysis of energy inputs to freeze the sea buckthorn fruits in single-stage compression refrigerating machine.

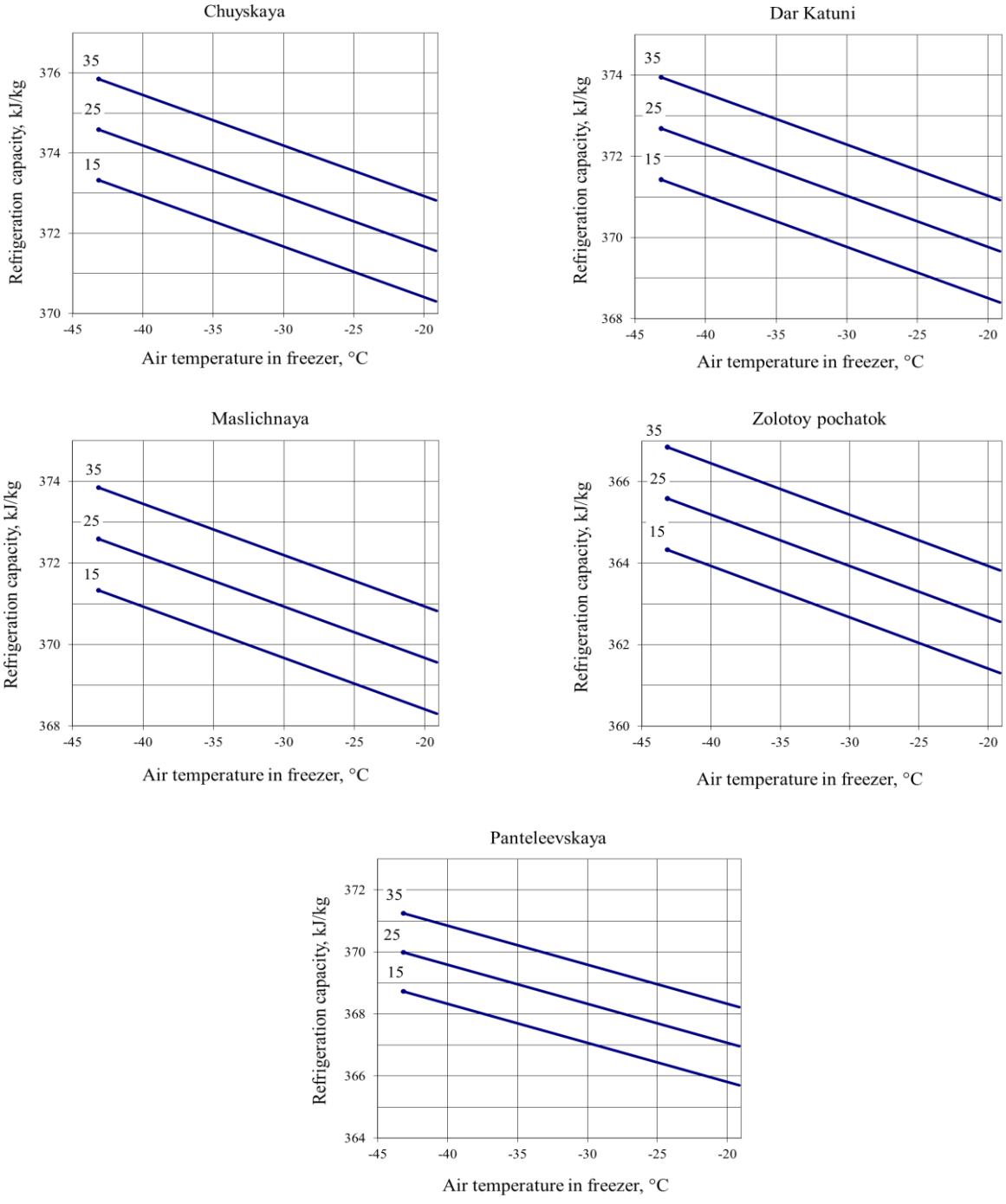


Fig. 4. Refrigeration capacity required to freeze 1 kg of the sea buckthorn fruits at temperature from 10°C to -18°C in relation to the temperatures of surrounding air and the air in quick freezy; 15°C, 25°C, 35°C are the temperatures of surrounding environment.

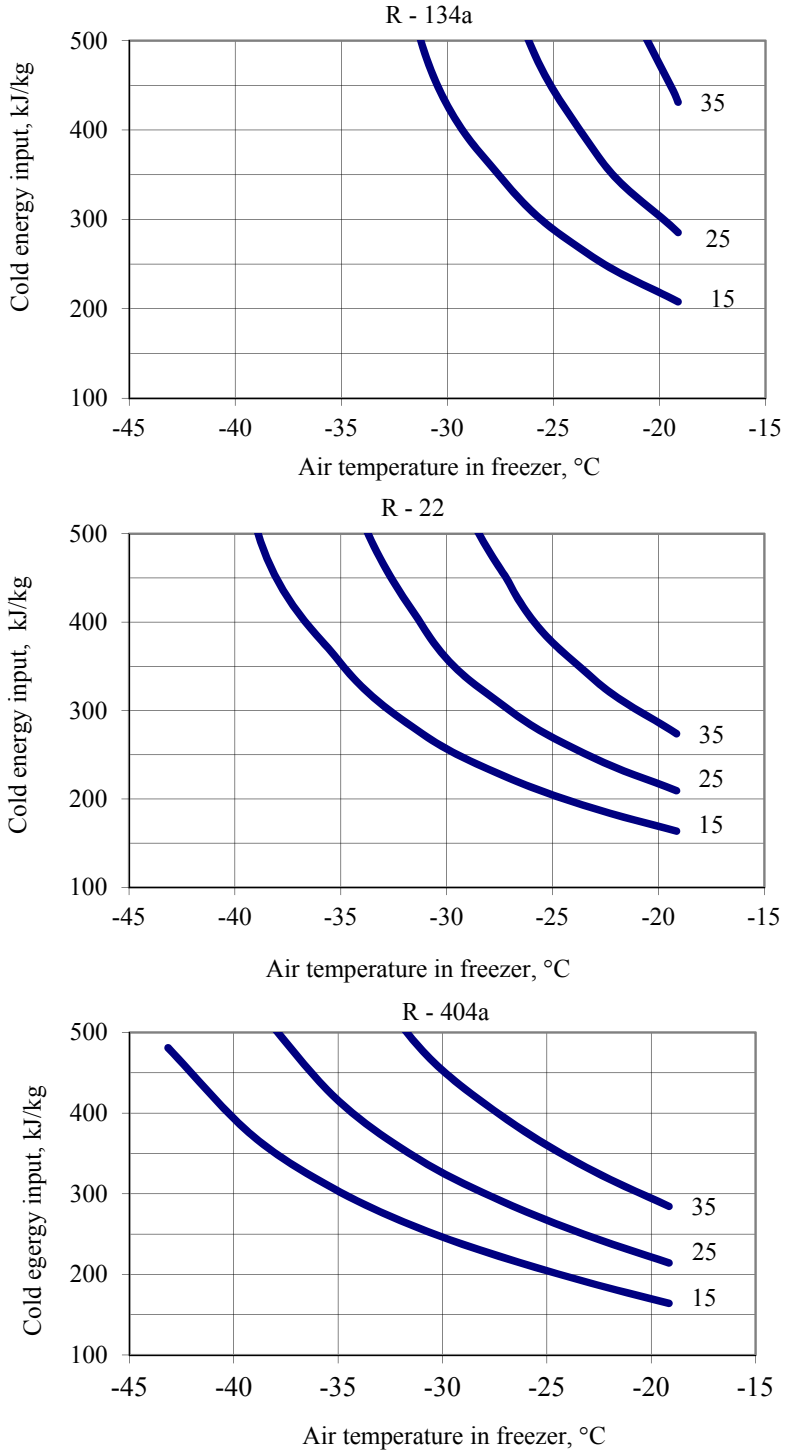


Fig. 5. Energy inputs to freeze 1 kg of the sea buckthorn fruits in single-stage refrigerating machine.

It is not desirable to use Freon R-134a in single-stage refrigerating machine because of excessive energy consumption.

Values of energy consumed to produce artificial cold with cooling unit temperature up to -25°C in single-stage refrigerating machines working on R-22 and R-404a are close. Further decrease of air temperature in quick freezer leads to a sharp rise in energy consumption for the refrigerating machine working on K-22. Single-stage refrigerating machine working on Freon R-404a can be used to attain -30°C temperature levels in quick freezer.

Fig. 6 shows the results of the energy efficiency analysis in relation to air temperatures in quick freezer and surrounding environment air when two-stage compression refrigerating machines used to freeze the sea buckthorn fruits.

Drawing upon the obtained results, application of Freon R-134a is not desirable for quick freezing in two-stage refrigerating machines either. Energy inputs are significantly higher compared to other refrigerants.

Energy efficiency of the refrigerating machines that use R-717 и R-22 as refrigerants, are approximately similar. Two-stage ammonia refrigerating machine has

insignificant advantage over two-stage refrigerating machine that works on R-22 within temperature range up to -35°C. Having said that, the latter is more efficient than ammonia refrigerating machine at the temperatures lower than -35° C. However, Freon based refrigerating machine has better performance and costs less in comparison with ammonia refrigerating machine. Hence, it appears to be more efficient to utilize refrigerating machines using R-22 than ammonia-based.

Energy efficiency rates of the R-404a two-stage refrigerating machine are lower than those of the refrigerating machines working on R-22 и R-717.

Calculations for the energy consumed by cascade refrigerating machines to produce cold for quick freezers are shown in Fig. 7.

From the all studied cold producing circuits the cascade refrigerating machines perform best in terms of energy efficiency. However, practical application of the scheme is more complex.

With the volume refrigerated to attain temperatures ranging to -30°C the amount of energy used by cascade and two-stage refrigerating machines working on R-22, vary insignificantly. Freezing and operational performance of two-stage refrigerating machines being more straightforward and reliable, their application (with R-22) is advisable to the temperature values of -30°C. To reach temperature levels lower than -30°C the cascade refrigerating machines should be preferred.

Our study revealed that the highest energy efficiency of quick freezing processes can be achieved with optimal air velocity value that relate to geometrical and thermophysical parameters of the objects being treated by low temperatures. Change in the velocity of air medium in quick freezers, both and increase and decrease of the optimal values, causes energy cost of the low temperature treatment process to rise.

The lower the air temperature in quick freezer is, the higher heat transfer efficiency is. At the same time, it is accompanied by growth in energy consumption towards refrigerating machine drive. Fig. 8 shows the impact of the combined actions of these two factors on energy inputs required to freeze the sea buckthorn fruits.

Tables 2 and 3 offer the most efficient sea buckthorn fruits freezing regimes, the cold supplied by cascade and two-stage refrigerating machines.

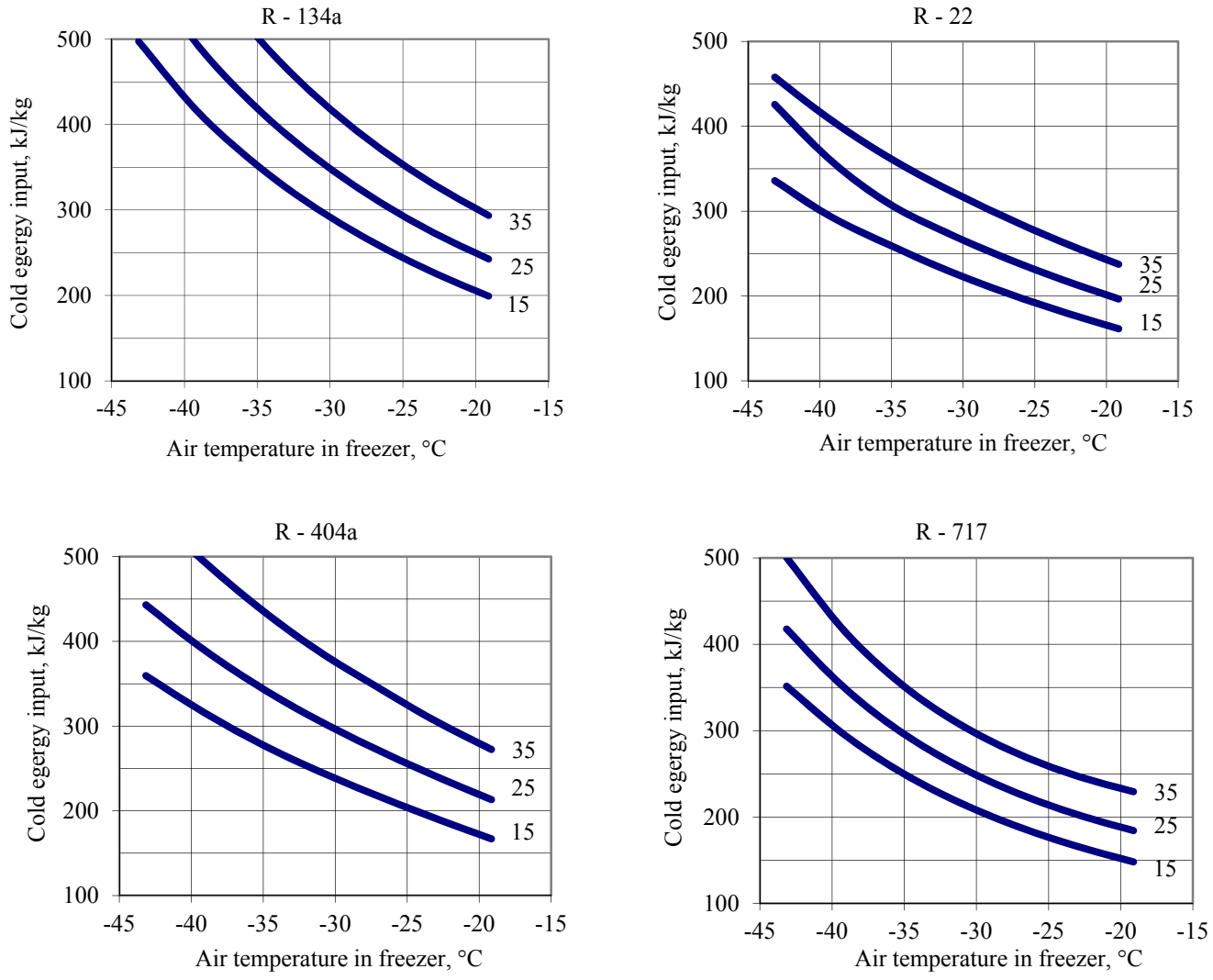


Fig. 6. Energy consumed to freeze 1 kg of the sea buckthorn fruits in two-stage refrigeration machine in relation to the temperatures of air in quick freezing unit and surrounding environment.

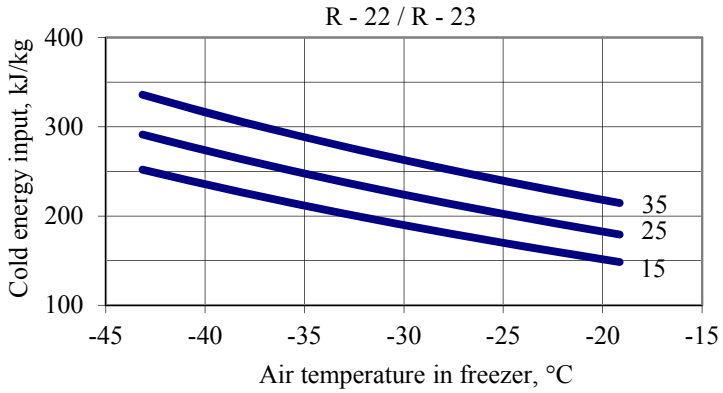


Fig. 7. Energy consumed to freeze 1kg of the sea buckthorn fruits in cascade refrigerating machine working on Freon R-23 in the lower branch and R-22 in the upper branch of cascade for variable air temperatures in quick freezer and of surrounding environment.

Table 2. Optimal freezing regimes (energy efficiency) for the fruits studied in quick freezing unit of cascade refrigeration machine

Sea buckthorn cultivar	Temperature of surrounding air, °C											
	15	25	30	15	25	35	15	25	35	15	25	35
	Air temperature in the machine, °C			Air flow velocity, m/s			Energy consumption (kJ) per freezing of 1kg of berries			Freezing time, s		
Chuyskaya	-39	-39	-39	4.4	4.4	4.4	410.1	449.4	493.6	678.9	678.9	678.9
Panteleevskaya	-35	-35	-35	2.8	2.8	2.8	358.3	395.5	437.8	1321.6	1321.6	1321.6
Dar Katuni	-39	-39	-39	2.8	2.8	2.8	392.4	431.5	475.5	995.9	995.9	995.9
Maslyanichnaya	-39	-39	-39	4.4	4.4	4.4	418.4	457.5	501.5	624.4	624.4	624.4
Zolotoy pochatok	-39	-39	-39	5.2	5.2	5.2	412.8	451.2	494.3	593.2	593.2	593.2

Table 3. Optimal freezing regimes (energy efficiency) for the fruits studied in quick freezing unit of two-stage refrigeration machine

Sea buckthorn cultivar	Temperature of surrounding air, ° C											
	15	25	30	15	25	35	15	25	35	15	25	35
	Energy cost per unit during freezing (kJ/kg)			Freezing time, s			Air velocity, m/s			Air temperature, °C		
Chuyskaya	464.9	511.3	565.1	893.5	893.5	893.5	4.4	4.4	4.4	-31	-31	-31
Panteleevskaya	401.4	447.2	500.4	1527.8	1527.8	1527.8	2.8	2.8	2.8	-31	-31	-31
Dar Katuni	440.3	486.5	540.0	1313.2	1313.2	1313.2	2.8	2.8	2.8	-31	-31	-31
Maslyanichnaya	475.3	524.4	578.0	714.6	829.8	829.8	4.4	4.4	4.4	-35	-31	-31
Zolotoy pochatok	471.6	516.9	569.4	780.0	780.0	780.0	5.2	5.2	5.2	-31	-31	-31

Optimal sea buckthorn fruits freezing regimes greatly vary depending on the cultivars. Presumably, this can be explained with the significant differences in geometrical parameters of fruits harvested from different cultivars.

The least energy consuming regime for cascade refrigerating machine appears to be freezers air temperature of -39°C, and for two-stage refrigerating machine it appears to be -31°C. The optimal air flow velocity in quick freezer ranges from 2.8 m/s for the fruits of cultivars “Dar Katuni” and “Panteleevskaya” to 5.2 m/s for the fruits of cultivars “Zolotoy pochatok”. The smaller air flow velocities correspond to the fruits with more oblong shape. The sea buckthorn fruits freezing times in the most energy efficient regime at the temperature of -39°C were determined to range from 22 minutes for the cultivar “Panteleevskaya” and 17 minutes for the cultivar “Dar Katuni” to 10 minutes for the cultivar “Zolotoy pochatok”. At temperature of -31°C the freezing time will vary from 25 and 22 minutes (“Panteleevskaya” and “Dar Katuni” respectively) to 12 minutes for the cultivar “Zolotoy pochatok”. On average, in terms of energy efficiency the use of cascade refrigerating machine for the sea buckthorn fruits freezing is 13.8% more productive when compared with two-stage refrigerating machines.

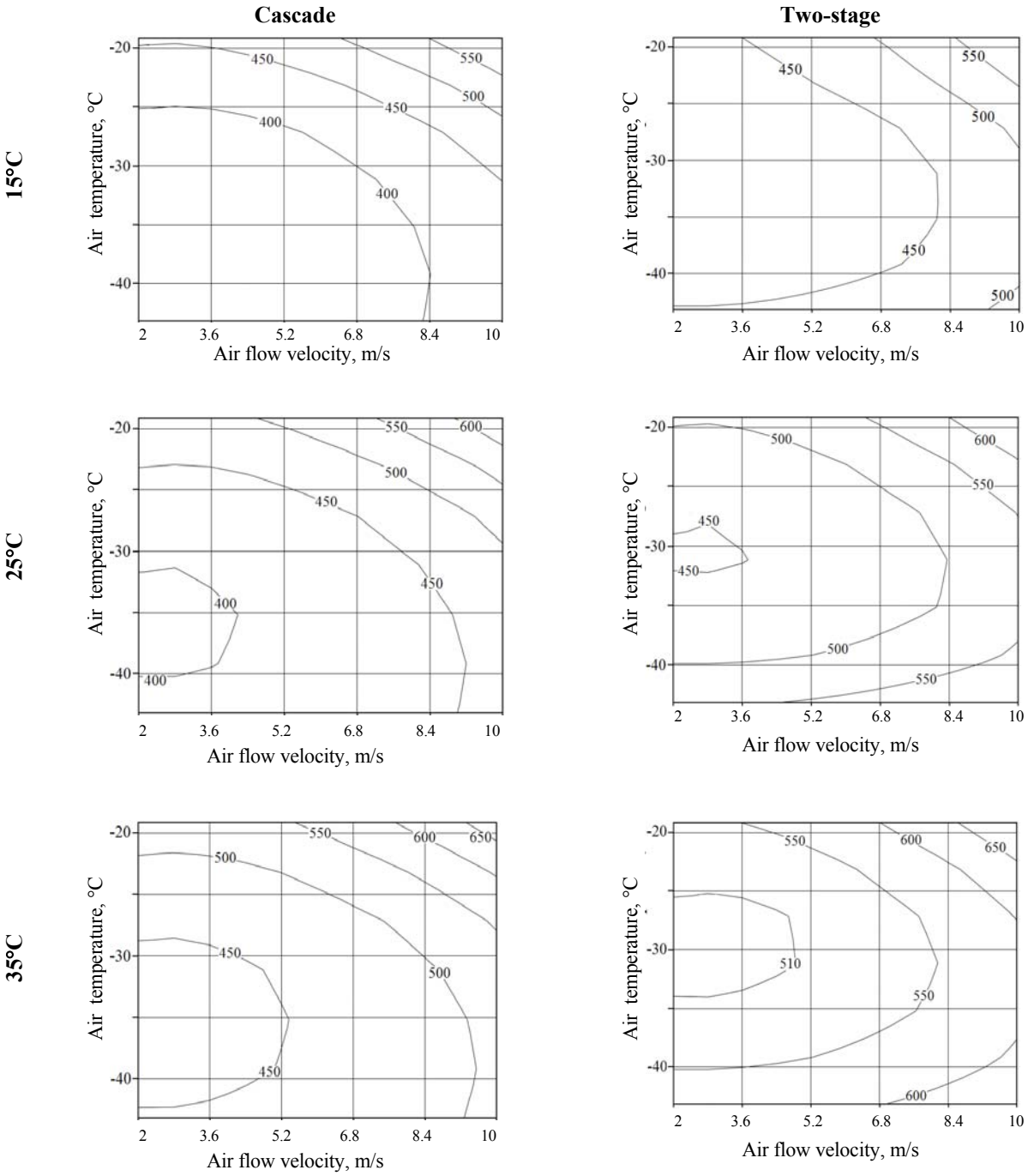


Fig. 8. Energy cost per unit (kJ/kg) in the process of freezing the sea buckthorn fruits of the cultivar "Panteleevskaya" in quick freezer receiving cold from cascade (R-23/R-22) and two-stage (R-22) refrigerating machines.

CONCLUSION

Thus, the performed calculations and analysis of the obtained data demonstrate that the use of cascade refrigerating machine for the sea buckthorn fruits quick freezing is significantly more energy efficient in comparison with other types of refrigerating machines. Moreover, sea buckthorn fruits quick freezing in the freezers that receive cold from cascade machines occurs under lower temperatures, which significantly accelerates low temperature treatment process and hence, improves the quality properties of the frozen fruit.

Cascade refrigerating machines has a higher price tag than two-stage machines. Their assembly and maintenance costs more, it requires more qualified staff to service them. However, increase in the equipment productivity due to the freezing time reduction and lower energy inputs to their drive in cascade refrigerating machines makes them more attractive economically for quick freezing of fruits and berries.

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Please cite this article in press as: Korotkiy I.A., Korotkaya E.V., and Kireev V.V.Energy efficiency analysis of the sea buckthorn (*Hippophae rhamnoides*) fruits quick freezing. *Foods and Raw Materials,* 2016, vol. 4, no. 1, pp. 110–120. doi: 10.21179/2308-4057-2016-1-110-120.

STUDY OF CALCIUM ROLE IN COLLOIDAL STABILITY OF RECONSTITUTED SKIM MILK UNDER RENNET COAGULATION CONDITIONS

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Received February 07, 2016; Accepted inrevised form March 20, 2016; Published June 27, 2016

Abstract: The role of calcium in rennet coagulation of milk is unquestionable in production technology of many cheeses. Therefore, understanding the possible mechanism of calcium influence on the colloidal stability of casein micelles may be the key to control the process of milk coagulation. It is evident that calcium ions are involved in maintenanceof milk coagulation stability, but the molecular mechanism of how these ions influence micellar caseins system is not fully known. Thus, the role of calcium in maintenance of the colloidal stability of milk is quite an urgent problem. Methodologically, the research was based on analysis of coagulation process of reconstituted skim milk, enriched with ions of calcium, magnesium and sodium. Milk whey separated from the clot after coagulation was investigated for sodium, magnesium, calcium and phosphorus. A simple quantitative model, which includes kinetic description of the proteolysis process and the thermodynamics of the dissociation process of the functional groups of micellar caseins, was worked out to analyze experimental results. Kinetic and thermodynamic methods of describing the process of stability loss in micellar system were combined in one model, using the concept of solvent quality which is defined by the second osmotic virial coefficient. The experiments showed that calcium and magnesium ions chemically connect to casein micelles. Using reasonable assessments for thermodynamic and kinetic parameters, we managed to get quite adequate description of the experimental data on the coagulation of reconstituted skimmed milk enriched with calcium and magnesium ions. It was stated that the equilibrium constants for the dissociation of magnesium and calcium caseinates should differ by more than two orders of magnitude. The authors demonstrated principal possibility of using the model to describe the rennet, acid and mixed acid-rennet clotting of milk.

Keywords: Reconstituted skim milk coagulation, casein micelles, colloidal stability of milk, the second virial coefficient

DOI 10.21179/2308-4057-2016-1-121-128 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 121–128.

INTRODUCTION

Coagulation of milk is an important process in the production of many food products. The basis of this process is the coagulation of milk casein, which can be caused by various reasons, for example by acids, proteolytic enzymes, certain salts, alcohols, or high temperature. Coagulative stability of milk and its change in the production process, of course, is due to features of the structure and functional properties of milk as a colloidal system. Understanding these features is the basis for the development of dairy products technology within the modern approach [1].

It is known that during rennet coagulation soluble calcium salt is added into milk with a reduced activity of calcium ions in order to produce clot of necessary density [2]. It is evident that calcium ions are involved in maintenance of milk colloidal stability, the molecular mechanism of how these ions influence micellar caseins system is not fully known. Most researchers suggest that charge destabilization of micelles is the basis of calcium influence on the coagulation process [3, 4]. Native casein micelles have an electric charge arising in the dissociation or recombination caseins of different functional groups. In general, this charge has a negative sign, which manifests itself in a negative value ζ-potential of micelles in milk. ζ-potential value is associated with the ion composition of milk whey [5, 6]. Changing of ζ-potential may be due to the fact that ions dissolved in milk can either chemically connect to functional groups of caseins, altering the charge of micelles, or they form an electric double layer close to the surface of micelles without contacting them chemically, but shielding their electrostatic interaction.

The aim of this work is to study possible ways how calcium ions influence the stability of the colloidal micelle casein in reconstituted skimmed milk.

OBJECTS AND METHODS OF STUDY

The experimental part

The object of research is reconstituted skim milk. To get it 90 g of skim milk powder was dissolved in 910 ml of distilled water and then it was thoroughly stirred.

Calcium, magnesium, and sodium were added to reconstituted skimmed milk in the form of solutions of their chlorides. $MgCl_2$ and $CaCl_2$ solutions with a concentration of 1 M andNaCl at a concentration of 3 M were used. Thus, 1 ml of solutions of calcium and magnesium chlorides contained 1 mmol of calcium and magnesium. The concentration of sodium chloride was chosen 3 times higher in order to get solutions of equal ionic strength.

After preparation the samples were kept at 6 ± 2°C for 18 hours. Activity of calcium ions was measured potentiometrically immediately after preparation and after storage in all samples by ion-selective electrode ELITE-041Ca (NICO Analyt).

Crystal microbial chymosin CHY-MAX (Chr. Hansen) was used as an enzyme preparation for coagulation of milk. To prepare the solution of the enzyme preparation 0.1 g of dry powder was dissolved in 100 cm^3 of distilled water.

Coagulation of milk samples was at 30°C in thermostatted cell volume of which was 130 ml. Duration of clotting time was determined by time between adding of 1 ml of the enzyme preparation and the start of milk coagulation.

Our own thermographic method, described in detail in [7] was used to determine the moment of milk coagulation. The principle of method is to increase the temperature difference between heated and unheated thermometers immersed into milk during the process of forming a gel-like structure.

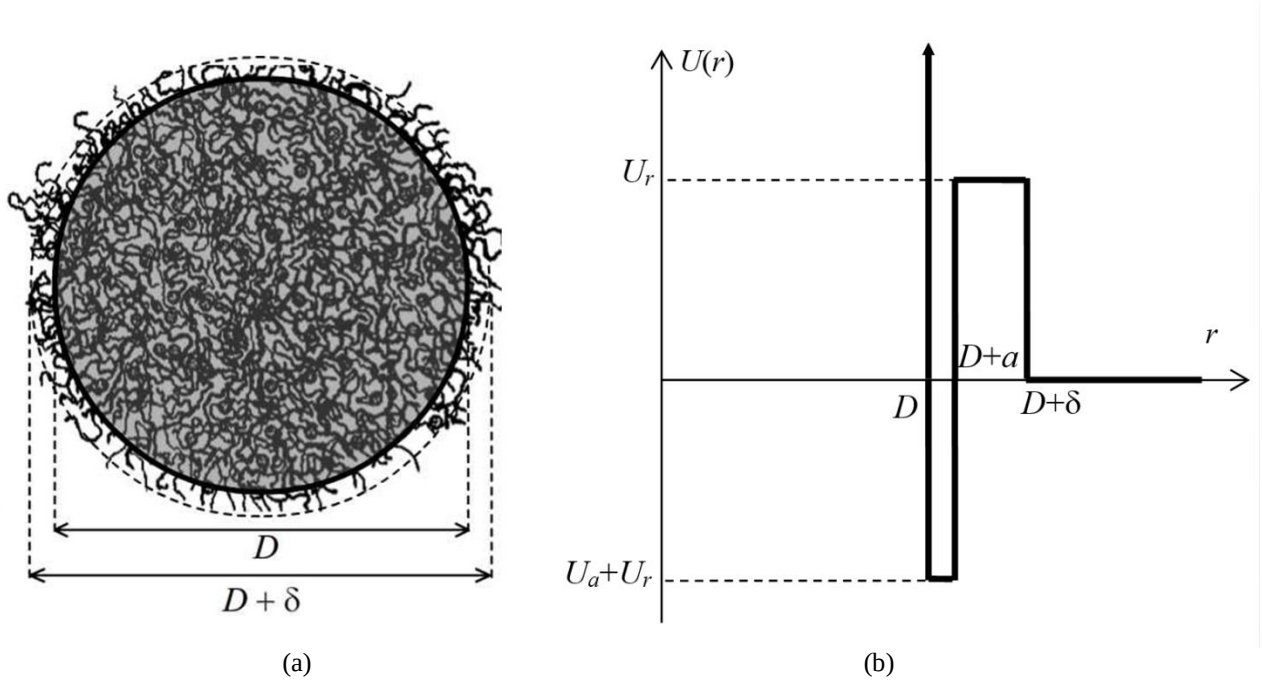
Indirect method for analyzing the concentration of calcium and magnesium in milk whey was used to determine the amount of added into the milk calcium and magnesium which were connected with the casein micelles. After milk coagulation clot was separated from milk whey in tubes of 50 ml with Centrifuge 5430 (Ependorf) for 25 minutes at 7800 rpm. Then 50 ml of the whey samples were dried in Mini Spray Dryer B-290 (BUCHI). The elemental composition of the obtained powder was spectroscopically investigated using an atomic emission spectrometer with inductively coupled plasma iCAP 6500 Duo LA (ThermoFisher Scientific).

Equipment for centrifuging and drying of samples was provided by the Research Institute of Biotechnology of Kemerovo Institute of Food Science and Technology(University).

Determination of the elemental composition of samples of dried whey was carried out on the basis of Kemerovo Center for collective use, Kemerovo Research Center, Siberian Branch of the Russian Academy of Sciences.

The theoretical part

To simulate the experimental results we used the model of binary interaction of micelles based on the model of sticky hard spheres [8, 9] with two additional square potentials [10]. The total potential energy of binary interaction of micelles is shown schematically in Fig. 1.



(a) (b)

Fig. 1. Schematic micelle image (a) and interaction potential (b) depending on distance *r* between centers of two micelles.

It includes a completely solid wall at a distance D between the centers of micelles, a rather deep narrow "attractive" well of a-width and repulsive step of δ-width:

$$U(r) = U_w(r) + U_a(r) + U_r(r), \qquad (1)$$

where

$$U_w(r) = \begin{cases} +\infty, & r \le D \\ 0, & r > D \end{cases},$$

$$U_a(r) = \begin{cases} -U_0 + U_{add}, & r \le D + a \\ 0, & r > D + a \end{cases}, \qquad (2)$$

$$U_r(r) = \begin{cases} U_r, & r \le D + \delta \\ 0, & r > D + \delta \end{cases}.$$

The repulsion, characterized by the potential energy of U_r, is due to the presence of elastic hairy layer of δ- depth, consisting of macro-peptide residues of κ-casein,on the surface of micelles.

The attraction, characterized by potential energy – U_0, includes various interactions (van der Waals attraction, hydrophobic interactions, hydrogen bonds, etc.), which provide micelles adhesion at direct contact of surfaces.

Additional potential U_{add} describes the repulsion of micelles as a result of similar electrical charge originated from dissociation of micellar calcium caseinate. This repulsion obviously has a short-range character due to strong Debye shielding of protein molecules by ions contained in milk whey. At a sufficiently high degree of dissociation of calcium caseinates the potential of intermolecular attraction U_a can change the sign, becoming repulsive in this case.

Let's take the model of solvent quality, characterized by a second osmotic virial coefficient, to quantify the characteristics of the colloidal stability of the micellar system of skim milk:

$$B_2 = 2\pi \int_0^{\infty} r^2 \left[1 - \exp(-U(r)/kT)\right] dr$$

where$k$ is Boltzmann's constant and$T$ is absolute temperature.

In the case of potential energy form of (2) integration has following results:

$$B_2 = \frac{2\pi}{3} D^3 + \left[1 - e^{-(U_a + U_r)/kT}\right]\left[(D+a)^3 - D^3\right] + \left[1 - e^{-U_r/kT}\right]\left[(D+\delta)^3 - (D+a)^3\right]$$

As in [8, 9] let's have a look at the value of the specific excluded volume $\beta_2 = \frac{B_2}{V_{HS}}$,where $V_{HS} = \pi \frac{D^3}{6}$ is a solid sphere volume, and furthermore, we will neglect components containing $\frac{a^2}{D^2}$, $\frac{a^3}{D^3}$ and $\frac{\delta^2}{D^2}$, $\frac{\delta^3}{D^3}$:

$$\beta_2 = 4 + 12\frac{a}{D}\left[1 - e^{-(U_a + U_r)/kT}\right] + 12\frac{\delta - a}{D}\left[1 - e^{-U_r/kT}\right]. \qquad (4)$$

The colloidal solution becomes unstable under condition $\beta_2 \approx -6$ [11, 12].

Kinetics of coagulation is determined by the fact that the potential energies U_r and U_{add} depend on time. These relationships are determined by the kinetics of changes in corresponding charges: a negative electric charge of κ-casein hairs q_{CMP}, proportional to the concentration of dissociated macro-peptide residues and additional negative electric charge of the micelles q_{CAS}, proportional to the concentration of dissociated molecules of calcium caseinates.

In the frame of approach presented in [10], the change in these charges is described by the following expressions:

$$q_{CMP} = -e\frac{[CMP^-]}{[M]} = \frac{-e}{[M]}\frac{K_{CMP}[CMP]_0}{K_{CMP} + [H^+]}\exp(-k_{CMP} \cdot t), \qquad (5)$$

$$q_{CAS} = -2e\frac{[CAS^{2-}]}{[M]} = \frac{-2e}{[M]}\frac{K_{CAS}[CAS]_0}{K_{CAS} + [Ca^{2+}]}\exp(-k_{CAS} \cdot t). \qquad (6)$$

In the expressions (5) and (6) $e = 1.6 \cdot 10^{-19}$ C is an elementary charge; [M] is casein micelle concentration in milk; $K_{CMP}$is the equilibrium constantfor dissociation reaction of macro-peptide residues ofκ-casein ($CMP \leftrightarrow CMP^- + H^+$); K_{CAS} is the equilibrium constant for dissociation reaction of micellar calcium caseinates ($CaCAS \leftrightarrow Ca^{2+} + CAS^{2-}$); k_{CMP}isthe rate constant for proteolysis ofκ-casein by chymosin; k_{CAS}isthe rate constant for additional non-specific proteolysis of α- and β- caseinsegments, containing phosphoserine groups, by chymosin; $[CMP]_0 = [CMP^-] + [CMP]$ is total concentration of macro-peptide residues of κ-casein; $[CAS]_0 = [CAS^{2-}] + [CaCAS]$ is total concentration of phosphoserine groups ofα- and β-casein, which are capable of binding calcium.

When additional magnesium ions are added to milk the micelles charge associated with dissociation of native calcium caseinates, may also be reduced due to the shift of the equilibrium to the left in reaction $MgCAS \leftrightarrow Mg^{2+} + CAS^{2-}$. Denoting the equilibrium constant of this reaction K^*_{CAS} and taking into account the fact that the sum of the concentrations of charged $[CAS^{2-}]^*$ and uncharged [MgCAS] phosphoserine residues of caseins after adding magnesium is equal to concentration of charged residues associated with the dissociation of native calcium caseinates $[CAS^{2-}]$, we have the following expression for the charge of micelles after adding magnesium:

$$q^*_{CAS} = -2e\frac{[CAS^{2-}]^*}{[M]} = \frac{-2e}{[M]}\frac{K^*_{CAS}}{K^*_{CAS} + [Mg^{2+}]}\frac{K_{CAS}[CAS]_0}{K_{CAS} + [Ca^{2+}]}\exp(-k_{CAS} \cdot t). \qquad (7)$$

We will consider as a first approximation, as well as in [10] that potential energy associatedwith charges is proportional to squares of them:

$$U_r = Aq^2_{CMP}, \quad U_{add} = Aq^2_{CAS}. \qquad (8)$$

Constant A will be pre-estimated of the following considerations. If one macro-peptide residue of κ-casein has, in average, 40–50 nm^2 of micellesurface area [14], then their total number on the surface of micelle of radius 100 nm will be about 3000. Let's suggest that at neutral pH each of them has one elementary charge. Coagulation of casein in milk begins when the degree of proteolysis ofκ-casein is 80-90%. We assume that when the charge of micelles as a result of proteolysis reduced by 10 times the energy of molecular repulsion U_r becomes of order ofkT. This means that the maximum energy of micelles repulsion, associated with the elastic hairy layer, is about $100kT$. Then it follows from (8) that $kT \approx A(0.1 \cdot 3000e)^2$ or $A \approx \frac{kT}{10^5 e^2}$.

Similarly, we can estimate the equilibrium constant $K_{CMP}$for the dissociation reaction of macro=peptide residues ofκ-casein. If we assume that at $pH \approx 5$ repulsion energy is becoming of order ofkT, then the charge of the hairy layer is reduced by about 10 times. Then, according to (5) $\frac{K_{CMP}}{K_{CMP}+10^{-5}} \approx 0.1$. Then $K_{CMP} \approx 1.1 \cdot 10^{-6}$M. In this case, as it is seen from (5), at $pH \approx 7$ almost all macro peptide residues are dissociated.

For a preliminary estimation of K_{CAS} we assume that, in accordance with generally accepted notions [15], the micelle has a mass of about $5 \cdot 10^8$ Da and, on average, it consists of $2 \cdot 10^4$ casein molecules with mass $2.5 \cdot 10^4$ Da. If each molecule of casein has an average of 7.5 phosphoserine groups (from 1 forκ-casein and to 15 forα-casein) which can reversibly connect calcium ions, then their concentration is $[CAS]_0 = 1.5 \cdot 10^5 [M]$. According to our preliminary estimations, if the concentration of calcium ions in milk whey is approximately $[Ca^{2+}] = 10$ mM, then the additional stabilization effect disappears [10]. It means that an additional charge of micelles q_{CMP} decreases, as in the case of estimation ofq_{CAS}, lower than, approximately, 300e. Taking into account that each dissociated group has a charge equal in absolute value to 2e, we get from (6): $\frac{1.5 \cdot 10^5 K_{CAS}}{K_{CAS}+10^{-2}} \approx 150$. Then $K_{CAS} \approx 1 \cdot 10^{-5}$M. When the concentration of calcium ions in the whey decreases to $[Ca^{2+}] \approx$ 1mM, the additional charge of q_{CAS} micelles increases 10 times, and additional repulsion energy, according to (8), becomes $100kT$. As it is known from [16], with such values of the concentration of calcium ions coagulation of milk does not start even after the proteolysis of κ-casein micelles on the surface is completed. Therefore it is reasonable to estimate the depth of the potential binding $U_0 \approx 100kT$.

Let's estimate the rate constants for proteolysis previously as follows. The time during which the unit dose of chymosin leads milk to the beginning of coagulation process in case of a substantial excess of calcium ions in whey, i.e. the time of reaching the level of proteolysis ofκ-casein on the surface of micelles 90%, is about 5 minutes. Therefore, $\exp(-k_{CMP} \cdot 5) \approx 0.1$. Then $k_{CMP} \approx 0.2 \ln(10) \approx 0.5$ min^{-1}. Coagulation of milk deficient in calcium ions begins only in a few hours. For the initial estimation we assume this time equal to 200 minutes. If we assume this slowdown is due to non-specific proteolysis, reducing the additional charge of the micelles associated with the dissociation of calcium caseinates, then $\exp(-k_{CAS} \cdot 200) \approx 0.1$. Therefore, $k_{CAS} \approx 0.005 \ln(10) \approx 0.012$ min^{-1}.

These preliminary estimates may be corrected while simulatingthe experimental data.

RESULTS AND DISCUSSION

Fig. 2 shows the results of experiments on milk coagulation with different content of soluble calcium. A solution of calcium chloride was added so that the concentration of calcium added further was 4, 8, 16 and 32 mM. In all cases, the activity of calcium ions was determined by an ion-selective electrode immediately after preparation of milk samples and after their holding at a temperature of about 6°C for 18 hours. The accuracy of measurement of calcium ion activity was not high due to the complex composition of milk and the presence of interfering ions, especially Mg^{2+}. Perhaps a significant impact on the accuracy was due to the deposition of milk proteins on the measuring diaphragm. In any case, within the sensitivity of the method it was stated that the activity of calcium ion reached an equilibrium value within a few minutes after adding calcium chloride and remained practically unchanged after keeping milk for 18 hours.

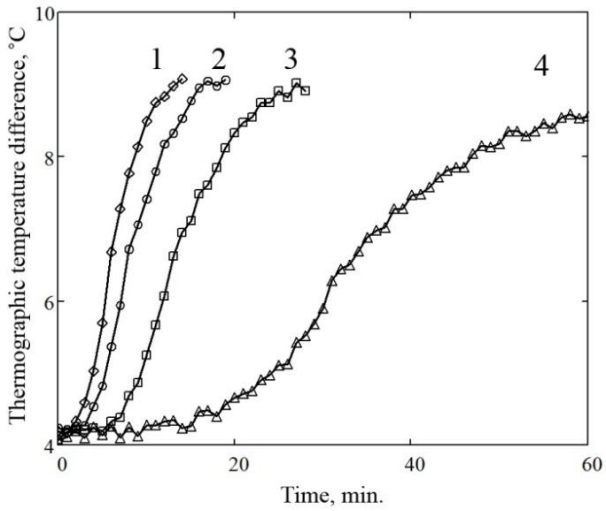


Fig. 2. Thermograms of rennet coagulation of reconstituted skim milk with additional Ca^{2+}. Curves: 32 mmol of $CaCl_2$ added to 1 *l* of milk sample (1); 16 mmol of $CaCl_2$ added to 1 *l* of milk sample (2); 8 mmol of $CaCl_2$ added to 1 *l* of milk sample (3) and 4 mmol of $CaCl_2$ added to 1 *l* of milk sample (4).

In this series of experiments, we also prepared samples of milk with different contents of sodium ions. The sodium chloride solution was added so that the concentration of additional sodium was 12, 24, 48 and 96 mM. These samples were also held at a temperature of about 6° C for 18 hours. None of the prepared samples of reconstituted skim milk coagulated under the influence of chymosin during an hour. Possibly reaction $Na_2CAS \leftrightarrow 2Na^+ + CAS^{2-}$ has a very high value of the equilibrium constant, thus sodium ions are hardly connected with caseins under normal conditions.

Fig. 3 shows the results of experiments on the milk coagulation with different content of soluble magnesium. Magnesium chloride solution was added so that concentration of added calcium was 8 and 16 mM. As it can be seen from the figures, coagulation stability of these samples is comparable to the stability of the milk samples enriched with calcium ions in the same ratio. However, the coagulation duration for samples with magnesium is observably longer than the coagulation duration with the same amount of added calcium. This fact makes it possible to estimate the value of the equilibrium constant for the reaction $MgCAS \leftrightarrow Mg^{2+} + CAS^{2-}$ slightly higher than for the reaction $CaCAS \leftrightarrow Ca^{2+} + CAS^{2-}$.

To determine the accurate proportion of added calcium and magnesium connected with caseins in milk samples, we measured the elemental composition of centrifuged whey for each sample. The results of whey spectroscopic studies showing the content of calcium, magnesium and phosphorus in whey samples are presented in Table 1.

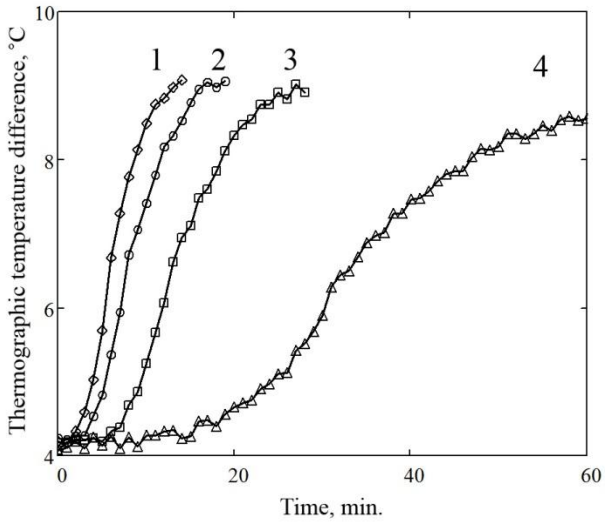


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Table 1. Content of Ca, Mg and P in serum samples

Conditions	Element	Content, mM					
Added	Ca	4.0 ± 0.1	8.0 ± 0.2	16.0 ± 0.4	32.0 ± 0.8	0	0
	Mg	0	0	0	0	8.0 ± 0.2	16.0 ± 0.4
Detected	Ca	5.6 ± 0.2	5.7 ± 0.2	6.5 ± 0.2	9.1 ± 0.2	4.3 ± 0.2	3.7 ± 0.2
	Mg	1.7 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	6.4 ± 0.1	8.9 ± 0.1
	P	7.4 ± 0.2	6.8 ± 0.2	5.9 ± 0.2	5.5 ± 0.2	6.7 ± 0.2	6.0 ± 0.2
Adapted	Ca	2.8	3.6	5.4	8.5	2.3	2.5
	Mg	1.4	1.5	1.4	1.5	6.1	8.7

As it is seen from the table, adding of a certain amount of calcium or magnesium does not increase additively their content in milk whey. This fact clearly confirms the interconnection of some part of added substances with casein micelles. It was noted above that the interchange of calcium between whey and micelles occurs quickly enough. This interchange is difficult to relate to poorly soluble colloidal calcium phosphate. In our opinion, it can reflect exactly the calcium and magnesium interchange between whey and casein according to the scheme described above.

It should be marked that the amount of calcium and magnesium detected in whey need some correction. The table shows that the detected amount of phosphorus in various samples clearly correlates with the amount of added soluble calcium or magnesium: the more ions Ca^{2+}или Mg^{2+} we add to the milk sample, the less of phosphorus we find in whey after its separation from the clot. Apparently, some part of detected phosphorus belongs to micelles left in whey as a result of incomplete coagulation. The obtained data were processed further to consider calcium and magnesium associated with such micelles. The corrected values are shown in Table 1 in the column "Adapted". Additional calcium remained in whey with non-coagulated micelles was considered as follows. The extrapolated value of the phosphorous concentration in whey at full coagulation of casein was based on experimental data evaluated as $[P]_0 \approx 5$ mM. The average ratio between calcium and phosphorus in dry milk is about 1.2:1.0. Therefore, some calcium connected with micelles was taken away from calcium concentration directly detected in whey: $[Ca^{2+}]_{adapted} = [Ca^{2+}]_{detected} - 1.2 \cdot ([P]_{detected} - [P]_0)$.

Similarly values of magnesium concentration was processed, but in this case the role of the correction was much smaller because of the significantly lower content of magnesium in micelles.

We should also mark that the clot quality was higher when adding equal amounts of calcium rather thenmagnesium. Indeed, the phosphorus content in

whey samples enriched in magnesium is higher, than in similar samples enriched in calcium. Probably, as it was mentioned above in the analysis of Fig. 3, it is connected with a greater tendency to magnesium caseinate dissociation.

We used processed values of calcium and magnesium concentrations in milk whey samples to simulate the experimental data in order to clarify the basic parameters of the model.

Fig. 4 shows the results of calculation of the second osmotic virial coefficient (4), which characterizes the colloidal stability of the micellar caseins system depending on time for different values of added soluble calcium. Potential energy calculations were carried out according to the formulas (5), (6) and (8). The parameters used for the simulation were: $A = \frac{4.8kT}{10^5 e^2}$, $K_{CMP} = 1.2 \cdot 10^{-6}$M, $[CMP]_0 = 3000[M]$, $[H^+] = 10^{-6.7}$ M, $[CAS]_0 = 1.5 \cdot 10^5 [M]$, $K_{CAS} = 1.7 \cdot 10^{-5}$M, $U_a = 70kT$, $k_{CMP} = 0.15\,\text{min}^{-1}$, $k_{CAS} = 0.014\,\text{min}^{-1}$.

It is easy to notice that the obtained parameters are not too much different from the estimations given in the previous section. However, calculations of the data presented in Fig. 4 are in satisfactory agreement with the experimental results in Fig. 2 for coagulation of skim milk samples of enriched in calcium. If we assume that the coagulation time t_C is achieved at the maximum value of thermal temperature difference rise rate, then according to the analysis of curves in Fig. 4 we can obtain following clotting time values: $t_C = 6 \pm 1$ min for the curve 1; $t_C = 8 \pm 1$ min for the curve 2; $t_C = 13 \pm 2$ min for the curve 3 and $t_C = 32 \pm 2$ min for the curve 4. On the other hand, as it was noted in the previous section, if we consider achieving value $\beta_2 = -6$ by the second osmotic virial coefficient as the moment of stability loss for colloidal system, then we can see a good match.

Similar calculations were made for data simulation, shown in Fig. 3. The calculation results of the second osmotic virial coefficient (4) using equations (5), (7) and (8) are shown in Fig. 5. All common parameters used for the simulation coincide with the parameters used in the previous case. Moreover, it was believed that the calcium ion concentration was the same for both samples and equal to the average of the data from Table 1: $[Ca^{2+}] = 2.4 \cdot 10^{-3}$ M. The obtained equilibrium constant for the dissociation of magnesium caseinate was equal $K^*_{CAS} = 1.15 \cdot 10^{-2}$ M. As it was expected, this value was significantly (almost 700 times) bigger than the equilibrium constant for dissociation of calcium caseinates.

We should mark a good coincidence of experimental and calculated values of coagulation time. Analysis of the rate of rise of thermal temperature difference for curves in Fig. 3 allows us to obtain the following values of the coagulation time: $t_C = 10 \pm 2$ minutes for the curve 1 and $t_C = 15 \pm 2$ minutes for the curve 2, which coincides with the results shown in Fig. 5.

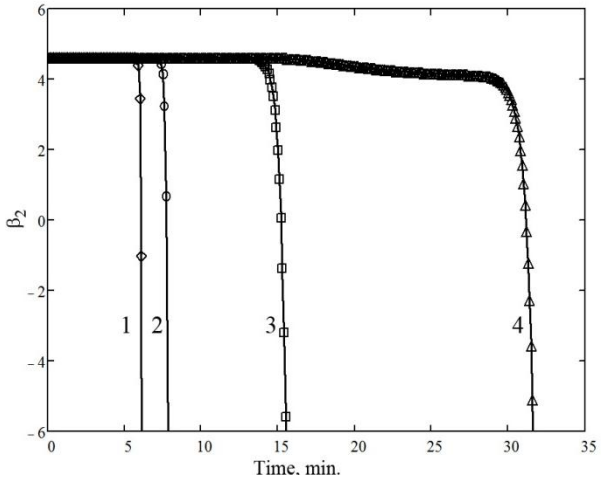


Fig. 4. Calculated values of β_2 resulted from fitting to curves 1, 2, 3 and 4 in Fig 2. See text for details.

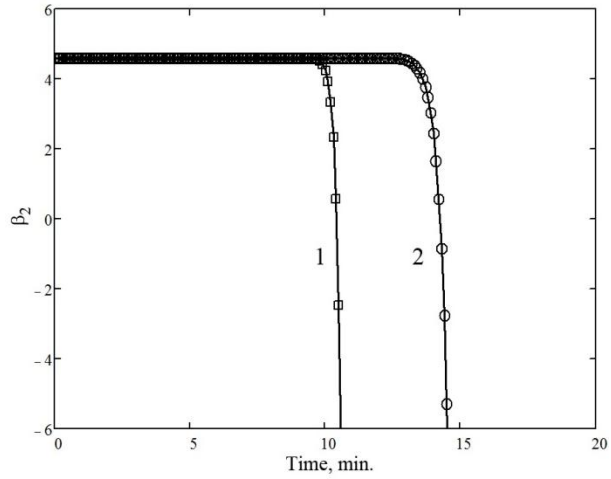
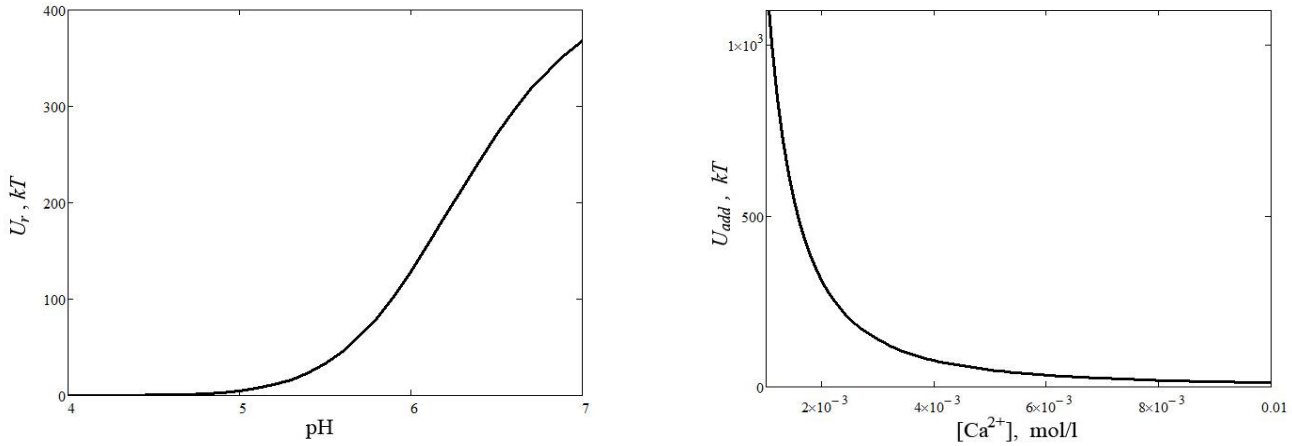


Fig. 5. Calculated values of β_2 resulted from fitting to curves 1 and 2 in Fig 3. See text for details.

We underline one more time that all the calculations for samples enriched in calcium and for samples enriched in magnesium were carried out in the framework of a unified model with the same set of parameters evaluated in the framework of obvious assumptions. Basically, this model easily explains the negative result of coagulation of milk with the addition of sodium chloride. If the equilibrium constant for dissociation of sodium caseinate is substantially higher than of magnesium, it is not possible to coagulate milk adding reasonable amounts of sodium chloride within a reasonable time.

Another advantage of this model is possibility to describe acidic and mixed acid-rennet coagulation of milk. It will be enough to add scheme characterizing the dependence of the solubility of colloidal calcium phosphate on $[H^+]$ concentration to the existing equations. For example, during acid coagulation the repulsive energy of elastic polyelectrolytic brushes from macro peptide residues of κ-casein on the micelles surface decreases with increasing

concentration of $[H^+]$ (Fig. 6a). On the other hand increase in acidity leads to the dissolution of the colloidal calcium phosphate and, consequently, to increase of ion $[Ca^{2+}]$ concentration. As a result (Fig. 6b) additional repulsion decreases, and with pH≈5 the colloidal stability of casein micelles in milk loses.



(a) (b)

Fig. 6. Dependence of U_r on pH (a) and dependence of U_{add} on $[Ca^{2+}]$ (b).

The parameters for the calculation of the curves in Figure 6 are the same as for the calculation of the curves in Fig. 4 and Fig. 5.

CONCLUSION

We have explained the possible mechanism of how calcium ions influence on the process of milk coagulation within the framework of a simple quantitative model which uses the concept of solvent quality, determined by the second osmotic virial coefficient. Using reasonable estimations for thermodynamic and kinetic parameters of the model we could obtain an adequate description of the experimental data on the coagulation of reconstituted skim milk enriched in calcium and magnesium ions. The difference in the effect of calcium, magnesium and sodium in the coagulation of casein micelles has been explained. The principal possibility of using the model to describe rennet, acid and mixed acid-rennet coagulation of milk has been shown. Basically, this calculation method may also be used to quantify the magnesium ion content in products produced by coagulation of milk enriched in magnesium ions for special nutrition intended for magnesium deficiency states.

ACKNOWLEDGEMENTS

The authors are grateful to C.G. de Kruif, professor of Applied Physical and Colloid Chemistry at Utrecht University and Research Strategist at NIZO food research, for his fruitful advices on solvent quality formalism.

We also appreciate our cooperation with S.A. Sozinov, Director of Kemerovo Center for Shared use of Scientific Equipment of Siberian Branch of Russian Academy of Sciences.

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Please cite this article in press as: Osintsev A.M., Syrtseva A.P., Kolmykov R.P., Braginsky V.I., Lapshakova O.Yu., and Osintseva M.A. Study of calcium role in colloidal stability of reconstituted skim milk under rennet coagulation conditions. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 121–128. doi: 10.21179/2308-4057-2016-1-121-128.

PHYSICAL AND CHEMICAL ASPECTS OF VACUUM DRYING OF BERRY RAW MATERIALS

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Received February 07, 2016; Accepted in revised form March 20, 2016; Published June 27, 2016

Abstract: The problem of production of functional products of the natural origin intended for daily regular consumption and having an effect on biochemical reactions and physiological functions through restoration of its microecological status is considered in this article. It is shown, that one of sources of the vital substances for an organism is berry cultures, which play an essential role in nutrition of population. Dry berries practically completely conserve all spectrum of vitamins and biologically active materials that makes them valuable raw materials in various food industry branches. The use of products on the basis of dry berries allows to compensate for the deficiency of some vitamins, edible filaments and other useful substances, and also to normalize an intestinal microflora of an organism. The research purpose consisted in selection of optimum physical and chemical and technological aspects of low-temperature vacuum drying of berries. At performance of tests both standard and original techniques of investigation of technological, physical and chemical, biochemical, microbiological and statistical methods of research of raw materials and finished products properties have been used. Physical and chemical aspects of vacuum drying of berry raw materials are studied. Optimum technological parametres of drying are picked up. It is stated, that drying at higher values of temperature (80°C) proceeds faster in the chamber, thus there is fuller moisture removal from a berry, which positively influences on product shelf life. However there is increasing of specific power inputs on drying of a product and decreasing of quality indicators.

Keywords: vacuum drying, berries, thermalphysic characteristic, drying parametres

DOI 10.21179/2308-4057-2016-1-129-134 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 129–134.

INTRODUCTION

Now one of vital issues of our country is population maintenance with a qualitative and safe foodstuff [1]. Deterioration of ecological conditions observed in last decades is the reason of pollution of a foodstuff pesticides, radionuclides, toxic metals (lead, zinc, mercury, copper, arsenic, cadmium), various nitro compounds (nitrites, nitrates and so forth), antibiotics etc.

Such situation extremely negatively affects the general state of health of the population of our planet. One of the consequences of this situation is disease growth, the speeded up cases of a newborns pathology, and also life expectancy decreasing – now in Russia this index is 65 years while in England and Japan the life expectancy is equal to 76 and 80 years accordingly [2].

A necessary condition for health preservation and level of working capacity of the person, increasing of his life duration is following the balanced diet that means reception by a human body a demanded complex of the nutritious ingredients balanced on a parity and quantity from food [3]. Thus, there is a necessity for restructuration and quality of population nutrition, working out of production enriched various biologically active materials, and also for expansion of possibilities of food consumption with high biological value [4].

In the light of the pointed problem the production of functional products of the natural origin intended for daily regular consumption and having an effect on biochemical reactions and physiological functions through restoration of its microecological status is extremely important [5]. One of the sources of vital substances for an organism is berry cultures which play an essential role in population nutrition.

Biological value of berries is defined not only by its energy value, but also by the high maintenance of pectic substances, macro- and minor components, and also the vitamins which maintenance plays an essential role in preventive maintenance of such diseases as a scurvy, Beriberi etc. [6]. A berry gets special value in northern widths where the nutrition consists mostly of products of acid character (bread, meat, eggs, fish, groats prevail), that results to surplus in an organism of the sour connections causing development of acidosis

against infringement of a metabolism. Berries and fruit are the best source of the alkaline bases, allowing to avoid similar diseases [7].

Among all ways of canning (cooking, freezing, degermination), drying is the most perspective method of preservation of products which advantages are: small weight of the exsiccated product, not expensive tare for packing, possibility of long-term storage and transportation without cold application, etc. [8].

Now the most perspective ways of drying are the vacuum drying which is passing at negative pressure, but above triple point of water and the freeze drying proceeding at pressure below triple point of water. Each of ways has advantages and disadvantages. Vacuum drying allows to apply cheaper equipment, to reduce process of drying at the expense of absence of a stage pre-award frosts, and also to reduce power inputs on removal of moisture of 1 kg. Freeze drying, despite lacking of the above pointed advantages, allows to receive a product with very high quality indicators, especially this question concerns preservations of vitamins.

The products received by this methods of drying, are characterised by the smallest contraction, good ability to dehydration and high storage times that is especially important by production of dry berries and berry powders adding into the formula of wide assortment of foodstuff in the edible industry, and also biologically active admixtures in a pharmaceutical industry.

The dry berries manufactured by method of vacuum and freeze drying, can be widely applied in dairy industry at manufacturing of curd cakes, fruit milk, yoghourts, cream, etc. In cookery they are used for kissels, sauces, stuffings, gravies, etc. In confectionery industry berries have found application in fruit and wafer productions as flavouring agents, dyes and stabilizers of fats.

Thanks to the high nutritional value berries represent an integral part of person nutrition. Fresh berries are an irreplaceable source of vitamins, mineral substances, organic acids and sugars that is vital for maintenance of high-grade health. Regular consumption of berries allows avoiding many diseases connected with avitaminosis, to reinforce immunity, and also to raise the general working capacity. Fresh berries contain about 75–90% of water, thus the most part of solid content (80–90%) is carbohydrates among which the most important are laevulose, glucose, sucrose, cellulose, hemicellulose and starch.

The basic share of berries nutrients makes the sugar, substantially influencing their flavour profiles and storage life. Among all presented sugars the sweetest one is laevulose, then – sucrose, and the last place belongs to glucose. Depending on a cultivar and conditions of cultivation the maintenance of sugars can vary on the average from 5 to 12% [9].

Drying is known to be now the most perspective method of product canning, including berries [10]. This way is based on supression of ability to live of organisms and retardation of all biochemical processes as a result of partial or full moisture removal from product. At product drying there is strengthening of substrate to such level at which there are no conditions for a high-grade cellular metabolism in product tissues. The way of drying allows not only to raise safety of qualitative and biochemical indexes of products, but also appreciably to raise their shelf life and to reduce the canning cost price.

Now many ways of drying based on various ways of warmth supply and moisture removal from product are developed. Every way has advantages and disadvantages. Among the basic methods of drying it is necessary to note such as convective, conductive, infra-red, microwave, acoustic, vacuum and sublimation.

Dry berries practically completely conserve all spectrum of vitamins and biologically active materials that makes them valuable raw material in various food industry branches. The use of products on the basis of dry berries allows to compensate for the deficiency of some vitamins, edible filaments and other useful substances, and also to normalize an intestinal microflora of organism. The pectic substances in dry berries help to deduce heavy metals (lead, mercury, cobalt, molybdenum, zinc), and radio nuclides (isotopes of strontium, caesium, yttrium, etc.), and also urea, cholesterol and other harmful substances from organism.

Now the production of fruit powders becomes more and more expanded. The basic advantage of such product is flash restorability at water addition. Application of fruit powders in the food-processing industry has given the chance to expand substantially assortment of manufactured production, and also to raise its biological value at the expense of the big maintenance of vitamins, macro- and minor components and cellulose which give the necessary relish and odour to products.

Berry powders are applied in such areas of food-processing industry as dairy, confectionery and bakery, and also in a pharmaceutical industry by production of biologically active admixtures [10]. Addition of dry berries in production of various products allows improving a consistence to optimise structural characteristics of weight, and also to save utilization of gelling agents.

Berry powders have received special distribution in dairy industry where they are used as fillers in curd cakes, yoghourts, dairy and cottage cheese creams, fruit milk, ice-cream, fruit and milk desserts, melted cheese, etc. Dry scraps of berries are also an irreplaceable component by scented tea production where the weight fraction of dry berries can reach 50%. Dry berries are used as a source of the natural cellulose used at manufacturing of cookies, candy sticks, dietary drugs, desserts, muesli, etc. They are raw materials for reception of aromatic substances, dyes and others biologically active materials.

The aim of the investigation is selection of optimum physical and chemical, and technological aspects of low-temperature vacuum drying of berries. Selection should be based on individual physical and chemical properties of various kinds of this production. At drying it is necessary to consider contents, the cryoscopic temperature, thermal and physical, physical and chemical properties, solid content, etc.

OBJECTS AND METHODS OF STUDY

Objects of study at various stages of work were:

– berries of strawberry, raspberry, black currant, cranberry. The fruit and berry raw material was got: in State Unitary Enterprise "Plodopitomnik-1" (Kemerovo, Russia) during the period from 2010 to 2012; in cities of Tomsk area of crops 2010–2012. In experimental researches the berries which have reached full maturity, healthy, not having bruises were applied;

– granulated sugar;

– laboratory and trial samples of dry berries.

At performance of tests both standard and original techniques of investigation of technological, physical and chemical, biochemical, microbiological and statistical methods of research of raw materials and finished products properties have been used.

Selection and preparation of hallmarks for the analysis, the total maintenance of organic acids, organoleptic indexes, chemical composition, vitaminized and mineral value of berries of strawberry, raspberry, currant black, cranberry were defined in laboratories of the scientific-educational centre and department "Bionanotechnology" of Kemerovo Institute of Food Science and Technology (University), and also in laboratories of the test centre of federal official establishment of the centre of agrochemical service "Kemerovo" (Kemerovo, Russia).

The determination of macro- and minor components was made by method of atomic-absorption spectrophotometry. The method principle is based on ability of the dissociated atoms of elements to capture light in a narrow spectral range.

Thermalphysic characteristics of berries were determined by the first buffer method of two temperature and time intervals [11, 12].

Microstructural researches of strawberry, raspberry, black currant, cranberry berries before drying were made in the institute "Coal and coal chemistry" of the Siberian Branch of the Russian Academy of Science (Kemerovo, Russia). Electronic-microscopic researches were carried out on raster scanning electron microscope JEOL JSM-6390 LA.

RESULTS AND DISCUSSION

Drying process consists in the following. The probed product is kept on pans which are positioned in drying chambers. The chambers are closed from above by caps. The refrigerating unit is switched on from operating console and within 10–15 minutes the installation turns on a winterizing mode. The winterizing mode is fixed on evaporator temperature (the temperature should make no more than minus 35°C). Then the vacuum pump is switched on and the drying condition begins. Owing to low pressure in chambers there is a vaporization of moisture from berries and their drying. The duration of drying process takes from 5 to 6 hours.

According to their properties berries represent colloidal capillary-cellular body. Walls of capillaries of such bodies are elastic and bulk up at moisture absorption. After dehydration such bodies shrink, become fragile and can be transformed into a powder. Physical properties of products appreciably influence on their qualitative characteristics, a storage ability and transportation. The list of these properties is wide enough and includes such indexes as weight, density, size, form, and thermal, structurally-mechanical, optical, electrophysical, sorption indexes, etc.

Weight, size and form are quality factors of many kinds of foodstuff, rationing of these parametres is manufactured for bakery, confectionery products, cheeses, cake cheeses, etc. The defined form and size of any fruit and berry correspond to each economic-botanical or pomological cultivar.

Thermal properties represent a complex of physical quantities defining character and speed of passing of processes of cooling and heating in product. Such properties are heat capacity, thermal conductivity, fusion point and freezing. Thermal characteristics are considered at degermination, cooking, batch, transportation and storage of products, they are basic sizes used by working out of technological modes of cold treatment and at research of influence of low temperatures on foodstuff.

In Table 1 the results of definition of thermal characteristics of probed berries are resulted.

The obtained data testify about authentic correlation between such indexes as solid content, heat capacity and cryoscopic temperature. The increasing of moisture content of a berry conducts to decrease the cryoscopic temperature and to increase heat capacity. So, for example, the strawberry characterized by the smallest solid content ($13.13 \pm 0.05\%$), and, hence, and the greatest moisture content, has shown the least cryoscopic temperature which has made minus 0.91 ± 0.02°C and the greatest heat capacity 3961 ± 15 J/kg·K of all probed berries.

The similar heat capacity has been positioned in cranberry (3820 ± 15 J/kg·K), however because of smaller moisture content its cryoscopic temperature has made minus 1.51 ± 0.02°C. The greatest solid content ($18.63 \pm 0.05\%$) was revealed in black currant that is the reason of its lowest cryoscopic temperature which has made minus 2.13 ± 0.02°C. However, despite it, heat capacity of raspberry (3654 ± 15 J/kg·K) has appeared lower than in black currant.

At drying point selection, experiments were made at the following temperatures: 60, 70 and 80°C, the size of residual pressure was 4–5 kPa and heat flux density was 5.5 ± 0.3 kw/m. According to the data received during experiments, schedules of relative weight change (Fig. 1), temperatures on a surface (Fig. 2) and in thickness of a berry (Fig. 3) depending on a drying time have been constructed. First 15 minutes the installation turns on operating duty, thus the cooling machine and a vacuum pump works depressing pressure in the chamber from atmospheric to pressure 4–5 kPa.

Table 1. Thermalphysic characteristics of berries

Kinds of berry	Solid content, %	Heat capacity J/(kg·K)	Cryoscopic temperature $t_{I<p}$, °C
Strawberry	13.13 ± 0.05	3961 ± 15	minus 0.91 ± 0.02
Raspberry	17.70 ± 0.05	3654 ± 15	minus 1.51 ± 0.02
Black currant	18.63 ± 0.05	3800 ± 15	minus 2.13 ± 0.02
Cranberry	12.81 ± 0.05	3820 ± 15	minus 1.45 ± 0.02

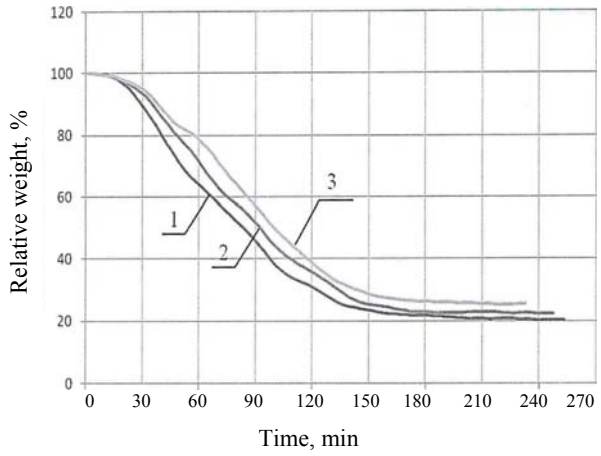

Fig. 1. Schedules of relative weight change of currant at temperatures in the chamber:
1–80°C; 2–70°C; 3 – 60°C.

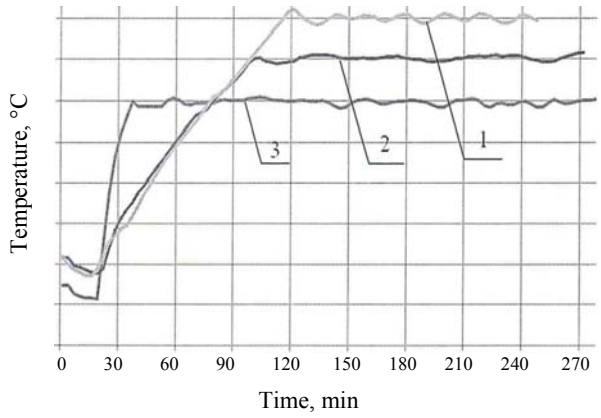

Fig. 2. Schedules of temperature change on a currant surface at temperature in the chamber:
1 – 80°C; 2 – 70°C; 3 – 60°C.

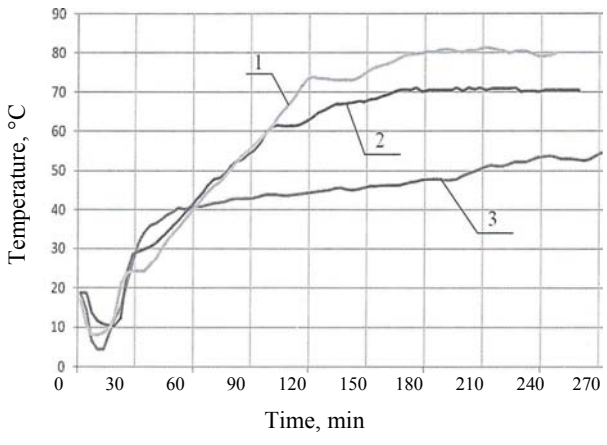

Fig. 3. Schedules of temperature change in thickness of a currant at temperature in the chamber:
1–80°C; 2–70°C; 3–60°C.

At the first stage there is a moisture removal, being in product macrocapillaries. The relative weight of a berry during this period of drying varies slightly – from 100 to 95% from initial weight. At the expense of depressing of residual pressure in the chamber the temperature on a product surface sharply falls on the average to 14–16°C (Fig. 2). The duration of the first stage makes about 15–20 minutes. At the second stage, characterized by constant speed of drying, infra-red lamps of heating are switched on, there is a removal of the basic part of moisture in the product – osmoticly connected moisture and moisture in microcapillaries.

In the experiment with temperature in the chamber 80°C the duration of the drying second stage was 145 minutes from the drying beginning. The relative weight of product by the end of the second stage was 26.2 % from the initial. The temperature on a currant surface reached 80°C in 114 minutes after the drying beginning (Fig. 2) while in depth of a berry the temperature reached this level in 180 minutes after the drying beginning (Fig. 3). Thus, duration of the second period of drying of currant was 100 minutes.

In the experiment with temperature in the chamber 70°C the speed of drying started to drop after 140 minutes from the drying beginning. The relative weight of currant by the end of the second stage was 32.7% from the initial. The temperature on berry surface reached 70°C in 100 minutes, and temperature in depth – in 165 minutes, after the drying beginning. The duration of the second stage of drying was 115 minutes. At drying with temperature in the chamber 60°C, the second stage came to the end in 135 minutes after the drying beginning. The relative weight of currant by that moment was 36.2% from the initial. The temperature on product surface reached 60°C in 54 minutes after the drying beginning. In thickness of product at the end of the second stage the temperature did not reach the necessary value and was 48°C.

Having got the demanded temperature in the chamber, radiant intensity of infra-red lamps drops, in this connection growth rate of a core temperature also decreases, that is shown in Fig. 3.

At the third stage the moisture of mono- and polymolecular adsorption is removed. This kind of communication is the strongest and at the further drying it is removed extremely slowly. The duration of the third stage of dehydration at temperature in the chamber 80°C was 60 minutes, for 70°C this time was 67 minutes, for 60°C – 75 minutes. The relative weight of the exsiccated currant at temperatures in the chamber of 80, 70 and 60°C was 20.5%, 22.4% and 25.4% from the initial accordingly.

In Table 2 the comparative characteristics of drying efficiency such as duration of process of dehydration, moisture weight fraction in a dry berry, specific power inputs on removal of moisture of 1 kg and an organoleptic estimation are resulted.

The presented data allow to make a conclusion, that drying at higher values of temperature in the chamber proceeds faster, thus there is fuller moisture removal from a berry, that positively affects product shelf life, however there is the increasing of specific power inputs on drying of product and decreasing in quality indicators. It is stated, that at currant drying the most rational temperature in the chamber is 70°C. The duration of drying at this temperature is equal to 209 ± 5 mines, the organoleptic estimation thus makes 34 points from 40, in the exsiccated berry the moisture weight fraction is 3.1 ± 0.1%.

The similar experiments with a drying point 60, 70 and 80°C have been made for other kinds of berries. Efficiency factors of drying of strawberry, raspberry and cranberry are presented in Table 3.

It is stated, that strawberry exsiccated at temperature 60°C, is characterized by a high organoleptic estimation – 36 points from 40 and rather low power inputs – 2.96 ± 0.05 kw/kg of a remote moisture. The increasing of temperature to 70°C conducts to falloff of quality indicators, thus duration of drying contracts all for 24 minutes and moisture content of product drops only on 0.3%. Thus, the rational drying point for strawberry was 60°C.

It is concluded, that the rational drying point of raspberry and cranberry is 70°C. Drying at lower temperature, despite increasing of an organoleptic estimation of raspberry and cranberry on 5 and 2 points accordingly, is characterized by longer duration of dehydration process, accordingly for 41 and 45 minutes. At temperature in the chamber 80°C the process duration of drying contracts, but quality indicators considerably drop: for raspberry on 7 points, for cranberry on 6 points. Thus there are hulls on many berries and the power consumption increases considerably on 10.7% for raspberry and on 14.1% for cranberry.

The optimum physical and chemical, and technological aspects of vacuum drying of berries are picked up. The selection is based on individual physical and chemical properties of various kinds of berry production. At drying the contents, the cryoscopic temperature, thermalphysic, and physical and chemical properties, solid content were considered, etc.

Table 2. Comparative characteristics of drying of black currant depending on a drying point

Temperature in the chamber, °C	60	70	80
Duration of drying, min.	223 ± 5	209 ± 5	186 ± 5
Moisture weight fraction, %	7.8 ± 0.1	4.8 ± 0.1	3.1 ± 0.1
Specific power inputs, kw/kg of a remote moisture	3.33 ± 0.05	3.45 ± 0.05	4.20 ± 0.05
Organoleptic estimation, points			
Smell	14	13	8
Consistence	14	12	10
Colour	9	9	8
Total	37	34	26

Table 3. Indexes of drying of berries at temperature selection in the chamber

Temperature in the chamber, °C	60	70	80
Duration of drying, min			
Rasberry	276 ± 5	235 ± 5	197 ± 5
Cranberry	286 ± 5	241 ± 5	212 ± 5
Strawberry	264 ± 5	233 ± 5	189 ± 5
Moisture weight fraction, %			
Raspberry	2.6 ± 0.1	2.5 ± 0.1	2.2 ± 0.1
Cranberry	4.8 ± 0.1	4.3 ± 0.1	4.0 ± 0.1
Strawberry	3.4 ± 0.1	3.1 ± 0.1	2.5 ± 0.1
Specific power inputs, kw/kg of a remote moisture			
Raspberry	3.79 ± 0.05	4.12 ± 0.05	4.56 ± 0.05
Cranberry	3.05 ± 0.05	3.32 ± 0.05	3.79 ± 0.05
Strawberry	2.96 ± 0.05	3.25 ± 0.05	3.44 ± 0.05
Organoleptic estimation, points			
Raspberry	34	29	22
Cranberry	37	35	29
Strawberry	36	27	21

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Please cite this article in press as: Petrov A.N. and Maslennikova G.A. Physical and chemical aspects of vacuum drying of berry raw materials. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 129–134. doi: 10.21179/2308-4057-2016-1-129-134.

STANDARDIZATION, CERTIFICATION, QUALITY AND SAFETY

DEVELOPMENT OF INTEGRATED MODEL OF RISK ANALYSIS IN MEAT INDUSTRY

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Received February 07, 2016; Accepted in revised form March 10, 2016; Published June 27, 2016

Abstract: The existing global approach to risk analysis takes into account risks to human health and veterinary risks. However, this approach does not consider and does not evaluate the technological process of food production as a significant stage for the identification and management of risks. In relation to meat foods, it is the technological process, which will be a key factor in management decisions in the identification, assessment, management and communication of risks. The proposed integrated model of risk analysis reflects the peculiarities of meat products production all across the chain 'from field to consumer'. Described is the mechanism of implementation model with respect to chemical risks, because there are no stages to eliminate chemical risks in meat industry, and on the contrary, some stages contribute to the emergence and introduction of this type of risks (introduction of sodium nitrite, smoking, etc.). Focused is the peculiarity of identification, assessment, management and communication of chemical risks at the stages of the 'raw meat - processing – ready-to-eat product' process. An approach to scientifically based risk communication procedure for the Russian Federation is suggested. The use of the proposed model will improve confidence of producers, government agencies, and consumers in product safety both in respect with human health and veterinarian matters. This mechanism can be used as the legislative basis in the field of food production development, both for the Russian Federation and the Eurasian Economic Union.

Keywords: risk, risk analysis, risk assessment, risk management, risk communication, meat industry

DOI 10.21179/2308-4057-2016-1-135-140 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 135–140.

INTRODUCTION

The priority of food legislation in most countries of the world is the concept of risk analysis, which is seen as a key mechanism for the strategy development determining the focused action aimed at maximum, economically viable reducing the negative impact of chemical, biological and physical hazards to health. The member states of the Eurasian Economic Union are also guided by the global trend to introduce a risk-based approach to building and implementing a supranational food and veterinary legislation [1, 2].

Foodborne diseases are a real and serious problem. About one third of the population of developed countries suffer from diseases associated with the consumption of food. The main causes of these diseases are microorganisms and chemicals. Moreover, chemicals can cause both acute poisonings and chronic ones providing significant harm to human life and health, including those in subsequent generations. New technologies such as genetic modification and nanotechnology put forward additional food safety issues that require evaluation and management, as well as the relevant information about risks [2].

As to the products of animal origin, not only the risk of harm to human health, but also veterinary risks should be considered. Agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) of the World Trade Organization (WTO) allow WTO members to install two versions of sanitary measures to protect against such risks. The established policies and regulations or more stringent requirements are adopted by the WTO with respect to the member states only on the condition that, the country will substantiate and prove this need by means of risk analysis. SPS Agreement urges the member states to base their sanitary and veterinary regulations on international standards, such as the World Organization for Animal Health (OIE) Terrestrial Animal Health Code and the documents of the Codex Alimentarius Commission, with the guidance documents on risk analysis [3, 4].

Meat and meat products occupy a leading position in the national diet. In 2014, the average consumption in the Russian Federation amounted up to 74 kg/year per capita [5].

In terms of security, meat and meat products should be considered not only as products, which if inappropriately processed can cause harm to human health, but also as potentially unsafe products in the veterinary regard. Meat products are known to be a source of proliferation because of a number of zoonotic diseases.

In world practice, meat products are considered as high-risk products, which are characterized by both biological and chemical hazards. The documents of the Codex Alimentarius and the OIE contain instructions for the use of the risk-oriented approach to products of animal origin. However, in the Codex Alimentarius they relate to a finished product and are considered with regard to human health and in the documents of the OIE, for the most part, – to the veterinary well-being of farm animals. Technological component is not highlighted in these documents, but it is this component which serves as an essential mechanism for managing both individual risks and their totality, which results in obtaining of a guaranteed security product within the expiration date, and security both in terms of human health, as well as with respect to its epizootic status .

In connection with this, the most important way to prevent alimentary human and animal diseases is the development of comprehensive risk analysis techniques specific to meat products in order to build, according to the analysis, the system of norm-setting, monitoring, production and state control of food products.

OBJECTS AND METHODS OF RESEARCH

As a research object, chemical risk analysis process was chosen, specific to meat industry.

The research was based on the system-modular approach to risk analysis.

RESULTS AND DISCUSSION

After analyzing the existing guiding documents, as well as identifying the missing link, which reflects the specificity of the meat industry we compiled the integration scheme of risk analysis approaches (Fig. 1).

The scheme is based on the FAO/WHO guidelines for assessing risk to health, as reflected in the documents of the Codex Alimentarius Commission and the OIE approaches. The proposed third block “technological risk analysis” includes the emphasis on the technological aspects of risk detection, identification, evaluation, management and communication. Moreover, “production technology” in this model is seen as a chain “from field to counter”, including, in particular, the evaluation of raw material sources.

In explication of the proposed scheme (Fig. 2) it can be seen that the addition of “technological risk analysis” block allows you to combine different recommendations for risk analysis and create a holistic approach, reflecting the specificity of meat products. Thus, the OIE approach underlies in the risk analysis of raw animal material, and the approach of the Codex Alimentarius – the finished product. For all that, the relationship can be traced to the information received at the stages of risk assessment on the OIE and the Codex Alimentarius, which underlies in the information used in the assessment of technological risk. In turn, the information obtained during the “risk management’ stage in the ‘technological risk analysis” block is the source of initial information for similar stages by the OIE and the Codex Alimentarius.

The unifying element of the whole scheme is the stage of risk communication, which provides the exchange of information between all the components of the line, at the same time correcting and correlating the results with each other, thus creating an effective tool for an integrated approach to risk analysis for meat products.

Provided that the management of chemical risks in the line of meat production is rather difficult, since there are no processing stages, allowing to reduce this hazard, the proposed model was examined on this form of risk too [7, 8].

The first “hazard identification” stage for chemical risks means the identification of a chemical element contained or allegedly contained in ready-to-eat meat products. For the identified element the analysis of available information on the possibility of its adverse effect on human health is performed.

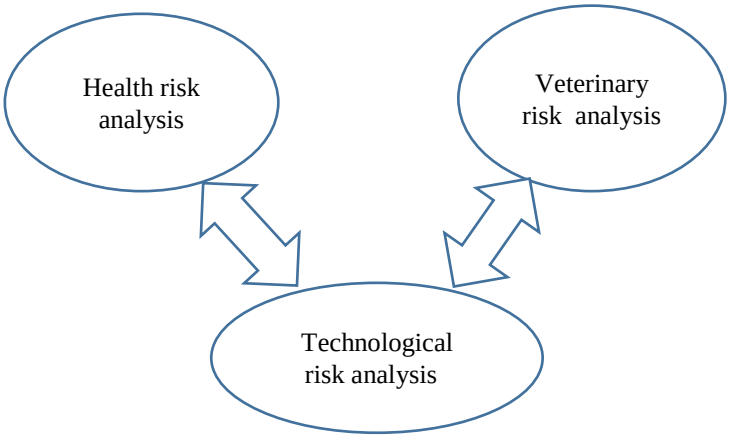

Fig. 1. Integration scheme of risk analysis approaches for meat products.

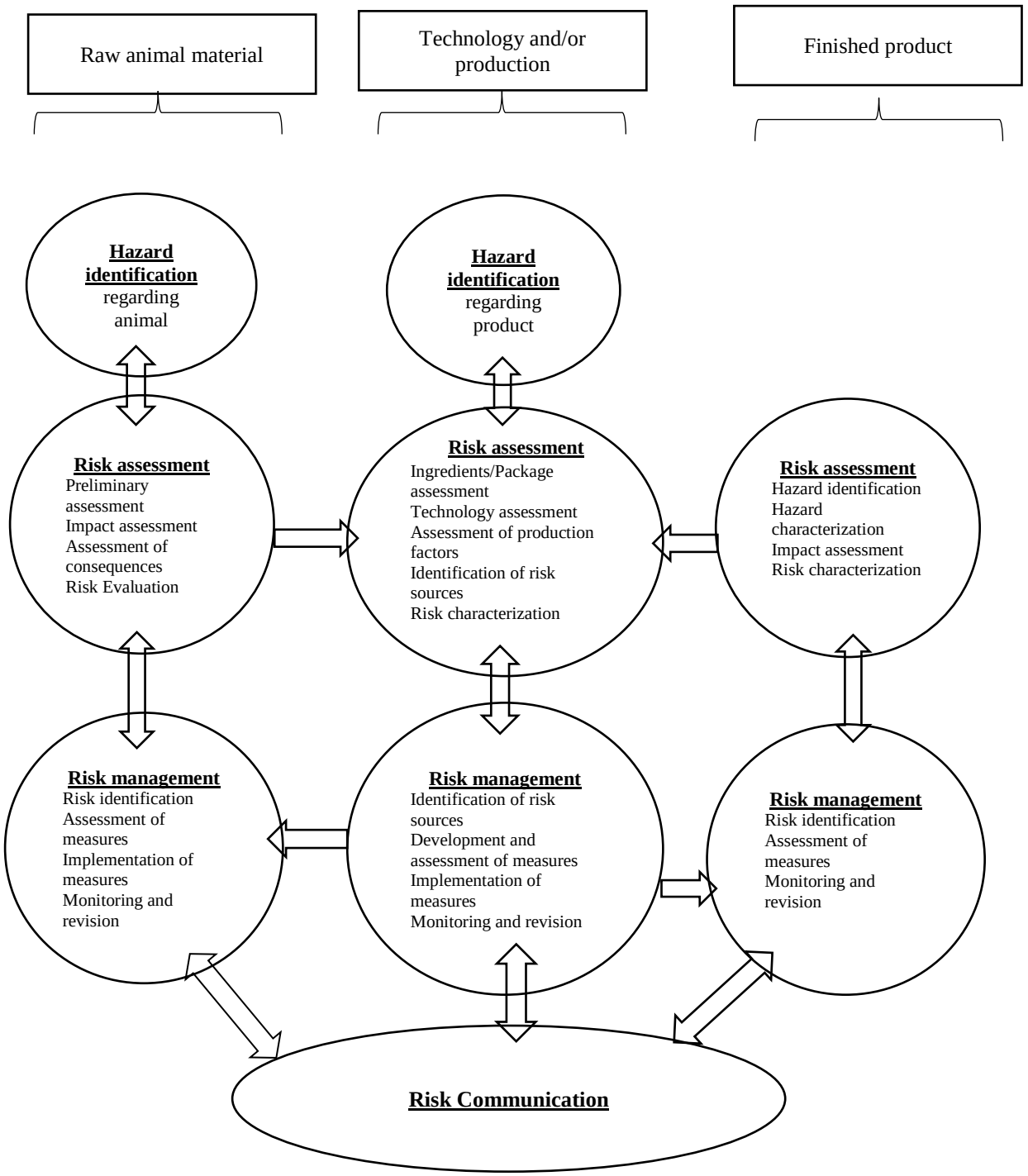

Fig. 2. Integrated model of risk assessment in meat industry.

The peculiarity of the use of this stage in relation to meat products is to identify not only the elements that directly have an adverse effect on human health, but also to identify the elements that contribute to the displacement of alimentary equilibrium of micro-, makronutrients entering the body. Besides it is necessary to consider substances – precursors of chemical compounds capable to form dangerous chemical complexes to health when processed or stored.

The second "risk assessment" stage consists of several substages. At the beginning "hazard description" is formulated, then the nature of an organic / inorganic chemical element, its environmental stability, prevalence, reactivity, etc. are examined. In particular, at this stage the known data on the dependence of adverse health effects from the doses of the substance are described. From the point of view of the technological component assessment it is necessary to identify the critical substages appropriate to the composition of raw materials, packaging technology of the product in respect to the estimated risk (introduction of nitrite, food additives, smoking, etc.).

In the analysis of information at the "identification of risk sources" substage (Fig. 3) the entire food chain "from field to counter" is considered to identify the chain stages in which chemical element in question falls into. Moreover it is important to remember that the first stage, in the case of analysis for meat products, is the information about the conditions of fodder cultivation. It is this stage that is one of the main sources of chemical risks entering the food chain. Equally important is the information on the epizootic status of region's livestock breeding and the degree of environmental pollution (with the identification of major pollutants), since it can serve as the information on the probability of inclusion of veterinary drugs and natural / man-made contaminants into the food chain.

Identification of risk sources, Analysis of food chain stages "from field to counter", Fodder cultivation region, Livestock breeding region, Lifestock processing technology, Product obtaining technology, Life and storage conditions.

At the technological stage of this analysis block the specific product and formulated risk are considered. Basically here attention is drawn to the commercially used chemicals which do not take part in the process of production directly (lubricants for equipment, detergents and disinfectants, chemicals for refrigeration units, boilers, veterinary or merchandising dyes, etc.).

Also are identified constituent components of the product which are characterized by the content of the chemical element (by-products, bones, fat, and other ingredients). Besides at this point packing is regarded as the source of chemical elements migrating into the product. Defined are technological stages and the conditions under which dangerous chemicals are formed.

At the "impact assessment" substage the determination of the product consumption level in the overall diet of the average consumer is carried out, and other products containing the chemical element in question are also identified.

"Risk characterisation" substage is the final in the "risk assessment" stage, and includes the analysis of the information obtained in previous substages and drawing up the risk profile.

Risk management, Preliminary risk management activities, Generation of control measures, Implementation (introduction) of control measures, Monitoring and assessment

"Risk management" stage (Fig. 4) is divided into several substages.

In the first substage setting a goal on analyzed risk management with respect to the reported chemical elements and generating of information on the existing control mechanisms are carried out, the analysis of their effectiveness (if possible) is also performed.

Next, control actions are formulated, the effectiveness of which is supposed to be sufficient to achieve the objective of risk management under consideration. Moreover alternative measures are identified, for their efficient use in the case of the ineffectiveness of the main events.

When forming the control measures, the assessment of cost-effectiveness correlation to use the event should be an important component, because in the case of too high costs for the event the effectiveness of its implementation is significantly reduced.

The implementation activities include the development or bringing in the changes to existing regulations, technical and regulatory documents, planning of monitoring studies and so forth.

At the next stage system monitoring and evaluating the effectiveness of control measures are carried out.

Risk communication, Scientific communication, Traceability, Emergency information.

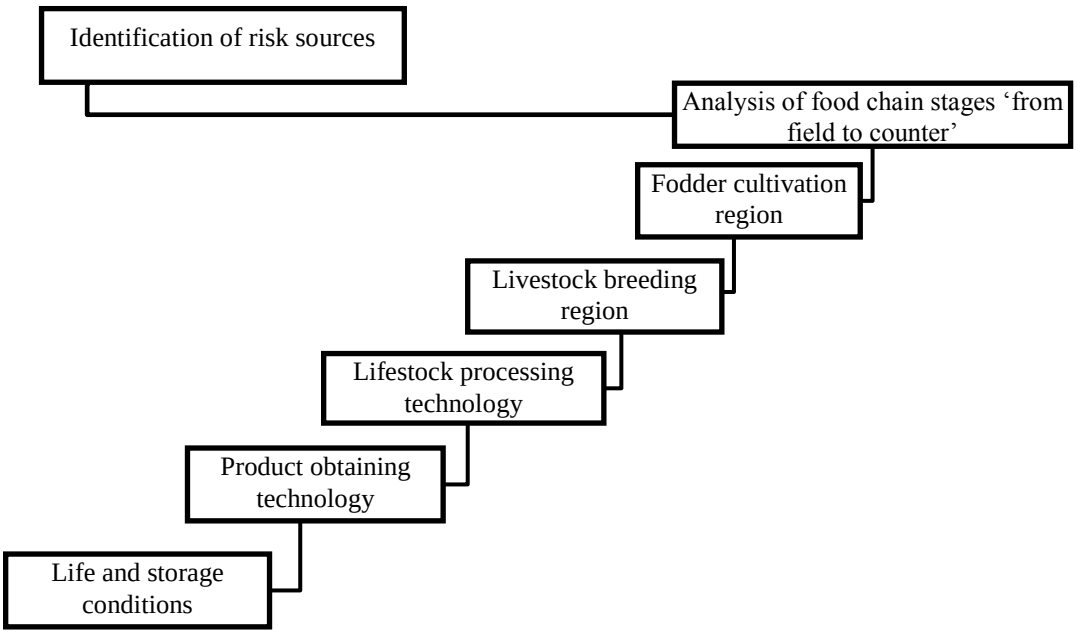

Fig. 3. Model of the "Identification of risk sources" stage.

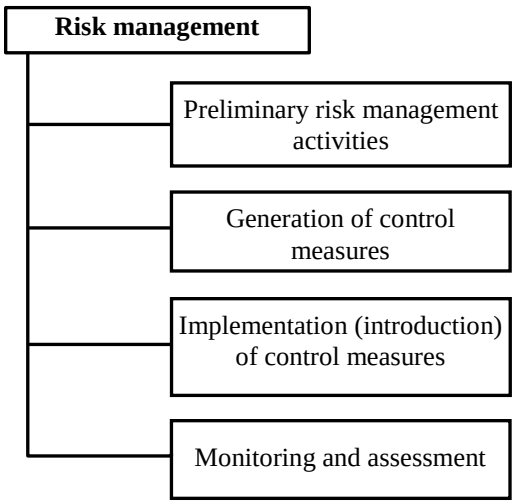

Fig. 4. Model of the "risk management" stage.

At "Risk communication" stage (Fig. 5), it is important to identify the stakeholders to obtain information about the risk, as well as the way of bringing this information to the notice.

Scientific communication is a key factor in the most effective risk analysis as it involves multidirectional research teams for a comprehensive study and obtaining of information about the risks. For meat production, science communication should take place between technological institutes, hygiene institutes in the field of public nutrition, and veterinary institutions. The principle of cooperation between instititutions is presented in Fig. 6.

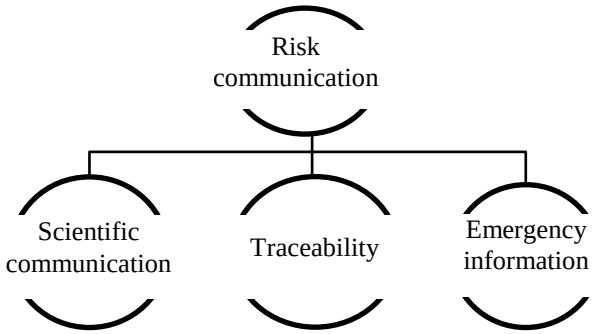

Fig. 5. Model of the "Risk communication" stage.

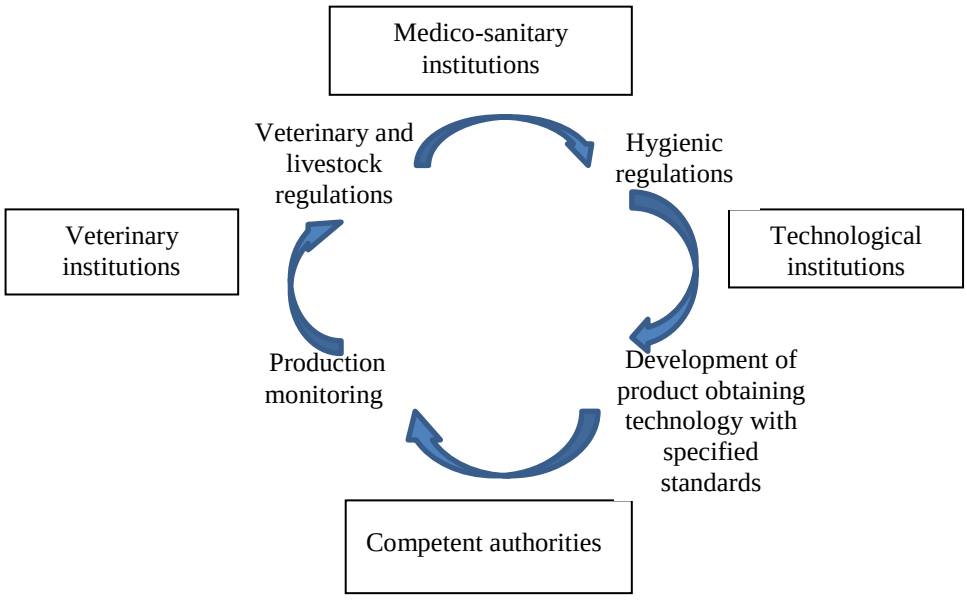

Fig. 6. Principle of scientific communication in the Russian Federation.

Veterinary and livestock regulations, Hygienic regulations, Development of product obtaining technology with specified standards, Production monitoring.

The next important substage in risk communication is the system of traceability. Obtaining objective information about any part of the food chain one can effectively monitor the status of chemical safety of the product in due course to identify the causes of certain excess chemical elements and to take corrective and preventive actions in time.

This approach has shown high efficiency in many countries, and is now the mandatory legislative requirement in most developed and developing countries, including the Russian Federation.

Development of an emergency notification system promotes coordinated work of all federal agencies in case of a threat to the population as a consequence of food consumption. This system is also based on the traceability system and is a well-proven mechanism in many countries around the world.

When carrying out the risk analysis, such factor as consumer behavior must be taken into account too, because under the influence of various social reasons typical consumption diet can vary, or separate consumer groups (vegetarianism, religious rejection of certain products, the transition to low cost products, etc.) may be formed. It can lead to the distortion of information at the stage of evaluation and risk specification.

An important influencing factor is also the establishment of standards at different levels: national, interstate, and international ones. In some cases, the established hygienic norms may differ, which also must be considered when evaluating staple food components.

CONCLUSION

The developed model allows to give full consideration of the risks specific to meat products and to establish effective management mechanisms both at the production and at the state level.

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Please cite this article in press as: Kuznetsova O.A. Development of integrated model of risk analysis in meat industry. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 135–140. doi: 10.21179/2308-4057-2016-1-135-140.

DETERMINATION OF THE CARBON ISOTOPE $^{13}C/^{12}C$ IN ETHANOL OF FRUIT WINES IN ORDER TO DEFINE IDENTIFICATION CHARACTERISTICS

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Received March 06, 2016; Accepted in revised form May 10, 2016; Published June 27, 2016

Abstract: In recent times we can notice a large number of counterfeit fruit wines in the market as it is difficult to discover exogenous alcohols in them. This is due to the fact that while producing table fruit wines it is allowed to add cane or beet sugar during fermentation to provide necessary alcohol conditions. Our measurements were carried out with the help of the method "Détermination du rapport isotopique $^{13}C/^{12}C$ par spectrométrie de mass isotopique de l'éthanol du vin ou de l'éthanol obtenu par fermentation des moûts concentrés ou des moûts concentrés rectifies" OIV-MA-AS312-06. Analyzing Russian crops of fruit and berries in 2015, with the exception of pomegranate (Azerbaijan), we have obtained the following results: black currant – minus 25.75 ± 0.08‰, cherry – minus 25.62 ± 0.06‰, chokeberry – minus 26.13 ± 0.26‰, pear – minus 27.04 ± 0.06‰, plum – minus 26.24 ± 0.41‰, apple – minus 27.58 ± 0.54‰ and pomegranate – minus 28.21 ± 0.22‰. These results suggest the following conclusions: using exogenous alcohols derived from plants C3 – photosynthesis type leads to a slight change in isotopic characteristics of carbon ethanol in fruit wines, while adding sugars or alcohols from plants C4 – type leads to an increase of the isotope ^{13}C, resulting in significant changes of the indicator $\delta^{13}C$. To establish significant differences of exogenous alcohols C3 – type introduced from the outside and obtained by fermentation of adding beet sugar, in some cases it is not enough to use the only one indicator δ^{13} C (‰). Therefore promising researches are connected with defining isotope ratios of other biophilic elements of fruit ethanol, namely, oxygen $^{18}O/^{16}O$ and hydrogen D/H.

Keywords: fruit wine, ethanol, isotope mass spectrometry, stable isotopes, the ratio $^{13}C/^{12}C$, isotopic characteristics

DOI 10.21179/2308-4057-2016-1-141-147 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 141–147.

INTRODUCTION

Currently the Russian market is characterized by a large number of counterfeit wines of both domestic and foreign production. In this regard objective quality assessment and identification of wine products throw this issue into sharp relief. During the formation of a civilized market it is important not only to know the quality requirements to wines, but also indicia of counterfeit, defective products and me-too products.

Standards of the Russian Federation have physical and chemical indicators, norms and testing methods. However, they are not always able to define wine authenticity and detect counterfeit, as indicators of the type standard "General specification" are primarily for the positioning of wine, referring it to a specific group of goods and formulating security requirements.

One of the main tasks currently facing the wine industry is to improve the quality of products with the help of modern technological methods. However, their implementation is often troubled due to unfair competition of counterfeit products producers, who sell wines at dumped prices. The greatest damage is done by ethyl alcohol. Its use is illegal in the production of table grape and fruit wines.

Until recently a large number of counterfeit products in the market was due to the lack of instrumental techniques which could detect presence of alcohols produced not from grape. However, in recent years science has made a significant step forward and today it is possible not only to determine wine composition, but also to detect the nature of raw materials used in their production.

Most biophilic elements (elements that make up the organic matter of living systems) are poly isotope elements and contain "light" isotopic atoms that make up the bulk of the element and "heavy" isotopic atoms – the minor part of the element (carbon ^{12}C and ^{13}C, oxygen ^{16}O and ^{18}O, hydrogen ^{1}H and ^{2}H).

Biochemical differences in photoassimilation of atmospheric carbon dioxide by plants are accompanied by significant differences in the isotopic characteristics of the synthesized organic material [1–2].

A relatively wide range of variation of carbon isotopes abundances is due to the discrimination of

isotope ^{13}C to ^{12}C, which is explained by different ways of assimilation and carbon fixation by plants.

The photosynthesis is the process of carbon dioxide assimilation by green plants and the formation of organic matter with the help of sunlight energy.

There are two ways of CO_2 assimilation:

– using enzyme ribulozodifosfat carboxylase/ oxygennase, which oxidizes ribulozodifosfat to phosphorglyceric acid;

– using phosphoenolpyruvate carboxylase enzyme, which synthesizes oxaloacetic acid from phosphorenolpyruvate, CO_2 and water.

Plants which have phosphoglyceric acid as the first product of CO_2 fixation are called C_3 plants, and those with synthesized oxaloacetic acid – C_4 plants.

Plants assigned to $C_3 – CO_2$ assimilation type have specific characteristics of the carbon isotopic composition of biomass. They are determined by the significant carbon isotope fractionation compared with atmospheric CO_2 as a primary carbon source. The value $\delta^{13}C$ characterizing the carbon isotopic composition of carbohydrates of these plants ranges from minus $31^0/_{00}$ to minus $24^0/_{00}$. Representatives of these plants are fruit trees, shrubs, and vineyards. In addition, each type of fruit is characterized by a well-defined narrower range. Maize, sugarcane and sorghum are plants with $C_4 – CO_2$ type assimilation. C_4 plants are characterized by a value $\delta^{13}C$ measured in a range from minus $15^0/_{00}$ to minus $9^0/_{00}$ [3–7].

High-precision measurement of the corresponding pairs of stable isotopes such as ^{13}C and ^{12}C showed that the substances are chemically identical, but produced differently. They have significant differences of isotope ratios. Methods of analysis of carbon stable isotopes, hydrogen, oxygen, allow us to obtain an isotopic label that characterizes raw material for products and production processes.

The analytical base of methods using isotope mass spectrometry is a measurement of natural abundances of stable isotopes of biophilic elements [8].

The standard PDB is taken as an international standard, representing the isotopic composition of carbon calcium carbonate of fossil Belemnitella Americana Late Cretaceous period from the formation PDB (South Carolina, United States). International comparison sample PDB is characterized by uniform isotopic composition. Currently, Viennese equivalent of the PDB-VPDB is used as an international standard.

The value $\delta^{13}C_{VPDB}$ is calculated by the formula (1)

$$\delta^{13}C_{VPDB} = \frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{VPDB}}{\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}} \cdot 1000, \quad (1)$$

where $\left(\frac{^{13}C}{^{12}C}\right)_{VPDB}$ is carbon isotope ratio of masses 13 and 12 in the comparison sample, equal to 0.0112372; $^{13}C/^{12}C$ is the ratio of carbon isotopes with masses 13 and 12 in the test sample; $\delta^{13}C$ is characteristic of the carbon isotopic composition of the sample with respect to the international sample, ‰.

The method of isotope mass spectrometry regarding $^{13}C/^{12}C$ stable carbon isotopes allows to detect with the required reliability spirits of non-grape origin in grape wine and brandy, to determine the nature of sugars in semi-dry, semi-sweet and sweet wines, to define the origin of carbon dioxide in sparkling wines [9–16].

In order to achieve this aim we have carried out a research to define a carbon isotope ratio range of ethanol in wines, produced from grapes grown in Russia. It was discovered that the wine made from grapes grown in the Krasnodar Region and Rostov Region contain alcohol, which isotopic characteristics ($\delta^{13}C_{VPDB}$ (‰)) are in the range from minus 26.2‰ to minus 28.9‰ (Table 1).

Additional research of alcohol contained in wines from Rostov region produced from grapes breed Sibirkovy and Krasnostop Zolotovsky showed ratio $\delta^{13}C$ in the range from minus 26.8‰ to minus 29.0‰.

However, variations of the $^{13}C/^{12}C$ isotope relations typical for major wine regions of Russian Federation were specified. To this end, samples of grapes were selected from various industrial viticulture production points during the harvest season in 2013.

Grapes were processed in mini winery and the obtained juice was fermented with pure cultures of wine yeast. Then this wine material was analyzed. The test results are summarized in Table 2.

Table 1. Carbon isotopes ratio ($\delta^{13}C_{VPDB}$, ‰) of ethanol in wines, produced from grapes grown in the Krasnodar Region and Rostov Region.

Year	Krasnodar Region		Rostov Region	
	Aligote	Cabernet Sauvignon	Aligote	Cabernet Sauvignon
2008	- 26.8 ± 0.1	- 27.6 ± 0.1	no data	no data
2009	- 26.9 ± 0.1	- 27.5 ± 0.1	- 26.2 ± 0.1	- 27.8 ± 0.1
2010	- 28.7 ± 0.1	- 26.6 ± 0.1	- 26.5 ± 0.1	- 27.5 ± 0.1

Table 2. The ratio of carbon ethanol isotopes ($\delta^{13}C_{VPDB}$, ‰) in wine samples obtained from grapes in major wine regions of the Russian Federation

Region	Geographical coordinates	Firm	Grape varieties	$\delta^{13}C_{VPDB}$, ‰
Krasnodar Region	44º45′07″ N 37º38′21″ E	"Villa Victoria"	Chardonnay Cabernet Franc	-26.55 ± 0.1 -26.70 ± 0.1
	44º45′07″ N 37º38′21″ E	"Myshako"	Riesling Cabernet Sauvignon	-27.01 ± 0.1 -27.14 ± 0.1
	45º11′26″ N 36º50′51″ E	Fanagoria	Aligote Cabernet Sauvignon	-26.48 ± 0.1 -26.59 ± 0.1
	45º11′25″ N 36º50′50″ E	"South Farm Firm"	Pervenec Magarach Cabernet Sauvignon	-26.83 ± 0.1 -26.52 ± 0.1
Rostov Region	47º40′55″ N 42º23′56″ E	"Wines of Tsimlyansk"	Krasnostop Zolotovsky Cabernet Sauvignon Tsimlyanskii Black	-27.11 ± 0.1 -27.24 ± 0.1 -27.05 ± 0.1
	47º40′55″ N 42º23′56″ E	Miller's winery Branch "Vederniki"	Rkatsiteli Cabernet Sauvignon Krasnostop Zolotovsky	-27.28 ± 0.1 -27.81 ± 0.1 -27.64 ± 0.1
Stavropol Region	44º44′20″ N 44º28′31″ E	"Levokumskoe"	Rkatsiteli Floral Cabernet Sauvignon Саперави	-27.97 ± 0.1 -26.87 ± 0.1 -27.85 ± 0.1 -27.61 ± 0.1
Republic of Dagestan	42º17′59″ N 47º39′51″ E	"Kirovskii"	Rkatsiteli	-26.38 ± 0.1
	47º40′55″ N 42º23′56″ E	"Tatlyar"	Rkatsiteli Agadai	-26.53 ± 0.1 -26.73 ± 0.1
	41º59′35″ N 48º09′53″ E	"Mugarti"	Aligote Chardonnay Cabernet Sauvignon	-26.68 ± 0.1 -27.14 ± 0.1 -26.81 ± 0.1

As we can see from Table 2, all the data are in the range from minus 26.38 ‰ to minus 27.97 ‰, i.e. they are within the range established by the interstate standard All-Union Standard 32710-2014 "Alcohol and raw materials for its production. Identification. The method of determining the isotope ratio of $^{13}C/^{12}C$ alcohols and sugars in juice and wine".

However, the problem of detecting the presence of exogenous alcohol in fruit wines remains unsolved. This is due to the fact that there is not enough research on $^{13}C/^{12}C$ ratio changes in fruit alcohols, depending on the geographical location of gardens and berry plantations, soil and climatic conditions, year of crop and on the fruit type. Moreover while producing table fruit wines it is allowed to add cane or beet sugar make before and during fermentation in order to provide the necessary alcohol conditions. The obtained alcohol consists of both fruit ethanol molecules which are endogenous component of wine and molecules of cane or beet ethanol, which on the one hand, are exogenous substance, but on the other hand they are allowed and their presence is justified by technological necessity. This fact makes it complicated to discover this type of fraud.

In 1997 C. Bauer-Christoph Back et al. studied isotopic characteristics of carbon ethanol of various origins, including those obtained by fermentation of fruit juice. [17] In their research the authors present the following results to determine $\delta^{13}C_{VPDB}$ (‰), which characterizes the ratio of carbon isotopes $^{13}C/^{12}C$ in fruit alcohols: ethanol apple – minus 25.90, pear – minus 26.16, Bartlett pear varieties – minus 26.84, cherry – minus 25.55, Mirabell – minus 25.71, plum – minus 26.11.

As we can see from the above data, $\delta^{13}C_{VPDB}$ indicator for ethanol produced from different fruit varies in a very small range – from minus 25.71‰ to minus 26.84‰.

For comparison they give data on some other alcohols and alcoholic beverages: grape spirit – minus 26.16‰, alcohol from grape husks – minus 27.09‰, grain (wheat) alcohol – minus 25.29‰, Scotch whiskey – minus 24.63‰, Bourbon whiskey (corn) – minus 13.48‰.

Using the method of isotope mass spectrometry Winterova R. et al. [18] determined isotopic characteristics of ethanol in fruit brandy (brandy) made from pears, apples, cherries, sweet cherries, plums and apricots. They were compared with the isotopic characteristics of beverages obtained from sugar beet, corn, sugarcane, grain, potato and synthetic alcohol. It was shown that isotopic characteristics (D/H) I distillates obtained from sugarcane, corn, and especially from synthetic materials are significantly

higher than the corresponding isotopic characteristics of fruit distillates. On the other hand distillates obtained from sugar beets, have lower isotope (D/H) I characteristic than fruit distillates. It was stated that distillates obtained from sugar cane and corn have value of $^{13}C/^{12}C$ from minus 13‰ to minus 11‰, which is significantly lower than in fruit distillates. According to the research the authors concluded that it was quite difficult to distinguish fruit spirits using only isotopic characteristics due to the imposition of $\delta^{13}S$ index within the same numerical range for alcohols from various fruits. On the other hand, the isotope parameters allow us to distinguish real fruit drinks from the beverage containing alcohol of non-grape origin (such as beet, cane or corn).

The aim of this work is to study the isotopic characteristics of ethanol obtained from domestic fruit and berries in order to give the possibility of using the method of isotope mass spectrometry while determining adulteration of table fruit wines.

OBJECTS AND METHODS OF STUDY

Objects of the research were fruit wines and ciders. Wine materials were obtained from seven types of fruits and berries (apple, pear, cherry, plum, pomegranate, black chokeberry (aronia), currants). Fermentation was performed at yeast race Cherry 33 without the addition of water and sugar. In order to study the possible impact of the variety of features and habitats of fruit on isotopic characteristics of biophilic elements, the research had several stages using fruits and berries of different breeds grown in different geographical regions.

All the measurements were made in accordance with standard All-Union Standard 32710-2014 “Alcohol and raw materials for its production. Identification. The method of determining the isotope ratio of $^{13}C/^{12}C$ alcohols and sugars in juice and wine”, based on the methodology of the International Organization of Vine and Wine (OIV) “Détermination du rapport isotopique $^{13}C/^{12}C$ par spectrométrie de mass isotopique de l'éthanol du vin ou de l'éthanol obtenu par fermentation des moûts concentrés ou des moûts concentrés rectifies” Résolution (Oeno 17/2001) OIV-MA-AS312-06.

The tool base for getting data on a carbon isotopic composition characteristics was a mass spectrometric complex Delta V Advantage of Thermo Fisher Scientific company (USA), providing precise analysis of the prevalence ratio of isotope $^{13}C/^{12}C$. Measurements of carbon isotopic characteristics were carried out on an international comparison sample V-PDB.

RESULTS AND DISCUSSION

Table 3 presents data on the carbon isotopic composition of domestic fruit and berry crops in 2014 and ethanol contained in obtained fermented wine materials. Investigations were carried out on five samples of each type of fruit and berries.

As it can be seen from Table 3, the carbon ethanol of fruit wine is noticeably lighter than carbon of organic components of fruits and berries. This is due to the fact that escaping carbon dioxide takes heavier carbon atoms with it [4]. As for the dependence of index values from the type of the original raw fruit, they are from minus 24.55 to minus 27.64‰ for fresh fruit and berries, and from minus 25.53 to minus 28.53‰ for ethanol of fermented wine.

Analyzing fruit and berry crops in 2015, grown in Russia, with the exception of pomegranate (Azerbaijan), the following results have been obtained: the black currant – minus 25.75 ± 0.08‰, cherry – minus 25.62 ± 0.06‰, chokeberry – minus 26.13 ± 0.26‰, pear – minus 27.04 ± 0.06‰, plum – minus 26.24 ± 0.41‰, apple – minus 27.58 ± 0.54‰ and pomegranate – minus 28.21 ± 0.22‰.

As we can see from these results isotopic characteristics of carbon ethanol in fruit wines obtained from different raw materials have similar values. For all fruits and berries it is possible to mark the total range of isotopic variations – from minus 25 to minus 28‰, which is consistent with literature data. However, the range may be quite narrow for wines obtained from separate raw materials.

Isotopic characteristics of ethanol in fruit wines were obtained through analysis of laboratory samples which had no exogenous sugars. In order to define possible differences industrial samples obtained from different manufacturers were analyzed. Physical and chemical parameters of analyzed wines are presented in Table 4.

Table 3. The ratio of $^{13}C/^{12}C$ isotope in fruit and ethanol in fermented fruit wine materials of the harvest in 2014

Raw material	$\delta^{13}C_{VPDB}$, ‰	
	Fresh raw material (direct combustion)	Fermented wine material (ethanol)
Strawberry	- 24.55 ± 0.08	- 25.92 ± 0.06
Raspberry	- 26.31 ± 0.32	- 27.46 ± 0.02
Blackcurrant	- 25.35 ± 0.08	- 26.24 ± 0.08
Cherry	- 25.13 ± 0.02	- 25.53 ± 0.07
Apricot	- 25.40 ± 0.55	- 25.54 ± 0.08
Apple	- 27.64 ± 0.01	- 28.53 ± 0.15
Chokeberry	- 25.69 ± 0.40	- 26.39 ± 0.12
Plum	- 25.71 ± 0.03	- 26.65 ± 0.24

Table 4. Physical and chemical parameters of fruit wines and the ratio of $^{13}C/^{12}C$ isotope in contained ethanol

Indicator name	Fruit wine "Blackcurrant"	Fruit wine "Chokeberry"	Fruit wine "Cherry"	Apple cider	Pear cider (puare)
Ethanol volume ratio, %	12.4	12.6	12.5	4.7	4.6
Total sugars, g/dm^3	1.8	2.6	1.7	1.0	1.2
Total titratable acids, g/dm^3	10.0	6.1	6.9	5.8	4.7
Total residual extract, g/dm^3	18.8	53.3	26.8	11.3	12.8
$\delta^{13}C_{VPDB}$, ‰	- 22.34 ± 0.10	- 19.07 ± 0.10	- 25.76 ± 0.10	- 21.74 ± 0.10	- 25.87 ± 0.10

As we can see from Table 4, δ^{13} C_{VPDB} (‰) in samples "Black currant", "Chokeberry" and apple cider significantly differs from the values of fruit ethanol. This is due to using the allowed method (sweetening with sugar cane before fermentation) or the banned use of glucose-fructose syrup and alcohol.

It is known that sugar beet and sugarcane belong to different groups according to the type of photosynthesis. Carbohydrates of sugar beets come in a range of carbon values from minus 24.5 to minus 26.0‰. Carbohydrates of sugar cane are enriched by "heavy" isotope ^{13}C due to the peculiarities of photosynthesis and have a carbon isotopic characteristics of minus 10 to minus 12‰.

During fermentation of carbohydrates of vegetable raw metabolic carbon dioxide, as it was mentioned previously, takes "heavy" isotopes of carbon with it, therefore, ethanol formed after fermentation contains less ^{13}C isotope than carbohydrates do.

In order to determine isotope ratios of ethanol biophilic elements formed as a result of fermentation of sugars of various origins, we prepared distillates from beet and cane sugars in laboratory conditions. Ethanol from sugar beet had $\delta^{13}C_{VPDB}$ (‰) in the range from minus 26.86 to minus 27.00, and from cane sugar the range was from minus 11.86 to minus 12.30.

According to the research results carbon isotope characteristics of ethanol samples from sugar beet practically coincide with $\delta^{13}C_{VPDB}$, typical for ethanol of fruit wines from various types of raw materials. Thus, this indicator does not always objectively and reliably detect falsification of products if exogenous sugars or alcohols derived from sugar beet are added. At the same time, the ethanol indicator $\delta^{13}C$ from sugar cane significantly differs from isotopic characteristics of beet and fruit ethanol.

We conducted research in order to determine processes of carbon isotopic characteristics change when alcohols of different exogenous origin were added in fruit wine materials. Spirits were added to fruit wine materials. Under laboratory conditions we prepared two samples of cider with added corn and barley spirits with isotopic characteristics of minus 13.8‰ and minus 24.59‰ respectively. Spirits were added to apple wine material substituting 15%, 25% and 50% of native spirits by exogenous ethanol. The test results are shown in Fig. 1.

According to the results presented below, adding 50% of alcohol from corn considerably changed isotope characteristics of carbon ethanol and went beyond determined ranges for apple ethanol. Barley alcohol alters isotopic characteristics of the product, but the ratio of carbon isotopes does not reveal the presence of exogenous alcohol, even if the amount of it is substantial.

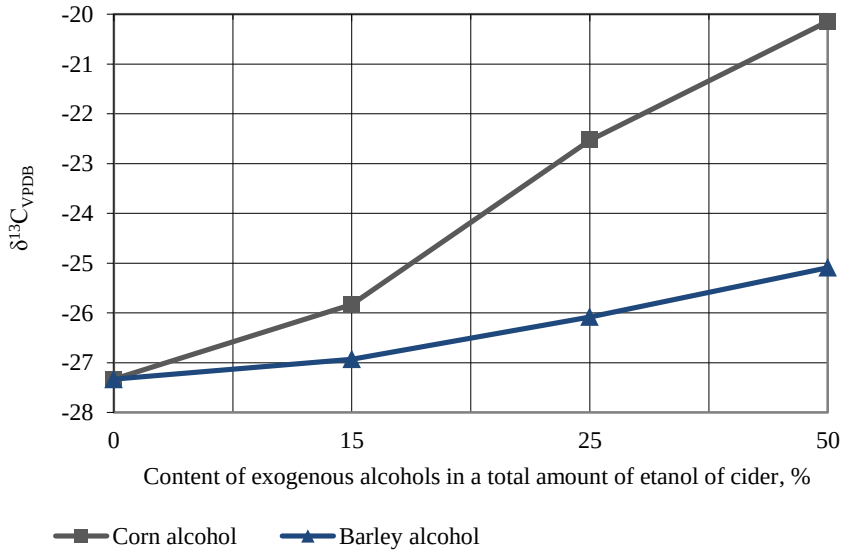

Fig. 1. Dependence of $\delta^{13}C_{VPDB}$ ‰ ethanol in cider depending on the amount of added (exogenous) spirits.

The current regulatory and technical documentation for fruit wines and cider allows adding sugar-containing components of various origins in their production. Under laboratory conditions we prepared fruit wine samples by adding various concentrations of cane and beet sugar in order to define possible fractionation of carbon isotopes, hydrogen and oxygen ethanol when adding sugars of various nature.

The work was carried out on the example of cherry wine. 6 samples of cherry wine with 15%, 25% and 50% sugars of various origins were prepared. Adding sugar replaced 15%, 25% and 50% of native sugar pulp. Control was made with the help of wine material without adding exogenous sugars. The initial mass concentration of sugars in cherry is 117 g/dm^3. Fermentation of the pulp was carried out on yeast Saccharomyces cerevisiae strain WET 136, at the rate of 0.25 g per 1 kg of raw material. Fermentation temperature was 20 ± 2°C, the fermentation process lasted 9 days. As exogenous sugars we used beet and cane sugar with their known isotopic characteristics: beet sugar with carbon isotopic characteristics minus 26.86‰; cane sugar with carbon isotopic characteristics of minus 12.3‰. Table 5 shows the main physical and chemical parameters and isotopic characteristics of ethanol in cherry wine.

Table 5. Basic physical and chemical parameters and isotopic characteristics of ethanol in cherry wine

Sample	Indicator name		
	Ethanol volume ratio, %	Total sugars, g/dm^3	$\delta^{13}C_{VPDB}$, ‰
cherry pulp + 15% of cane sugar	6.8	1.7	-25.20 ± 0.1
cherry pulp + 25% of cane sugar	6.7	2.1	-22.20 ± 0.1
cherry pulp + 50% of cane sugar	6.4	2.1	-19.32 ± 0.1
cherry pulp + 15% of beet sugar	6.9	1.1	-26.01 ± 0.1
cherry pulp + 25% of beet sugar	6.8	1.6	-25.94 ± 0.1
cherry pulp + 50% of beet sugar	6.7	1.7	-25.87 ± 0.1
control	6.9	1.1	-26.38 ± 0.1

As we can see from the data presented in Table 5, adding exogenous sugars leads to the shift of isotopic characteristics of ethanol in produced wine. This effect of re fractionation of stable biophilic isotopes is particularly noticeable when adding sugar from sugar cane. In this case adding even 15% of sugar increases "heavy" carbon isotope more than one unit.

The obtained results lead to the following conclusions:

– Carbon isotope characteristics of ethanol in fruit wines produced from different raw materials, have similar values. For all fruits and berries it is possible to mark the total range of isotopic variations of minus 25 to minus 28‰. The range may be quite narrow for wines obtained from separate raw materials.

– Using exogenous alcohols obtained from plants C3 – photosynthesis type results in a slight change of isotopic carbon characteristics of ethanol in fruit wines. When adding alcohols obtained from plants C4 – type the indicator $\delta^{13}C_{VPDB}$ changes noticeably; that allows us to detect their presence even in small amounts.

– In production of fruit wines and ciders when according to technology it is not allowed to add sugar to increase alcohol level, this method can detect exogenous alcohols from plants C4 type.

– To define significant differences between exogenous C3 alcohols introduced from the outside and obtained by fermentation of added beet sugar, in some cases it is not enough to use the only one indicator $\delta^{13}C_{VPDB}$ (‰). Therefore researches connected with determining isotope ratios of other biophilic elements of fruit ethanol, namely, oxygen $^{18}O/^{16}O$ and hydrogen D/H are promising.

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Please cite this article in press as: Oganesyants L.A., Panasyuk A.L., Kuzmina E.I., and Kharlamova L.N. Determination of the carbon isotope $^{13}C/^{12}C$ in ethanol of fruit wines in order to define identification characteristics. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 141–147. doi: 10.21179/2308-4057-2016-1-141-147.

CHEMISTRY AND ECOLOGY

EVALUATING AND IMPROVING THE EFFICIENCY OF THE USE OF ACTIVATED CARBONS FOR THE EXTRACTION OF ORGANOCHLORINE COMPOUNDS IN WATER TREATMENT TECHNOLOGY

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Received March 08, 2016; Accepted in revised form May 20, 2016; Published June 27, 2016

Abstract: Removing small amounts of organochlorine compounds from aqueous solutions is important for the water treatment and wastewater treatment. Sorption methods with the use of carbon sorbents proved to be very successful in some cases. The paper studies the adsorption equilibrium in the systems carbon sorbent (active carbons KAU, AG-OV-1, SKD-515, semi-coke) – aqueous solutions of chloroform, chlorophenol and carbon sorbent – aqueous solutions of mixtures of these organochlorines. The research holds that the known adsorption equations can be used to describe the adsorption equilibrium and to calculate the main parameters of sorption. The paper discusses the possible mechanisms of chloroform and chlorophenol sorption by active carbons from aqueous solutions. It identifies those brands of activated carbons, which most effectively extract chloroform and chlorophenol from the treated water. The authors examine the possibility of the reagent modification of the sorbents by acid and alkali solutions in order to increase the adsorption capacity.

Keywords: active carbon, chloroform, chlorophenol, adsorption, equilibrium, modification

DOI 10.21179/2308-4057-2016-1-148-153 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 148–153.

INTRODUCTION

Currently, the level of pollution is so high, that the ecosystem and people's health are endangered. This also applies to the natural water that is used for industrial and household needs. Over the last 10 years, the number of sub-standard samples for chemicals in the natural water sources, used for the centralized water supply in the Kemerovo region, has increased, and this indicates that the quality of the natural water has deteriorated. According to the data of the Kemerovo Centre of hydrometeorology and monitoring of environment (Kemerovo, Russia) – the branch of Western-Siberian Department of Sate Meteorological Service (Novosibirsk, Russia), the hazardous substances, including organochlorine compounds and a number of other substances, are found in the river Tom' and in the drinking water [1]. Summation indices show that such substances in the river water are up to 5 times greater than permissible levels; and up to 1.3 times higher in the drinking water [1]. In the preparation of drinking water, there occurs the partial transformation of its organic impurities and the formation of even more hazardous compounds than those that were originally present. This is why in Kemerovo, highly toxic organochlorine compounds in concentrations that exceed the permissible levels from 1.3 to 10 times (based on summation of the action) were found in the drinking water that was sterilized by active chlorine [2].

Adsorbents of different nature, including carbon material, are used to extract small amounts of substances from the aqueous media. In some cases their use in water purification was very successful. This paper studies the possibility and effectiveness of the use of activated carbons that are derived from different raw materials, and that have different physical and chemical properties, and surface structure, to extract organochlorine compounds (chloroform and chlorophenol). Chloroform and chlorophenol are the main water pollutants that are being formed when water is disinfected with the use of chlorine or chlorine-containing disinfectants.

OBJECTS AND METHODS OF STUDY

The paper examines the systems which include active carbons of KAU, AG-3, AG-OV-1, SKD-515, semi-coke (SC), as well as model aqueous solutions with varying content of chloroform (trichloromethane), chlorophenol (4-chloro-1-oksibenzol) and their mixtures [3]. The pre-treatment of active carbons and

semi-coke was carried out according to the method described in [4]. The sorbents were placed in distilled water for one day, then rinsed with fresh water to get rid of dust fractions, then they were dried to achieve the air-dry state, and then they were heated at 105.0 ± 0.10°C for 3 hours (to remove the "external" moisture [5]). Dried samples were cooled in a desiccator and stored in a hermetically sealed container.

The detection of chloroform in the aqueous solution was done by the method of gas chromatography as described in [6], and the detection of chlorophenol was done by the method described in [7].

The adsorption equilibrium in the system *aqueous solution – active carbon* was studied under static conditions by placing a constant accurate sample of sorbent in the aqueous solutions of chloroform, chlorophenol or their mixtures, with a given concentration; then the system was shaken for sufficient time to achieve the adsorption equilibrium [8]. The adsorption equilibrium time in the system *sorbent – solution* was determined in a previous series of experiments, and it did not exceed 20 hours. Thus, the duration of the contact between the *sorbent* and the *solution* was 24 hours for all samples.

Then the weighed amount was filtered off with a paper filter, and the concentration of the organic component was detected in the filtrate.

The amount of the equilibrium adsorption (a, mmole/g) was calculated using the formula:

$$a = \frac{C_0 - C_p \cdot V}{m}, \quad (1)$$

where C_o and C_p are the initial and the equilibrium concentration of the substance in solution, accordingly, mmole/dm^3; V is the volume of the solution from which the absorbtion is performed, dm^3; m is the mass of the sorbent sample, g.

The absorbtion isotherms were determined based on the experimental data. We used the Freundlich equation to describe the absorption equilibrium of the studied systems [9]:

$$a = \beta C_p^{\frac{1}{n}}, \quad (1)$$

where β represents the value of the absorption under the equilibrium concentration of the adsorptive, which equals 1 mole/dm^3; 1/n characterizes the degree of approximation to the isothermal line.

Langmuir equation [9]:

$$a = \frac{a_{\max} K \cdot C_p}{1 + K \cdot C_p}, \quad (2)$$

where a is the value of the adsorption at equilibrium concentration of C_p; a_{max} is the value of the limiting adsorption; K is the adsorption equilibrium constant.

BET equation [9]:

$$a = a_m \frac{K}{\left(1 - \frac{C_p}{C_s}\right) \cdot \left(1 + K - 1 \cdot \frac{C_p}{C_s}\right)} \cdot \frac{C_p}{C_s}, \quad (3)$$

where a_m is the adsorptive capacity of the saturated monolayer, which is determined by the size of the "landing" area of the solute molecule, mmole/g; K is the constant of the equation of the multilayer adsorption; C_p and C_s represent the concentration of the equilibrium and saturated solutions, respectively, mmole/dm^3, and the equation of Dubinin-Radushkevich to calculate the adsorption of substances from aqueous solutions [10]:

$$\lg a = \lg a_\infty - 2.303 \frac{R^2 T^2}{\beta^2 E^2} \left(\lg \frac{C_s}{C_p}\right)^2, \quad (1)$$

where T is temperature, K; β is the coefficient of similarity; E is the characteristic energy, kJ/mole; C_s and C_p represent the concentration of the equilibrium and saturated solutions, mmole/dm^3.

RESULTS AND DISCUSSION

We studied the equilibrium adsorption of chloroform and chlorophenol from unicomponent (for organic substance) aqueous solutions. The absorbents were active carbons and semi-coke. We used a broad range of the concentrations of the extracted components. The purpose was:

– to determine if the known absorption equations can be applied to describe the sorption behaviour of the studied systems;

– to calculate the basic sorption parameters;

– to study establishing adsorption mechanisms;

– as well as to measure the changes in the absorption mechanisms as the concentration increases during the transition from a low concentration to the limiting solubility of the components in water.

Experimental isotherms of the chloroform absorption are shown in Fig. 1.

Based on their shape, the isotherms of the chloroform absorption can be referred to the isotherms of L-type under the classification [11]. This allows us to suggest the physical nature of the interaction *sorbent – sorbtive*.

We found that the above equations satisfactorily describe the adsorption isotherm of chloroform. Hence, these equations can be applied for the calculation of the basic adsorption parameters needed to create the sorption technology of the extraction of contaminants from water. The adsorption isotherms have a classic form, and the maximum adsorption capacity increases in the following sequence:

SC < AG-OV-1 < AG-3 < SKD-515 < KAU.

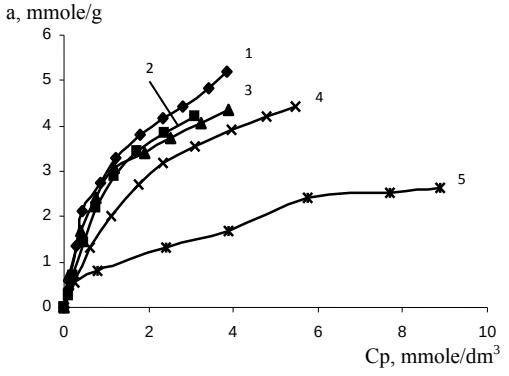

Fig. 1. The isotherms of the absorbtion of chloroform from aqueous solutions: 1 – KAU; 2 – SKD-515; 3 – AG-3; 4 – AG-OV-1; 5 – SC.

The data presented in Fig. 1 show that the preference in the choice of the brand of the activated carbon used to extract the chloroform should be given to the KAU. However, given the cost of raw materials for the production of this type of coal (the shell of pits from fruit trees) and the lack of the industrial capacity for its production, the recommended brands of active carbons to extract chloroform must be SKD-515 and AG-3, which have similar sorption characteristics with respect to chloroform.

We calculated the basic parameters for the adsorption of sorbents, that are supposedly effective for the chloroform extract from aqueous solutions (Table 1).

Table 1. The parameters of chloroform adsorption

sorbent Brand	Types of equations									
	Dubinin-Radushkevich			Freundlich		BET			Langmuir	
	a_{max}, mmole/g	E, kJ/mole	W, dm^3/kg	β	1/n	a_{max}, mmole/g	K	Q, kJ/mole	a_{max}, mmole/g	K
SKD-515	8.0	8.5	0.9	2.3	0.7	5.5	33.5	8.8	6.3	0.7
AG-3	5.9	10.5	0.6	2.6	0.4	4.6	58.7	10.1	5.3	1.1
SC	3.5	7.0	0.4	1.5	0.7	3.2	9.9	5.9	5.0	0.1

Due to the fact that chlorophenol often accompanies chloroform in purified water, and, as a rule, the concentration of chloroform is higher than chlorophenol, to extract both components from the purified water and to study the equilibrium sorption chlorophenol, we selected active carbons SKD-515 and AG-3. The interaction between the semi-coke SP and chlorobenzene were of particular practical interest. It is not only because it is significantly less expensive and is manufactured in Kuzbass, but because after the adsorption, it can be used as fuel. Fig. 2 shows the experimental adsorption isotherms of chlorophenol from aqueous solutions by the activated carbons SKD-515, AG-3 and SC.

The sorption behaviour of chlorophenol with these brands of adsorbents is similar to the behaviour of chloroform. The earlier mentioned adsorption equations can be used to describe isotherms of adsorption and for calculating the basic parameters of adsorption. The chlorophenol sorption parameters are shown in Table 2.

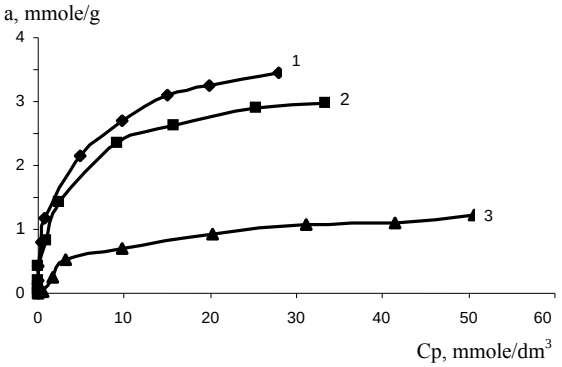

Fig. 2. The isotherms of the absorbtion of chlorophenol from aqueous solutions: 1 – AG-3; 2 – SKD-515; 3 – SC.

Table 2. The parameters of the chlorophenol adsorption

sorbent Brand	Types of equations									
	Dubinin-Radushkevich			Freundlich		BET			Langmuir	
	a_{max}, mmole/g	E, kJ/mole	W, dm^3/kg	β	1/n	a_{max}, mmole/g	K	Q, kJ/mole	a_{max}, mmole/g	K
SKD-515	2.9	14.1	0.3	1.0	0.3	2.7	138	12.2	3.1	0.4
AG-3	3.6	13.5	0.4	1.1	0.4	3.0	186	13.0	3.5	0.6
SC	1.7	8.0	0.2	0.1	0.7	1.0	66.9	10.4	1.4	0.1

The analysis of the values of the adsorption volume W, presented in Tables 1 and 2, which are in the range of 0.2–0.4 dm^3/kg for the chlorophenol adsorption and 0.4–0.9 dm^3/kg for the chloroform adsorption, leads to the conclusion that the adsorption of chlorophenol and chloroform from individual aqueous solutions is subject to micropore volume filling mechanism. The characteristic energy values E of the chloroform adsorption (Table 1) and chlorobenzene (Table 2) indicate that the adsorption takes place mainly in the micro- and meso-pore adsorbents [12].

The heat of the adsorption of chloroform by the tested sorbents' brands does not exceed 10.13 k/mole (Table 1), which indicates that the adsorption of chloroform is caused by the van der Waals forces (non-specific interaction). The heat of the chlorophenol adsorption by the tested sorbents is significantly higher than of the chloroform absorption (Table 2). This indicates that in addition to the dispersion of nonspecific interactions, we observe a specific physical adsorption. This is due to the fact that the oxygen-containing surface functional groups of activated carbon can form hydrogen bonds with the substituents of the aromatic ring of chlorophenol. In addition, the aromatic ring of chlorophenol is an acceptor of the electron density. This allows us to assume the formation of donor-acceptor complexes of the type *dipole – dipole* attraction, as the donor is able to

provide an electron pair to bond formation; whereas the acceptor (chlorophenol) does not have a vacant orbital. During the chlorophenol adsorption, the oxygen of surface carbonyl groups plays role of an electron donor, whereas the π-electron system of the aromatic ring plays role of an acceptor [13].

Thus, the study of the equilibrium of the adsorption of chlorophenol and chloroform from individual aqueous solutions by the tested brands of carbons allowed us to calculate the basic adsorption characteristics and to determine that the carbon brands SKD-515, AG-3 are the most effective abstractors of organochlorines from aqueous solutions. In the case of chloroform, semi-coke SC has a low adsorption capacity, compared with SKD-515 и AG-3. In this regard, we did not proceed with the further research of the sorption behaviour of the semi-coke.

We studied the equilibrium sorption of a mixture of chloroform and chlorophenol from an aqueous solution with the range of concentrations used in the actual practice of water treatments with chloramines for disinfection (Fig. 3 and 4). We tested the adsorption of chlorophenol and chloroform from their mixtures in the components' ratio 200 : 1 (mmole/dm^3) using SKD-515 and AG-3. Having compared the adsorption isotherms of chlorophenol and chloroform from their aqueous mixtures with the adsorption isotherms of the aqueous solutions of the individual components (Fig. 3 and 4), we found that the absorption is weaker when the two components are combined. In this case, the adsorption of chloroform is higher than the chlorophenol adsorption, probably due to its lower solubility in water ($C_{Schlorophenol}$ = 210.8 mmole/dm^3, $C_{schloroform}$ = 68.67 mmole/dm^3). It is also lower due to its smaller (compared to chlorophenol) van der Waals' molecule size (0.64 nm chloroform; 0.8 nm chlorophenol).

When extracting organic compounds from their aqueous mixtures we observed the change of the isotherm shape for chlorophenol (Fig. 4, curves 3 and 4) from L to S_4 [9]. That may indicate a change in the nature of the components' interactions in the solution and with the surface of the AC. Changing forms of adsorption indicates a fierce competition between the molecules of chlorophenol, chloroform and water for room in the AC's pores.

We found that the Freundlich and BET's equations don't work for the description of the adsorption equilibrium in the system *water – chlorophenol – chloroform – AC* due to the large deviation of the calculated adsorption isotherms from the experimental ones. The adsorption isotherms based on the equations Dubinin-Radushkevich and Langmuir coincide with the experimentally obtained isotherms. Table 3 represents the equilibrium sorption parameters calculated using this equation.

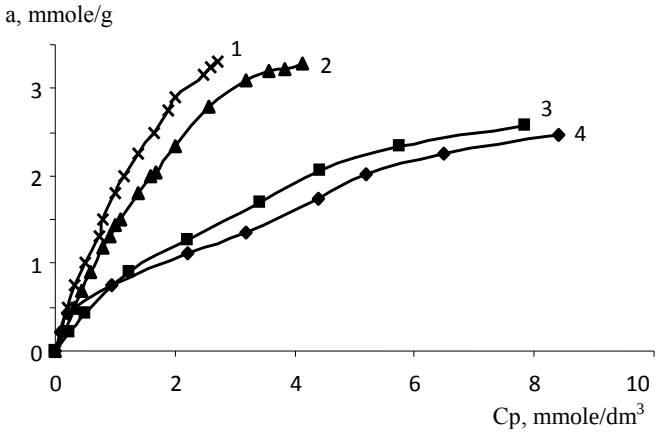

Fig. 3. The adsorption isotherms of chloroform from an aqueous solution: 1 – SKD-515; 2 – AG-3; 3 – SKD-515 (in the presence of chlorophenol); 4 – AG-3 (in the presence of chlorophenol).

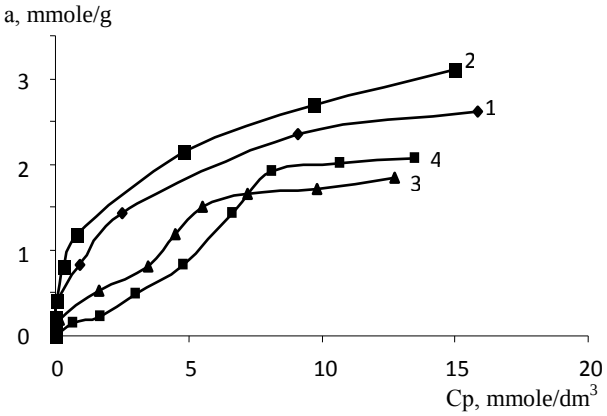

Fig. 4. The adsorption isotherms of chlorophenol from aqueous solutions: 1 – SKD-515; 2 – AG-3; 3 – SKD-515 (in the presence of chloroform); 4 – AG-3 (in the presence of chloroform).

Table 3. The parameters of chlorophenol and chloroform adsorption from aqueous solutions

Sorbent Brand	Type of equations									
	chlorophenol adsorption in the presence of chloroform					chlorophenol adsorption in the presence of chloroform				
	Dubinin-Radushkevich			Langmuir		Dubinin-Radushkevich			Langmuir	
	a_{max}, mmole/g	E, kJ/ mole	W, dm^3/kg	a_{max}, mmole/g	K	a_{max}, mmole/g	E, kJ/ mole	W, dm^3/kg	a_{max}, mmole/g	K
SKD-515	8.0	8.5	0.9	6.3	0.7	2.9	14.1	0.3	3.1	0.4
AG-3	5.9	10.5	0.6	5.3	1.1	3.6	13.5	0.4	3.5	0.6

The limit value for the adsorption volume W of active carbon SKD-515 and AG-3 vary from 0.3 to 0.4 dm^3/kg (for chlorphenol) and from 0.6 to 0.9 dm^3/kg (for chloroform). This allows us to suggest that the adsorption of chlorophenol and chloroform, when they are both present in the solution, is subject to

the volumetric filling of the micropores. The characteristic energy values indicate that the chloroform and chlorophenol sorption at their simultaneous presence in the solution occurs mainly in the meso- and micropores. The values of the heat adsorption indicate a non-specific dispersion interaction of chloroform with the sorbent. As for chlorophenol, the values of the heat adsorption also indicate a certain contribution to the specific physical adsorption on active centers. The type of AC defines the distribution of macro-, micro- and mesopores, which, in its turn, determines their availability for the molecules of sorbed substances [13]. For chloroform, sorbed due to the non-specific interaction, the only option is the adsorption in the pores. For chlorophenol, the adsorption on oxygen-containing groups is possible, as evidenced by the data in Fig. 2 and Fig. 4, from which it follows that the chlorophenol sorbed by AC AG-3 is better than by AC SKD-515. We found that the surface of AC-3 is more oxidized and it contains a variety of oxygen-containing groups, which significantly contributes to the overall amount of adsorption.

Thus, based on the equilibrium sorption studies, we established the main parameters of the chloroform and chlorophenol adsorption by active carbons from the individual aqueous solutions and aqueous mixtures of these components. We analyzed the adsorption mechanisms which determine the adsorption capacity and efficiency of extraction. This allows us to identify the brands of activated carbons which possess the optimum extraction properties.

The modification of the sorbents

The major principle of the development of the adsorption technology is to increase the adsorption capacity of sorbents. This can be achieved by changing the structure and surface of the adsorbent through the use of various types of modifiers. The modification of carbon materials is widely used to increase their lyophilic and adhesive properties, as well as their reaction and ion-exchange capacity. The solutions of mineral acids, bases, organic reagents, as well as various types of oxidizing agents are used as modifiers. We studied the liquid phase reagent modification.

In [14] it is noted that the presence of iron salts in the active carbon catalytically decomposes some organic adsorbing substances, so it should be removed from the surface of active carbon during the adsorption studies.

To found that during the AC treatment with diluted hydrochloric acid and sodium hydroxide solutions, with a concentration of 1.5–2.0 mole/dm^3, the iron ions (III) are intensely washed off. This is evidenced by the ash content of the respective carbons, which is reduced on average by 18% [2].

We found that the treatment of active carbons with solutions of hydrochloric acid and sodium hydroxide reduces the adsorption capacity of chloroform, as evidenced by the data in Fig. 5.

The decrease in the adsorption of chloroform in the carbons treated by reagents, may be associated with the transition of part of the micropores into mesopores. We found that the reagent processing changes the state of the surface of the adsorbent, which may occur due to the formation of additional oxygen-containing functional groups of the acid type, which is evidenced by the potentiometric titration by Bem [15] (Fig. 6).

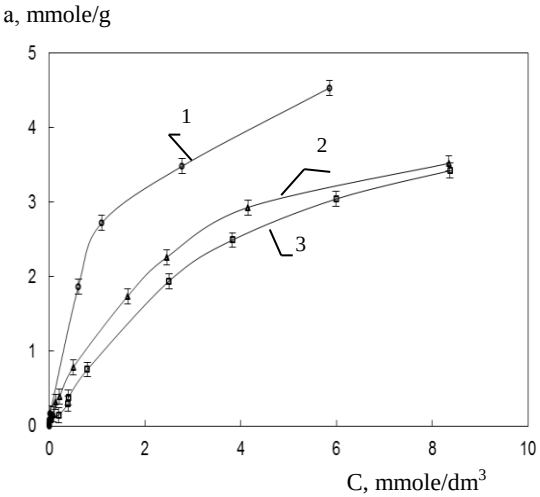

Fig. 5. The adsorption isotherms of chloroform on the SKD-515: 1 – Original; 2 – processed by HCl; 3 – treated by NaOH.

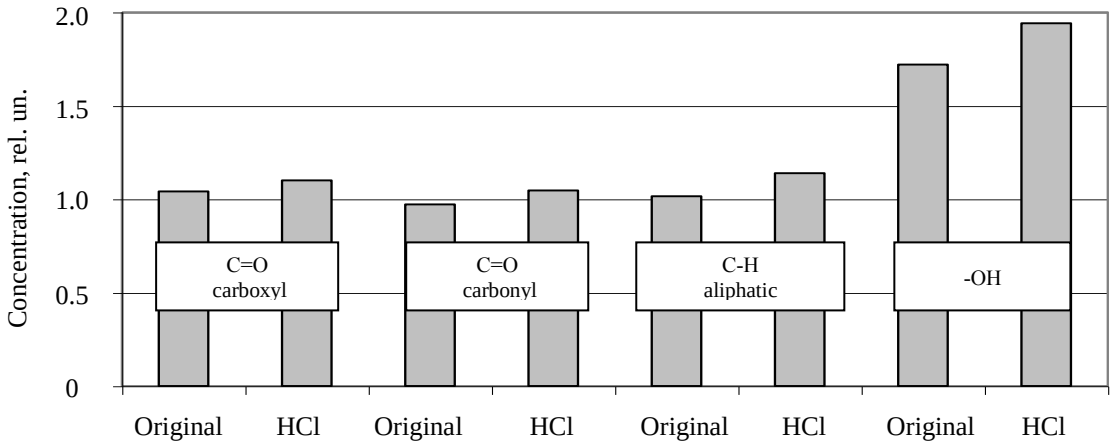

Fig. 6. The effect of reagent treatment on the surface condition of AC SKD-515.

The AC reagent treatment with hydrochloric acid or sodium hydroxide leads to an increase in the sorption capacity with respect to chlorophenol, which is connected, as shown above, with the ability of chlorophenol to the specific interaction with the surface formed after the treatment with oxygen-containing functional groups. In this regard, we should be selective in the treatment (modification) of AC, used for the extraction of chloroform and chlorophenol from aqueous solutions. We should take into consideration the concentration ratio of the components in the solution, since an increase in the sorption capacity for one component of the test mixture is accompanied by a decrease in another.

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Please cite this article in press as: Kirsanov M.P. and Shishkin V.V. Evaluating and improving the efficiency of the use of activated carbons for the extraction of organochlorine compounds in water treatment technology. *Foods and Raw Materials,* 2016, vol. 4, no. 1, pp. 148–153. doi: 10.21179/2308-4057-2016-1-148-153.

ECONOMICS

MODELING OF THE AGRIBUSINESS ENTERPRISE ACTIVITY ON THE BASIS OF THE BALANCED SCORECARD

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Received February 07, 2016; Accepted in revised form March 20, 2016; Published June 27, 2016

Abstract: In contemporary economic conditions, when enterprises function in the environment of uncertainty, the search of new approaches to strategic management of enterprises becomes the objective of the management level. The formation of the mechanism of enterprise management improvement, which allows adapting management system to the changes of external environment, providing their high performance and competitiveness, is a very topical objective. The article substantiates the advisability of applying the balanced scorecard (BSC) for strategic enterprise management. The paper determines the sequence of using the balanced scorecard to assess the effectiveness of the strategies implementation of agribusiness enterprises. The authors have designed the economic-mathematical model of activity of manufacturing enterprise in the form of a multi-parameter problem of linear optimum management. It allows evaluating the strategy of its development considering the peculiarities of the agribusiness enterprise and the concept of BSC. The specified model has been approved by the example of an operating enterprise. With the help of automated software product numeric experiments have been conducted, describing various scenarios of the development of the agribusiness enterprise on the basis of the multi-parameter analysis of a number of key components of BSC, with the aim to reveal their mutual connection in the optimum regime. The authors draw conclusions about the advisability of accounting revealed parameter correlations and regularities while making a strategic map of BSC. It is stated that the application of BSC may be the basis for methodology of development and administrative decision making both at the present moment, and in the strategy of agribusiness enterprises management taking into account the specificity of their development.

Keywords: balanced scorecard (BSC), math modeling, strategic management, food resources (food)

DOI 10.21179/2308-4057-2016-1-154-162 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 154–162.

INTRODUCTION

Contemporary conditions of economic management, necessity to implement the policy of import substitution in the area of food security, caused by sanctions which were introduced by the West towards Russia, require reconsideration of established practice of managing agribusiness enterprises, condition the necessity of working-out scientifically substantiated recommendations to improve the efficacy of their manufactory-commercial activity. Worn-out state of capital assets, the lack of financial resources, the use of outdated technologies, low level of management are characteristic to the majority of contemporary agribusiness enterprises, that hampers significantly their development.

The improvement of agribusiness enterprise management is one of the primary objectives of effectiveness increase of their commercial activity. It is conditioned firstly by the competition strengthening on the part of foreign commodity producers, and also by the high dynamics of business environment development. The economy management in market conditions requires from business management the assimilation of mechanism of the development and strategy realization, adaptation of the current activity to strategic goals, search of new means of efficiency evaluation of effectiveness of business entities functioning.

In this connection in front of the heads of the enterprise the solution of the problem of the mechanism formation of management improvement,

which allows adapting the management system to the changes in the external environment in the conditions of uncertainty, providing their high performance and competitiveness, is very topical.

According to the authors this problem may be solved by applying the balanced scorecard (BSC) in management. It presents an innovative approach to management and allows defining the goals of enterprise development more precisely for short-term and long-term periods, coordinating all subjects' interests, which operate in the agricultural sector, and making more informed and qualitative managerial decisions.

The methodology of effectiveness assessment of organizations' strategies should be based on tools, which provide the strategy implementation in the course of a current activity. The balanced scorecard is the classical tool of practical realization of strategies [1, 2, 3]. Clearly, BSC as the technology of strategy realization is considered neither the only true, nor reliably effective, but it is a very widely spread and has become one of the standard solutions. BSC includes several key components – structuring the organization activity as a single system in four interconnected prospects (financial, cliental, internal processes, teaching and growth), building a strategic map, which reflects strategic goals in the context of these prospects and interconnection between them, the formation of corresponding goals of administrative indicators [1, 3].

BSC is applied at all stages of a management cycle – starting from targeting and planning and finishing with the analysis, control that allows using it to assess the effectiveness of strategies realization. BSC reflects an integral approach to managing a complex system. It is in the fact that a clear logic of interconnection of strategic goals and indicators is built, the factors and conditions of final results achievement become clear. The most important feature of BCS, from the authors' point of view, is not only the presence of goals and indicators in various aspects of activity, but cause-effect connections between them. They show what and in what sequence should be done (and also what shouldn't be done, since it doesn't contribute to the achievement of strategic goals). It allows putting all actions in their places, determining their significance and priority. While designing a strategic map and selecting indicators it becomes obvious what strategic goals as if "are hanging in the air", not being backed with the goals of underlying perspectives, and also what underlying goals don't make contribution into the achievement of final results [4].

In contemporary economic conditions of management, the balanced scorecard is more frequently used for management of organizations, since an effective management based only on financial indicators becomes impossible. BSC allows uniting and varying financial and nonfinancial indicators of effectiveness [5]. This system may reflect all basic business processes and include internal indicators of the organization activity, through which one can regulate the market changes, the degree of consumer satisfaction, and also evaluate the development strategy of organization and its realization in the real scale of time. Despite positive moments of application, BSC requires specification and highlighting the specificity for each sector of economics. The activity of agribusiness enterprises, as a rule, is characterized by seasonality and high risks, and in consequence, the introduction of BSC requires a specific algorithm of application [6].

Representing a perspective model of strategic management, BSC allows: transferring the development strategy of agricultural organization in the system of measurable indicators and determining the sequence of actions in achieving their target values; influencing the system of personnel motivation, since formulated goals for the personnel have an impact on their behavior. At a correct goal definition, the personnel begin to understand their contribution into the achievement of company's strategic goals, thus the possibility of realization of the developed strategy is increased.

The system of key indicators of effectiveness is a tool of dataware of the process of managerial decision-making, the determination of the organization's goals taking into account how much the achievement of the set goal increases the company's worth, the availability of information system, which is the source of data and the base for defining the key indicators of effectiveness. When evaluating the effectiveness of structural units, it is necessary to reconsider the principles of stimulation of structural units, since the evaluation system of key indicators of effectiveness is limited to the assessment of the activity of each of them [7, 8, 9].

It should be noted that BSC isn't the instrument for strategy development, and is used only for the realization of an existing strategy [10, 11]. On the basis of verbal or another presentation of a ready strategy the strategic map is built, which is the foundation for BSC. If the organization strategy itself doesn't have a balanced character, it doesn't reflect various aspects of achievement of final goals, it is impossible to correct this shortcoming by means of applying BSC [4].

The main problem of effectiveness evaluation of strategy implementation based on BSC is to form data support of the calculation of indicators. BSC, according to its authors, causes essential difficulties in part of selection, in a certain sense, nonstandard, innovative indicators, which were not used in the management practice earlier (to the greatest extent it concerns leading indicators of perspectives "training and growth", "internal processes"). There are no ready patterns or tools in this sphere (at least, in the press). Besides, the appearance of new indicators requires the presence of new data in the information-communication model.

On the basis of examined approaches to defining the composition of indicators [12, 13, 14], we can draw conclusions that while developing and introducing BSC in the agribusiness enterprises of any section, one shouldn't limit oneself only by one area of activity (management accounts, personnel, the quality management system), the complex approach is necessary. The selection of indicators depends on specialization of the enterprise activity, and also on its management goals.

In general, the process of introduction of BSC in an enterprise includes the implementation of the following stages:
(1) Goal setting, the achievement of which will contribute to the realization of mission and enterprise strategy (balance).
(2) The determination of indicators, for measuring the level of achievement of each goal.
(3) Working-out activities that should provide a desired level of indicators (cascading).
(4) Introduction of BSC in the current enterprise activity.

The strategic goals of the company are "decomposed" in the set of company indicators and on this basis the strategic map of BSC is built [15]. The strategic map of the balanced scoreboard of the agribusiness enterprise is the scheme of strategy description as an iterative process, which allows an organization's specialist to formulate, specify, correct strategic goals and their modification with the help of intraeconomic decomposition methods.

However, the practice of building a map of the balanced scorecard of the enterprise activities is associated with a number of interrelated problems: the selection of strategic indicators and their distribution according to perspectives, the detection of interconnections of indicators inside one and higher perspective, the detection of the strength of interconnection between indicators; building the map in a science-based manner, but not approximately "by eye", relying often only on the practical experience.

We propose to highlight the following levels of solutions of above mentioned problems:
(1) Development of the system of indicators and detection of their target values.
(2) Multi-parameter analysis of enterprises activity taking into account BSC with the aim to reveal their mutual connection optimally.
(3) Detection of the interconnection strength between indicators and the balance of the cost according to perspectives of BSC.
(4) Building the strategic map and development of the support systems of decision making at the strategic enterprise management based on the BSC.

The consecutive solution of enumerated problems will allow working out science-based approach to building the map of BSC of the enterprise, developing typical maps of BSC and carrying out the support of decision making at strategic enterprise management of various sector orientation.

The recommended compositions of indicators of BSC for agricultural enterprises and the food industry enterprises are reflected in the works [7, 15, 16, 17] and may serve as the foundation for the solution of the second level problem, which is presented in the experimental part of our research. It should be noted that the positive solution of the second level problem may allow detecting the cost balance according to perspectives, the strength of interconnection between the indicators of BSC, that will give the opportunity to build typical strategic maps based on the objective, automated instrument.

OBJECTS AND METHODS OF STUDY

The research object of this paper is the agribusiness enterprise. The subject of the research is the efficiency evaluation of functioning of the enterprise under consideration from the point of view of BSC according to the criterion of the maximum of the present value at a given planning horizon. The goal of the study is modeling of agribusiness companies based on the balanced scorecard for detecting their optimum parameters.

The paper uses the following methods of research:
(1) economic-mathematical modeling of activity of the manufacturing enterprise;
(2) numeric investigation of the built model with the use of the software package "KARMA", described in the work [18];
(3) construction of algorithms of detecting the potential, presence and the strength of interconnection between the indicators of activity and building the strategic map of industrial enterprise with the use of BSC.

Given the above, the following algorithm of the study is offered:
(1) to select target indicators according to perspectives;
(2) to describe real numeric values of financial indicators;
(3) to describe the activity of an industrial enterprise in the form of a multi-parameter problem of linear optimum management;
(4) to investigate the constructed model with the help of a financial-analytical automated information system;
(5) to interpret the received results.

The economic-mathematical model of the enterprise activity is designed considering the following prerequisites. The implementation of the principle of pure industries is supposed: one team of workers produces one kind of goods at one type of the fixed production assets. The profit is calculated as the balance of income (aggregate revenue from products sale) and expenditure (depreciation of fixed production assets, renumeration of labor, taxes, material and financial costs, advertising and personnel training). An expert specification of a number of indicators of activity and characteristics of external environment of the enterprise is supposed. To detect the potential of its activity the problem of optimum management in the form of linear programming problem is formulated.

Let's introduce the following nominations for the economic-mathematical modeling of the activity of the agribusiness enterprise:
PV – present value of the enterprise;
n – quantity of kinds of products; k=1,…,n;
x_k – quantity of production workers of k product, x_{n+k} – production output of k product, x_{2n+1} – volume of credit, x_{2n+2} – volume of grants;
c_k – value of k fixed production assets [monetary unit/unit of fixed production assets];
P_k – product cost, produced in k fixed production assets, [monetary unit/product unit];
T_k – time of beneficial use of k fixed production assets [time unit];
q_k – actual demand on the product, made in k fixed production assets, for the period T, [time unit/product unit];

θ_k – material costs for the production of the product unit of k type [monetary unit];
V_k – worker efficiency of k fixed production assets for the period T, [product unit/worker unit];
S_k – productive wage, working on fixed production assets of k type for the period T [monetary unit /worker unit];
α_i (i=1,2,3,4) – taxation rates on the added value, property, profit and insurance contributions in state extra-budgetary funds correspondingly;
β_1 – share of proceeds from industry sales, spent on advertising;
β_2 – share of proceeds from industry sales, spent on personnel training (retraining);
T_0 – credit term [unit of time];
r_0 – annual credit rate;
S_{max} – maximum credit sum [monetary unit];
Dot_{max} – maximum grant sum [monetary unit];
DS^0 – initial amount of a producer's capital [monetary unit];
T – planning horizon [unit of time];
r – discount rate;
R – net sales of all products [monetary unit].

Using the introduced nominations and indicators, mathematical model of the enterprise activity takes the form of the following multi-parameter linear programming problem:

$$\sum_{k=1}^{n}\rho_k x_k - (1-\alpha_3)\sum_{k=1}^{n}\sigma_k x_{n+k} - \frac{r_0\ 12T_0+1}{24}\ x_{2n+1} - x_{2n+2} \le DS^0, \quad (1)$$

$$x_{n+k} \le V_k x_k, \quad (2)$$

$$P_k\ x_{n+k} \le q_k, \quad (3)$$

$$x_{2n+1} \le S_{max}, \quad (4)$$

$$x_{2n+2} \le Dot_{max}, \quad (5)$$

$$x_m \ge 0 \qquad (m=1,\ldots,2n+2), \quad (6)$$

$$PV = \frac{T}{1+r_э}\left(-\sum_{k=1}^{n}\rho_k x_k + (1-\alpha_3)\sum_{k=1}^{n}\sigma_k x_{n+k}\right) \to max, \quad (7)$$

where $\rho_k = c_k(1-T/T_k)+(1-\alpha_3)\varphi_k$, $\varphi_k = c_k(\alpha_2+(1-\alpha_2)T/T_k)+$ $+(1+\alpha_4)S_k$, $\sigma_k = (1-\alpha_1-\beta_1-\beta_2)P_k - \theta_k$, ($k$=1,…,$n$),
$1+r_э = rT \Big/ 1-(1+r)^{-T}$,

r_e – efficient discount rate, considering dynamic peculiarities of the indicator of present value PV, in assumption of approximate uniformity of exercising expenses and receiving income per time.

Let's make some observations concerning correlations of the presented model. The inequation (1) reflects the condition of the enterprise solvency; (2), (3) – correspondingly the conditions of limitation of volumes of produced commodities to the level of the production capacity of fixed production assets (or, otherwise, the level of scientific-technical progress) and the level of cost demand on the production on the whole planning horizon. The inequations (4), (5) reflect limitations on the volumes of the enterprise activity financing, and (6) – natural restrictions of meaningful non-negativity of variables. The condition (7) maximizes the present value PV of the enterprise.

The model (1)–(7) is adapted to the analysis of the agribusiness enterprise activity. In particular, it contains parameters, considering:
– high material cost of agricultural production (through the parameters θ_k);
– high wage capacity (through the parameters S_k);
– increased lifetime of fixed production assets in agribusiness (through the parameters T_k);
– peculiarities of demand, being characterized by relative constancy, on the production of agricultural enterprises (through the parameters q_k);
– subsidized character of agribusiness enterprises (through the parameter $Dot_{max)}$. Besides, the possibility of application of special tax treatments is admitted, particularly, unified agricultural tax for agricultural enterprises, essentially decreasing the tax burden on agricultural commodity producers and primary processor of agricultural raw materials.

Thus, the model (1)–(7) connects key components of BSC, considers the interaction of flows of material (the number of workers and production volume) and nonmaterial (financial, advertising activity, personnel training activity) character. It allows accumulating personnel experience and knowledge, paying attention to financial stability, production development, the increase of the customers' quantity. Along with that, considering the multi-parameter character of the model (1)–(7), the decision maker has an opportunity to conduct practically unlimited number of numeric experiments according to the multi-parameter analysis of BSC.

RESULTS AND DISCUSSION

Let's examine the application of the model (1)–(7), from the point of view of BSC, by the example of the operating enterprise of an agricultural sector – pig complex, which produce two types of products (n = 2): meat in live weight on a pig farm and meat in carcass weight at slaughter hall. The approbation of the model has been done by the example of the activity of the limited company of agro-industrial complex "Chistogorskii", situated in the village Chistogorskii of the Novokuznetskii District, Kemerovo Region. The enterprise is the largest farm in the animal production and meat processing in the region. At present "Chistogorskii" Ltd. acts successfully as an industrial complex. It is built for growing 250 thousand pigs a year that makes more than 22 thousand tons of meat in live weight or 15 450 tons in carcass weight. It is the leading enterprise of the Western Siberia for breeding of highly productive breeds: Kemerovo and Krupnaya Belaya (have the status of stud farm), Landras and Dyurok (the status of breed reproducer). Since 2013 "Chistogorskii" Ltd. has been the first and the only one enterprise in Russia, which has been producing pure breeding of the super-meat pig breed "P'etren". The data for the model are taken from financial-economic accounting of the commodity producer for 2014 (Table 1).

Table 1. Target values of the balanced scorecard for "Chistogorskii" Ltd.

Perspective	Objective	Nomination of activity indicators	Method of calculation / comments	Indicators value at 1.01.2015 г.	Units of measurement
Finances	Maximization of the present value	PV – Present value	balance on incomes and costs, discounted on the whole planning horizon T at the rate r	r=30	thousand of rubles per cent
		α_i (i=1,2,3,4) – taxation rates on added value, property, profit and insurance contributions in state extra-budgetary funds correspondingly	α_1 – taxation rates on the added value, Chapter 21 of the Tax Code of the Russian Federation α_2 – taxation rates on property, Chapter 30 of the Tax Code of the Russian Federation α_3 – taxation rates on property, Chapter 35 of the Tax Code of the Russian Federation α_4 – insurance contributions in state extra-budgetary funds, Federal Law from 24.07.2009 No. 212-FZ "On insurance contributions into the Pension Fund of the Russian Federation, Fund of Social Insurance of the Russian Federation, Federal Fund of Compulsory Medical Insurance" *or* α'_3 – unified agricultural tax, Chapter 26.1 of the Tax Code of the Russian Federation	α_1=18 α_2=2.2 α_3=20 α_4=30 α'_3=6	per cent
		T_0, r_0, S_{max} –credit term, annual credit rate and maximum credit sum correspondingly	T_0 – at the request of the borrower r_0 – by agreement with the creditor S_{max} – according to the method of creditor	T_0= 5 r_0 = 30 S_{max} = 60 000	years, annual interest rate, thousand rubles
		Dot_{max} – maximum grant sum	according to the organization accounting	96 935	thousand rubles
		DS^0 – initial amount of a producer's capital	according to the owner's decision	2 000	thousand rubles
	Ensuring current solvency	Positive monetary flow	check of the non-negativity of the current amounts of finance means (profits, credits, grants, DS^0)	≥ 0	thousand rubles
Clients	Increase of marketing research of food market	β_1 – share of proceeds R from industry sales, spent on advertising	up to 5% from turnover – refers to cost; over 5% - after profits tax payment, chapter 25 of the Tax Code of the Russian Federation	no data	per cent
		Customers satisfaction from manufactured products: q_1 – actual demand on the product 1 for the period T; q_2 – actual demand on the product 2 for the period T	according to the data accounting of the organization	q_1 – 965 876 q_2 – 955 411	thousand rubles
Processes	Cost reduction	Product cost: P_1 – cost of product unit 1; P_2 – cost of product unit 2	marketing assessment and management accounting data, according to the data accounting of the organization	P_1 = 0.1 P_2 = 0.11	thousand rubles / product unit
		Material costs: θ_1 – material costs for the production of a product unit 1; θ_2 – material costs for the production of a product unit 2	accounting data and the analysis of business processes of the enterprise, according to the data accounting of the organization	θ_1 = 0.087 θ_2 = 0.092	thousand rubles
Personnel	Training of employees	β_2 – share of proceeds from industry sales, spent on personnel training (retraining)	according to the data accounting of the organization	no data	per cent
	Work efficiency	Worker efficiency: V_1 – worker efficiency of the pig farm for the period T; V_2 – work efficiency of the slaughter hall for the period T	the analysis of business processes of the enterprise, according to the data accounting of the organization	V_1 = 195.3 V_2 = 1 365	pigs on one worker centner on one worker

ISSN 2308-4057. Foods and Raw Materials, 2016, vol. 4, no. 1

To solve the task of application of BSC to the analysis of the agribusiness enterprise activity classical perspectives of BSC ("Finances", "Clients", "Processes" and "Personnel"), and also target values of the balanced scorecard, considering the specificity of the enterprise under examination, corresponding to them parameters and some methods of their calculation in the presented model are highlighted in the chart.

It should be noted that in the proposed model there is the balance between strategic and operating levels of management, the elements of all components, used in the BSC, are considered:

(1) the perspective "Processes" (optimum quantities of workers and manufacture of products are defined, the indicators of product cost, material expenses vary and etc.) is described with the help of generally accepted methods of assessment of manufacturing projects efficiency;

(2) the perspective "Finances" is described through the variables, determining the optimum volumes of loan resources, grants, whereas the condition of nonnegativity of equity capital guarantees the enterprise solvency on the whole planning horizon;

(3) the perspective "Clients" is described through the restrictions of production volumes with the level of consumer demand, and also the level of costs on the product advertising;

(4) the perspective "Personnel" is described through the parameters of costs on personnel training and labor efficiency.

The authors have conducted the theoretical and numeric model analysis (1)–(7). In particular, it is proved that there is the existence of solution in the presented model in all capacities of the change of its variables and parameters. The numeric analysis has been executed with the use of software package, described in the article [9]. The mentioned package represents the complex of automated information system of entering, processing and analysis of entrance information, solver and multi-parameter curve analyzer of solutions of one- and many criteria problems of linear programming.

Software package is oriented on various users. It allows a specialist mathematician to create and correct mathematical models in the form of many criteria problem of linear programming, and also to control the accuracy of entering information. It allows an economist-analytic, businessman to create one's own project configuration in a friendly manner (highlighting blocks of assets characteristic, products, project external environment, financial block and etc.), to put down entrance statistic and expert information, to present results of calculations both in the form of charts of multiparameter dependencies, and in the form of Pareto-sets in the criteria space (two or three criteria). To the significant advantages of the package one may refer the speed of calculations, which allow applying it for the operative analysis of socio-economic phenomena and systems in the framework of situation centers of expert managerial decision making.

Below there are examples, illustrating the results of optimization analysis and revealing the interconnection of parameters of activity of the chosen agricultural enterprise taking into account BSC.

In Fig. 1 you can see curves of dependencies of the current present value *PV* of the enterprise on the share of proceeds from product sale, used for personnel training (retraining) β_2, and changes of the average demand level *q* on the products. The analysis of the figure lets the decision maker to answer several questions. Firstly, evaluate the potential of the enterprise activity (in the form of its present value *PV*) on the planning horizon, depending at once on two (significant in BSC) parameters – changes of the costs levels on the personnel training and demand levels on the product. Secondly, from the figure, the optimum threshold values $\beta_2 \in (0 \div 0.12)$ are visually defined for the costs parameter on the personnel training, which may suit or not suit the decision maker and be the basis for the support of current and strategic decisions of allocating financial resources on the mentioned cost item. The similar analysis, conducted, for example, according to the parameter β_1 (costs on product advertising), has shown the optimum range $\beta_1 \in (0 \div 0.07)$.

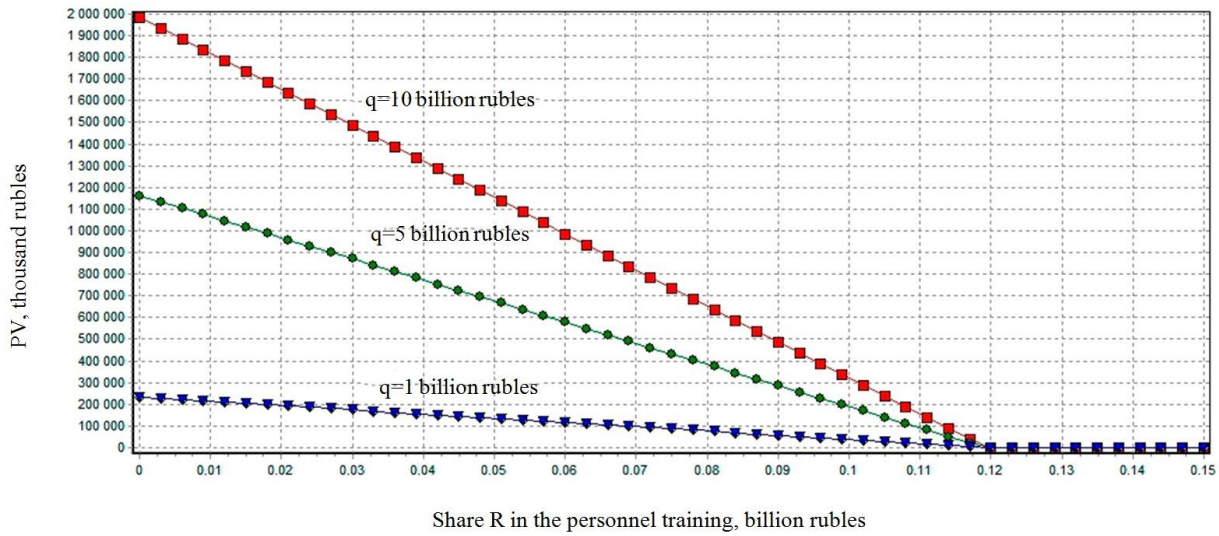

Fig. 1. Dependencies of the present value of the enterprise on the share of proceeds, spent on personnel training, and middle level of demand on product.

In Fig. 2 and 3 the results of joint analysis of the enterprise activity according to two parameters of BSC – the average demand q on the product and average worker performance V are given. From the figure 2 the decision maker has an opportunity to evaluate clearly the potential of the enterprise activity according to the parameter q, depending on a number of values V (for example, at $q \approx 8.7$ bln. rubles and $V = 16000$ the value is $PV \approx 250$ mln. rubles), and from the figure 3 – the values PV, depending on the parameter V and a number of values q. Besides, the figure 2 allows evaluating clearly the upper borders of demand, at which the further growth of its values doesn't lead to the increase of the potential of the enterprise activity in the form PV (that may be explained by achieving the maximum possibilities of the fixed production assets according to production or, in other words, the level of scientific-technical progress). The figure 3 also allows evaluating clearly the upper border of performance ($V \approx 15500$), lower which the enterprise activity loses its economic meaning ($PV = 0$). The comparison of the presented figures gives a rich information about the strategic enterprise activity in the three-parameter case of the chosen parameters of BSC.

In the Fig. 4 the results of the calculations of dependencies of the present value PV of the enterprise on the average product cost P at two systems of its taxation – general (low curve) and simplified, while applying the unified agricultural tax (upper curve), are presented. The corresponding sets of the used rates of tax and nontax costs are given in the figure. From the figure one can evaluate the quantitative level of occurring differences, reflecting the enterprise advantage from the application of tax allowances on the whole planning horizon. In particular, at $P \approx 1\,00$ (monetary unit / product unit) the enterprise advantage according to the parameter PV may make $(1600–1100)/1100 \approx 45\%$. The figure 4 also allows evaluating visually the thresholds of product cost, at which the enterprise activity loses its economic meaning ($PV = 0$).

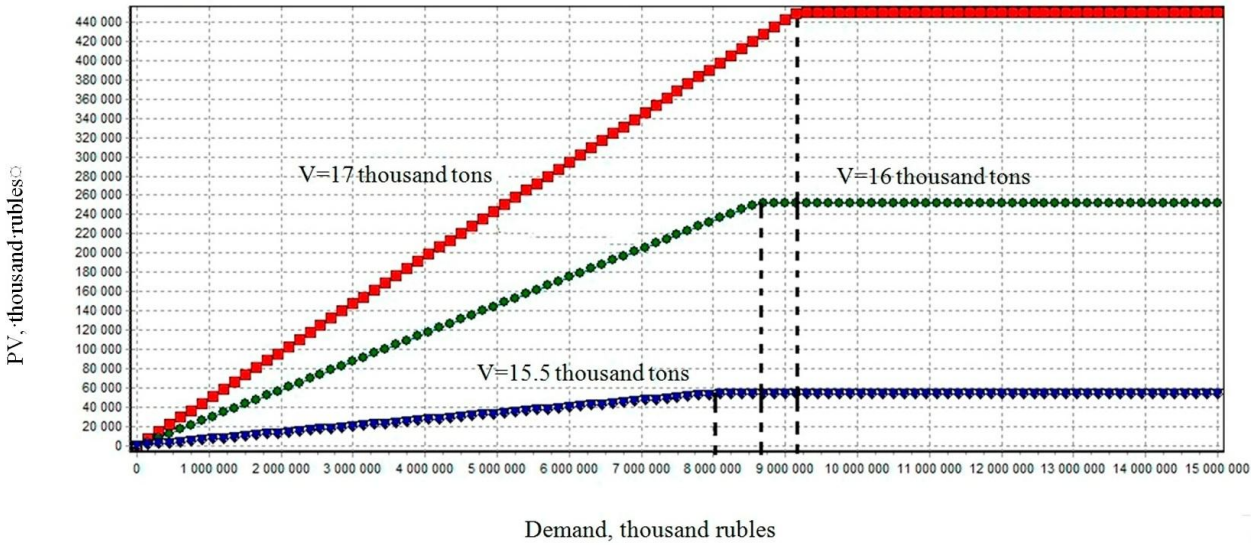

Fig. 2. Dependencies of the present value on product demand and average worker efficiency.

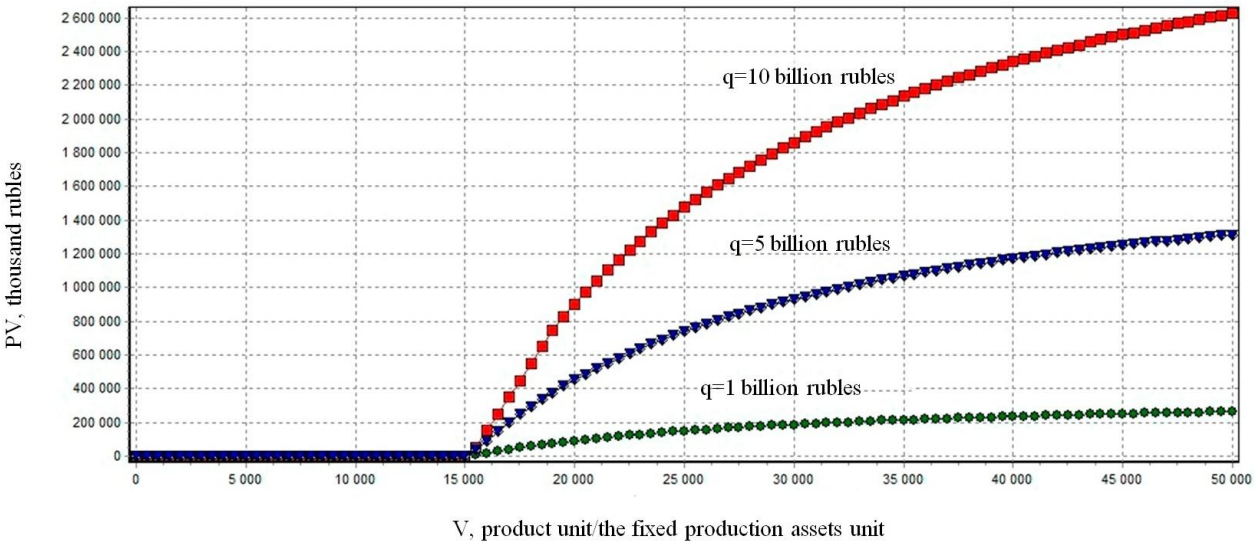

Fig. 3. Dependencies of the present value on the average worker efficiency and product demand.

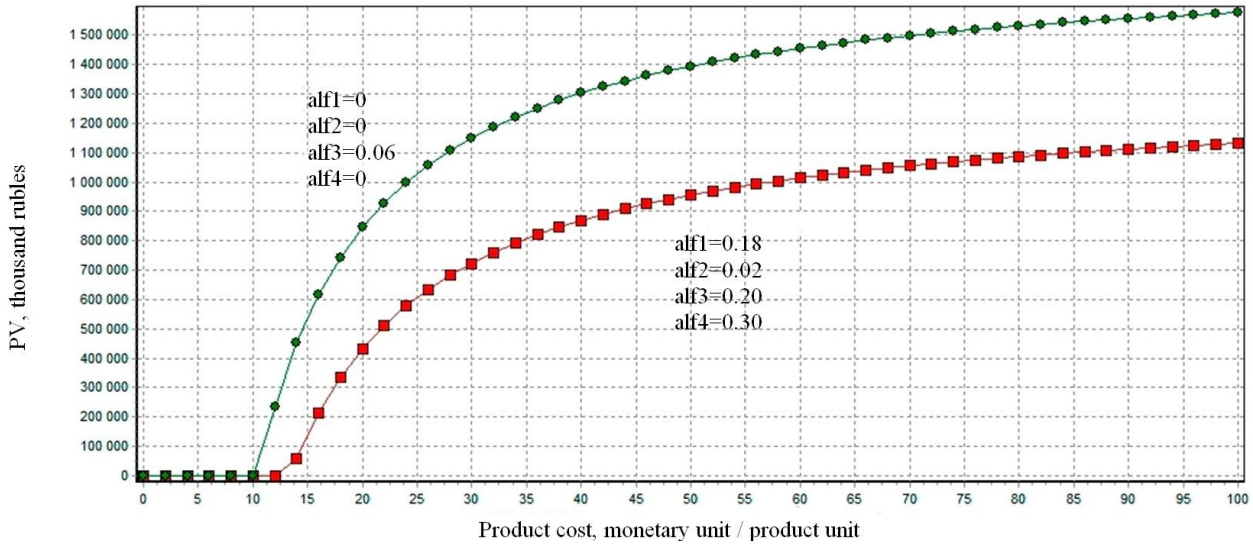

Fig. 4. Dependencies of present value on the product cost of the applying taxation system.

The results of modeling and numeric analysis of the activity of "Chistogorskii" Ltd. let us make the following conclusions, which are advisable to consider while designing a strategic map of BSC:

– the present value of the enterprise (perspective "Personnel") depends both on share of proceeds, spent on personnel training, and the average demand level on the product (perspective "Clients"); at the same time the optimum level of costs on the personnel training for the enterprise is no higher than 12 per cent from the volume of recoverable revenue;

– the present value of the enterprise (perspective "Finances") depends both on the share of costs on the product advertising (perspective "Clients") and the average product demand level (perspective "Clients"); at the same time the optimum level of costs on the product advertising for the enterprise is no higher than 7% from the volume of recoverable revenue;

– the present value of the enterprise (perspective "Finances") depends both on the average demand level on the product (perspective "Clients") and the average worker efficiency (perspective "Personnel"); in this case the threshold values of average productivity and average level of demand, which have an influence on the decision making in the management of the studied agribusiness company under study, are clearly determined from the graphical analysis;

– the present value of the enterprise (perspective "Finances") depends both on the average product cost (perspective "Processes") and the applied taxation system (perspective "Finances"); at the same time in the current market prices, on the planning horizon of 10 years, the discount rate 30%, the effect according to the parameter of the present value of the enterprise, while using a simplified taxation system, reaches 45%.

The proposed model, algorithm tools, based on BSC, may be used to solve more difficult problems of strategic management of agribusiness enterprises in future – revealing optimum cost balance according to perspectives BSC, strength of interaction between indicators, building a strategic map and developing the support system of managerial decision making. Furthermore, in our opinion, one may expect to obtain quantitative parameters of optimum distribution of BSC components, which automatically detect socio-economic potential of the enterprise functioning, groups of companies, branches, areas of economic activity at the local, regional and national levels.

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Please cite this article in press as: Fedulova E.A., Medvedev A.V., Kosinskiy P.D., Kononova S.A., and Pobedash P.N. Modeling of the agribusiness enterprise activity on the basis of the balanced scorecard. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 154–162. doi: 10.21179/2308-4057-2016-1-154-162.

METHODOLOGICAL ASSESSMENT OF TERRITORIAL COMPETITIVE POSITIONS: CONSUMER GOODS AND SERVICES INDUSTRIES AND MARKETS INFRASTRUCTURE IN KEMEROVO REGION CASE STUDY

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Received February 15, 2016; Accepted in revised form March 25, 2016; Published June 27, 2016

Abstract: The priority tasks of the modern times include challenges in assessment of the industrial competitiveness. Today the problem of assessing the competitiveness of regions is the main and the first. When competition for investments, innovations and new technologies between regions increases, the role of their key competitive positions start to go up too. A development of methodological approaches to assessment of territorial competitive positions of a region is a primary focus of the article. Consumer goods and services industries and markets infrastructure of Kemerovo region were selected to be an object of the current study. The results of our earlier works presented three groups of competitive positions to assess competitiveness of a given region: territorial competitive positions of a region (TCP), industrial competitive positions of a region (ICP), territorial and industrial competitive positions of a region (TICP). The object of more detailed research is some positions of the first group. The TCP has been divided into two subgroups: basic TCP (based on geographical location and general resource potential) and controllable TCP (built up by a region proactively in a targeted manner). Today the region market infrastructure becomes one of the most important controllable TCP. It is academic and practical interest to work out assessment methods to evaluate a rate of its development and contribution into economic performance of a region. Market infrastructure was classified by three characteristics: functional and industrial, services markets, hierarchical. To evaluate development rate of consumer goods and services markets infrastructure in Kemerovo region we defined its components and suggested three groups of indicators: absolute and relative, flow data, overall. Sets of additional indicators further determine each of the above groups. Through the detailed analysis, the overall indicators were used to assess Kemerovo region economic structure and how industries and consumer goods and services markets infrastructure contribute to its GRP. We analyzed changes that occurred in economic structure of the region during the last years and presented macroeconomic, aggregated, enlarged model of Kemerovo region. The study generally concludes that there is a need to further strengthening of territorial competitive position in service sector.

Keywords: competitiveness of a region, market infrastructure, regional economic model, consumer goods production sector, service sector

DOI 10.21179/2308-4057-2016-1-163-170 *Food and raw materials*, 2016, vol. 4, no. 1, pp. 163–170.

INTRODUCTION

The priority tasks of the modern times include challenges in assessment of the industrial competitiveness. Today the problem of assessing the competitiveness of regions is the main and the first [1]. In times of crisis and sanctions imposed by European Union competition grew inside the sector of consumer goods and services. Companies compete not only for markets to sell their products but also for investments, innovations, technologies.

The interest revealed by the prominent international researchers Peter Ferdinand Drucker [2], Michael Mescon [3], Michael Porter [4] and Russian scientists A.P. Pankrukhin [5], R.A. Fatkhutdinov [6] to the issue proves it to be one of the today's urgent topics.

OBJECTS AND METHODS OF STUDY

In the most general sense, competitiveness of any subject or object refers to a set of properties and values providing advantage in competition.

Competitive advantages of a given company can be attributed to by competitive possibilities and potential of a country, area, region, municipality and territories of market agents' location and functioning.

However, competitiveness of a territory can be assessed as based on the cooperation between territories along with assessment based on their competition.

Different types of competitive positions ensure competitiveness of a territory. We divided them into three major groups [7, 8, 9]:

– common territorial or given territorial competitive positions (TCP);

– industrial competitive positions (ICP);
– territorial and industrial competitive position (TICP).

We'll study the first group. Taking into account two aspects of territory definition the TCP can be further divided into two subgroups [10]: basic and controllable. There exist other groups: in-depth and shallow; basic and developed; tangible and intangible; economic determinant and strategic determinant.

Basic TCP will include:

– T_1CP – geographical location (distance from administrative-territorial borders, size and shape, neighboring territories);
– T_2CP – neighboring territories cooperation potential;
– T_3CP – territory accessibility;
– T_4CP – mineral resources (proven, exploited);
– T_5CP – climate conditions;
– T_6CP – environment pollution rate;
– T_7CP – rational distribution of production forces.

Controllable TCP will include:

– T_8CP– administrative potential;
– T_9CP – business climate favorability;
– T_{10}CP – education and qualification level of human resources;
– T_{11}CP – intellectual, scientific and technological potential;
– T_{12}CP – level of implementing technical progress achievements and innovative decisions;
– T_{13}CP – agglomerations, clusters, business territories, incubators, industrial parks businesses, special economic zones potentials;
– T_{14}CP – industrial potential;
– T_{15}CP – financial potential;
– T_{16}CP – tax potential;
– T_{17}CP – foreign economic potential;
– T_{18}CP – rate of infrastructure development (energy, transport, logistics, information and communication, market, social);
– T_{19}CP – economic structure;
– T_{20}CP – status potential (administrative center, distribution center, international business representation, innovative activity center, information (communication) network hub, international cultural center, tourism center);
– T_{21}CP – intangible assets (attractive image and brand, official territorial symbols, positive reputation, historical and cultural heritage).

Basic and controllable territorial competitive positions can interact in creating synergy effects.

In the above case competitive positions dependent on geographical location give a way to competitive positions that territories create themselves continuously in the process of their development. For example, gain in competitive advantages can be achieved by building up the capacity of market infrastructure.

Building up the modern market infrastructure is an important way to ensure efficient production, investment and social activities. For a given region to increase its competitiveness, the market infrastructure should include an extensive network of different structures serving demands of market economy participants, in particular, intermediary, trade and sales companies, finance and credit institutions, companies providing information and legal support. Efficiency in business highly depends on reliable market infrastructure, on the understanding that dynamic and quite complex market relations put every investor and entrepreneur in the position when their success is impossible without coherent relations within operating cycle and its financial, credit and marketing support.

Before evaluating market infrastructure development rate it appears reasonable to classify it by the following features:

– function within the sector;
– service markets;
– hierarchical (ranking).

Function within the sector feature differentiates between the following functions of infrastructure [11] (Table 1):

Table 1. Regional infrastructures by function

Infrastructure function	Composition
Trade infrastructure	Wholesale, wholesale intermediaries, commodity exchange, trading houses, retail companies, wholesale and retail fairs, exhibitions, retail booths
Procurement/purchasing infrastructure	Procurement intermediaries, purchasing enterprises, agricultural products purchasing companies
Finance and credit and insurance infrastructure	Commercial banks, non-banking credit and finance institutions, currency and stock exchange, insurance firms, factoring firms
Information technology infrastructure	Data processing center, telecommunication networks, information technologies service firms
Real estate sales infrastructure	Real estate agencies, real estate purchase and exchange centers, intermediary firms selling real estate abroad
General commercial activity infrastructure	Marketing firms, consulting firms, advertising agencies, business centers, chambers of industry and commerce
General economic legal infrastructure	Arbitration courts, consulting legal firms, notaries and lawyers offices

We assessed the market infrastructure of Kemerovo region by the feature above and determined the following entities to be representative of the regional infrastructure:

(1) Trade infrastructure of the region comprises wholesale enterprises, wholesale intermediaries, wholesale and retail enterprises, retail enterprises, foreign trade organizations, distribution centers, trade houses, wholesale fairs, exhibitions and booths, commercial centers, specialized distribution centers and warehouses;

(2) Purchasing infrastructure mainly includes purchasing intermediaries, purchasing enterprises, wild plants procurement enterprises;

(3) Finance and credit (investment) and insurance infrastructure is represented by commercial banks, non-banking credit financial institutions (not licensed by the Russian Federation Central Bank), insurance firms, factoring companies;

(4) Information technology infrastructure of the region comprises data processing service firms and telecommunication networks;

(5) Real estate infrastructure includes: real estate sales and exchange centers, real estate sales and rental agencies;

(6) The infrastructure of general commercial activity comprises consulting firms, advertising agencies, business centers, incubators, science and technology park, chamber of industry and commerce;

(7) Economy related legal infrastructure is represented in the region by arbitrary courts, consulting legal firms, lawyers and notaries offices.

Regional infrastructure classification by types of infrastructures in terms of service markets and hierarchy (ranking) is shown on Fig. 1.

By services, markets infrastructures of the region can be presented by the following types:

– consumer goods and services markets infrastructure,
– industrial and technological products markets infrastructure,
– financial markets infrastructure including securities market,
– labor markets infrastructure,
– real estate markets infrastructure,
– information markets infrastructure, etc.

From hierarchical perspective of subordination and management (ranking) a region can display the following formats of infrastructures: international, national, interregional, regional, municipal (city, district), local.

To evaluate development rate of consumer goods and services markets infrastructure we suggested a set of indicators that includes three groups (Table 2):

(1) Absolute and relative indicators;
(2) Flow data;
(3) Overall.

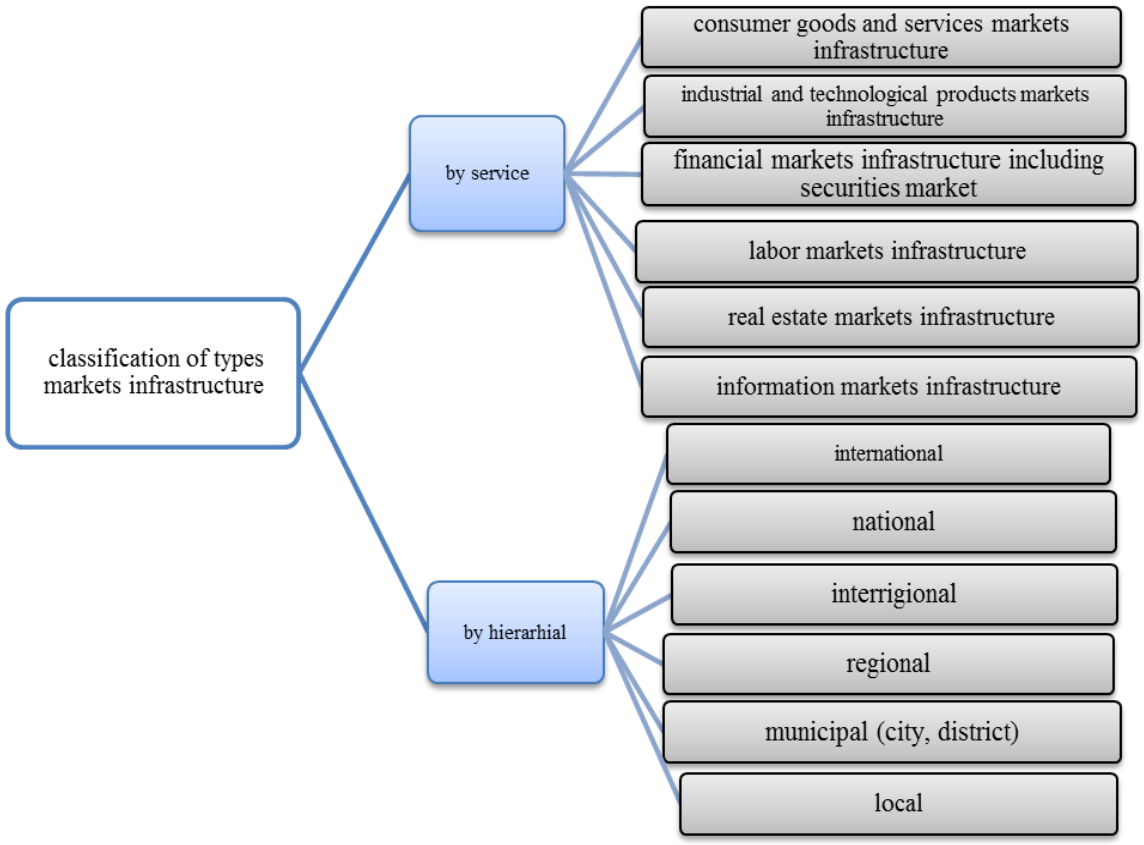


Fig. 1. Regional infrastructure classification by type.

Table 2. Market infrastructure development assessment indicators

Groups of indicators	Evaluation criteria
Absolute and relative	– by capital funds, – by number and size of businesses, – by output, – by density, – by number of personnel, – by economic efficiency;
Flow data	– dynamic indicators, – compared reporting and basis periods indicators;
Overall	– regional share in GRP, – regional share in GVA, – share in total capital investment cost, – share in total number of employed.

Let's apply the above set of indicators to evaluate the development rate of goods and services consumer markets infrastructure.

First and foremost, it must be noted that consumer goods and services market infrastructure plays a major role in formation and functioning of the whole market infrastructure of a region. Consumer goods and services market consists of the following aggregated segments: food market, durable goods market (household appliances, consumer electronics), consumer services market, real estate market.

The group of absolute and relative indicators of consumer market infrastructure development rate should include:

– costs of capital funds and capital investing into their development in total, by formats, by segments and their share in total volume;

– total number of enterprises, their distribution by segments, formats;

– trade enterprises size in terms of floor space, number of seatings including stores, shopping malls, booths, kiosks, warehouses, public catering and service enterprises;

– trade enterprises customers capacity that is average number of buyers per a selected unit of time, turnover or revenue per a given enterprise, turnover or sales per square meter, average number of employees per a given enterprise;

– density coefficients of trade enterprises: a number of enterprises per square unit of a region, average floor space per 10.000 residents;

– number of employees: total, on average per one enterprise, on average per square meter of a floor space, revenue per employee, etc.;

– economic performance: of infrastructure as a whole, by aggregated segments, formats and enterprises.

This study offers to expand the group of indicators by adding the flow data. The latter includes time series of absolute and relative indicators, and compares given indicators in reporting period with the same indicators in base (reference) period using various mathematical statistic calculations.

Finally, the group of overall indicators can be applied to describe general quantitative and qualitative characteristics [12, 13] of the role consumer market infrastructure and its aggregated segments play in regional economics. They represent contribution, share of the infrastructure in GRP, total capital investments and total number of employed in regional economics. Thus, a proportion of the consumer market infrastructure gross value added in regional GVA demonstrates the role of this infrastructure in the formation of present indicator. Ratio of a number of employed growth trend reveals that consumer market infrastructure attracts labor force that becomes available from manufacturing and other industries in the process of regional economic restructuring.

The state of the regional economic structure and its planned restructuring is a core competitive position, which predetermines actual opportunities for industries and market infrastructures to occupy its niche in regional, interregional and international commercial and economic relations. Regional economic structure predetermines segments and local market capacity, main directions of consumer goods and services by import and export.

RESULTS AND DISCUSSION

Industries contributions into gross regional products (GRP) define regional economic structure.

The study analyzed regional economic structure by the example of Kemerovo region. In 2012 GRP of the region was 717.7 billion roubles. It included 15 industries, 6 of which participate in production of goods and 9 render services (Table 3).

Table 3. Kemerovo region economic structure in 2012 [14]

Industries	2006 year		2008 year		2012 year	
	Current price, billion rubles	GRP breakdown, %	Current prices, billion rubles	GRP breakdown, %	Current prices, billion rubles	GRP breakdown, %
Mining	73 550.0	21.8	260 130.4	34.6	192 405.3	26.8
Manufacturing	66 334.5	19.6	103 163.6	13.7	113 529.1	15.8
Wholesale and retail sale, repair of motor vehicles, bikes, household and personal goods	47 455.6	14.0	89 154.3	11.9	78 128.0	10.9
Transportation and communication	31 172.2	9.2	56 949.3	7.6	58 780.5	8.2
Real estate operations, rental and services	21 884.3	6.5	50 899.8	6.8	67 438.2	9.4
Power generation and distribution of energy, gas, water	20 694.3	6.1	32 304.2	4.3	29 729.4	4.1
Construction	18 508.2	5.5	38 150.3	5.1	40 050.2	5.6
Public health and social services	14 351.9	4.2	30 053.3	4.0	34 766.0	4.8
Agriculture, hunting and forestry	12 633.6	3.7	23 922.5	3.2	20 680.7	2.9
Education	11 392.5	3.4	21 198.1	2.8	23 843.9	3.3
Public administration and defense, compulsory social security	10 494.3	3.1	30 941.5	4.1	40 108.3	5.6
Hotels and restaurants	4 580.2	1.4	4 788.0	0.6	7 232.9	1.0
Other social, personal services and utilities	4 242.3	1.3	6 425.5	0.9	7 976.8	1.1
Financial activity	793.1	0.2	3 020.3	0.4	2 937.4	0.4
Fishing, fisheries	51.7	0.0	97.3	0.01	93.2	0.01
GROSS REGIONAL PRODUCT	338 138.7	100.0	751 198.4	100.0	717 700.0	100.0
Goods production	191 772.3	56.7	457 768.2	60.9	396 487.9	55.2
Service rendering	146 366.4	43.3	293 430.1	39.1	321 212.1	44.8

The largest contributors into GRP from good producing participants are the following:
– mining – 26.8%;
– manufacturing (including consumer goods) – 15.8%;
– construction (including housing) – 5.6%.

Total contribution of the above group into GRP – 55.2%.

The largest contributors into GRP from service rendering participants are as follows:
– wholesale and retail sale – 10.9%;
– real estate operations – 9.4%;
– transport and communication (including public transportation and communication) – 8.2%.

Total contribution of this group into GRP – 44.8%. Thus, industries and infrastructure of consumer goods and services significantly contribute to GRP.

During the last 10 years the major changes occurred in the economic structure of Kemerovo region. The share increased:
– mining by 7% (from 21.8% in 2006 to 26.8% in 2012);
– real estate operations by 2.9% (from 6.5% in 2006 to 9.4% in 2012).

During the same period the share decreased:
– manufacturing by 3.8% (from 19.6% in 2006 to 15.8% in 2012);
– wholesale and retail by 3.1% (from 14.0% in 2006 to 10.9% in 2012);
– power generation and energy, gas, water distribution by 2.0% (from 6.1% in 2006 to 4.1% to 2012);
– transportation and communication by 1.0% (from 9.2% in 2006 to 8.2% in 2012).

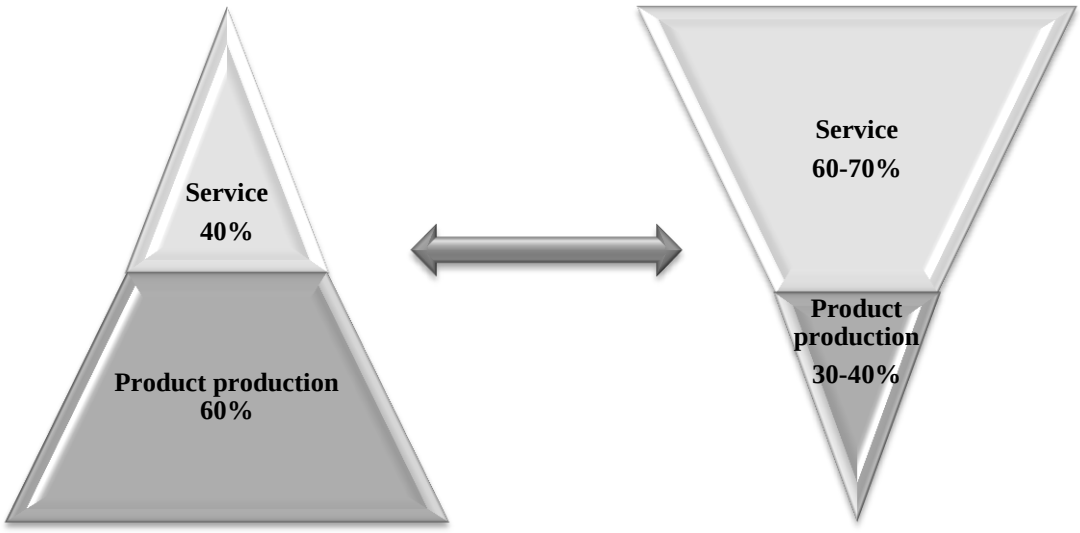
During the period the share of goods production in the regional economic structure fluctuated within a range of 55–60%, and share of services fluctuated within a range of 40–45%.

The given regional economic structure does not coincide with the structure of the developed economies. The latter "pyramid" is reversed: production of goods accounts for 30–40%, and rendering of services amounts to 60–70% (Fig. 2).

Further research provides insight into structural forming elements of regional economy. Aggregated macroeconomic enlarged model of the Kemerovo region economy can be presented as a "black box" pyramid (Fig. 3).

The model's point of entry shows raw materials and supplies totaling 1 247.1 billion rubles in 2012. At the exit the model shows total product for an overall amount of 1 964.8 billion rubles, current price. Gross regional product is formed inside the "pyramid" in the amount of 717.7 billion rubles. Total product output distribution: raw materials and supplies – 63.5%, GRP – 36.5%.

The ratio of resources used to produce output, value added and revenue by main types of activity is shown in Table 4.



Enlarged structure of economy of Kemerovo area

Enlarged structure of economy of developed countries

Fig. 2. Economic structures large-scale models comparison.

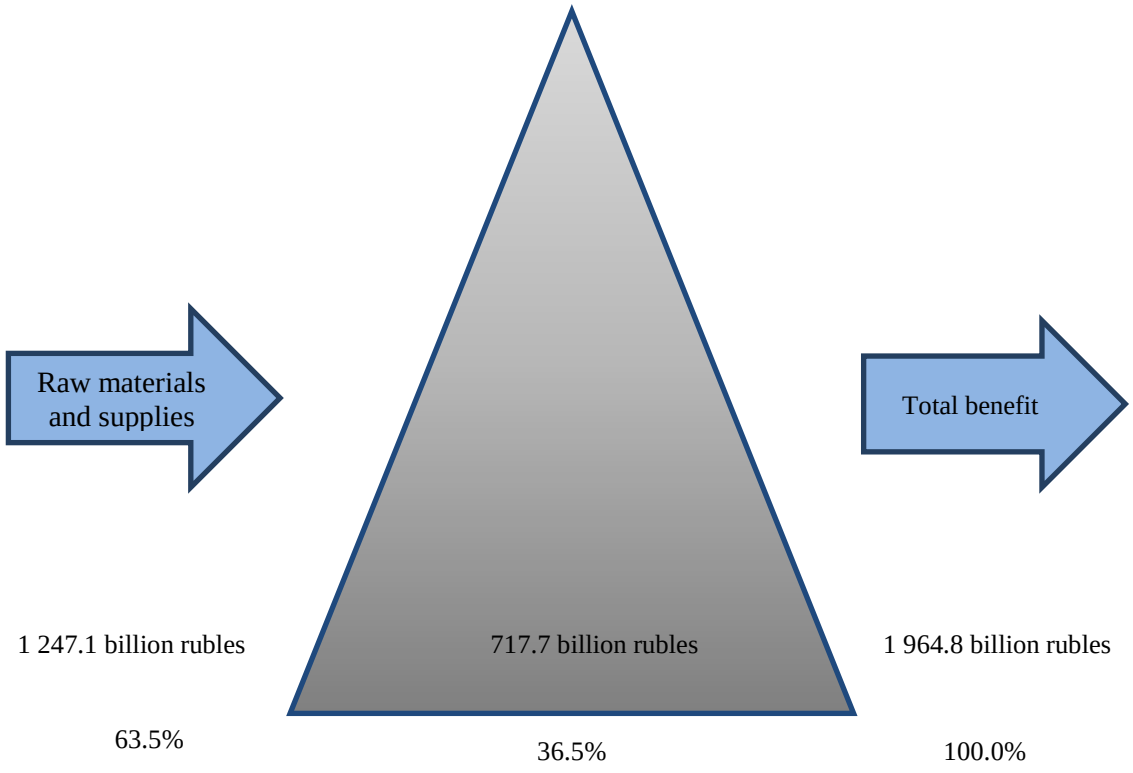


Fig. 3. Enlarged economic model of Kemerovo region.

Table 4. Ratio of resources used to produce output, value added and revenue by main types of activity [14]

Economic activity	Resources	Value added	Revenue	Share of value added in revenue, %	Share in GRP, %
Mining	319 908.7	192 405.3	512 314.0	37.5	26.8
Manufacturing	297 689.9	113 529.1	411 219.0	27.6	15.9
Construction	42 985.8	40 050.2	83 036.0	48.2	5.6
Power generation and distribution of electrical energy, gas and water	102 454.6	29 729.4	132 184.0	22.4	4.1
Wholesale and retail sales	516 737.0	78 128.0	594 865.0	13.1	10.9
Real estate operations, rental	1 927.8	67 438.2	69 366.0	97.2	9.4
Transport and communication	57 010.5	58 780.5	115 791.0	50.7	8.2
Utilities, social services	644.2	7 976.8	8 621.0	92.5	1.1
Hotels and restaurants	1 576.1	7 232.9	8 809.0	82.1	1.0
Total	1 247 118.0	717 700.0	1 964 818.0	36.5	100.0

The largest share of value added in revenue occurs in real estate – 97.2%, utilities and social services – 92.5%, hotels and catering enterprises – 82.1%. Wholesale and retail sale, power generation and distribution of electrical energy, gas and water have the least share – 13.1% and 22.4% respectively.

Reasoning from the value added share in gross regional product it appears possible to open "black box" model and reveal the leading industries of Kemerovo region economics both in production sector and service sector (Fig. 4).

CONCLUSION

Overall, Kuzbass industrial based economy strengthened during the last years. To ensure further development in the streamline of market economy, competitive positions in superstructure need to be strengthened too.

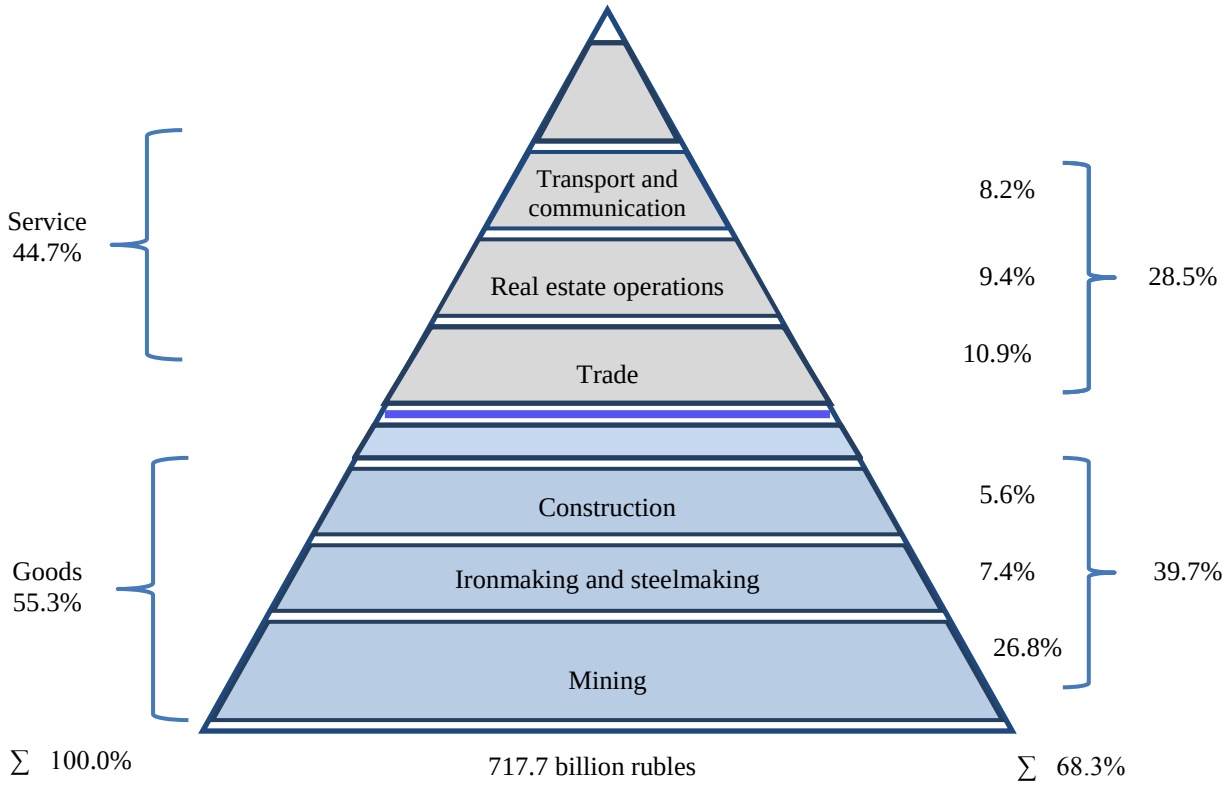


Fig. 4. Enlarged economic model of Kemerovo region.

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Please cite this article in press as: Lavrov A.M. and Polikarpova L.A. Methodological assessment of territorial competitive positions: consumer goods and services industries and markets infrastructure in Kemerovo region case study. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 163-170. doi: 10.21179/2308-4057-2016-1-163-170.

FOOD CONSUMPTION AS AN INDICATOR OF THE QUALITY OF LIFE OF THE POPULATION IN REGIONS

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Received February 07, 2016; Accepted in revised form March 20, 2016; Published June 27, 2016

Abstract: The quality of life, which is a complex characteristic of human existence, its level and conditions, in the research practice is measured by statistical and sociological methods. This characteristic reflects the degree of satisfaction with different needs and subjective perception of life and its individual aspects. In this work the statistical method is chosen to describe the quality of life. It aims to the indicators' analysis, which are connected with food consumption (using Kemerovo region since 2010 to 2014 as an example) and differentiated into two parts: standard of living and living conditions. The analyzed level (households' expenses share for food in overall consumer spending structure, food consumption structure, its nutrition and energy value) and conditions indicators (food prices, consumer price indexes, a minimum food set cost dynamics and its ratio with the average income, retail food trade turnover, its share in total turnover of the region, public catering turnover) have shown low life quality in the region in comparison with Russia in general, and also its decrease for the last one or two years, which is confirmed by traditional indicators of living standard and quality.

Keywords: quality of life, standard of living, living conditions, consumer behavior, food consumption, statistical analysis, indicators

DOI 10.21179/2308-4057-2016-1-171-180 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 171–180.

INTRODUCTION

The need for food is one of basic human needs, satisfaction of which provides his / her existence, allows to live, work; maintaining not only physical health, but also mental health. At the same time food is very different in different population groups (by quantity, assortment, and on content) and is capable in many ways to characterize some aspects of living standards. It is reported by researchers of food consumption problems [1–9].

Before showing position of food parameters in system of life quality criteria, it is necessary to define this concept, establish its internal filling and connection with other scientific categories of the corresponding subject field, and also specify approaches and methods of its measurement.

The category “living standard” and its empirical research are not deprived of scholars’ attention [10–25] in recent years, but complexity and diversity of this concept cause a rich spectrum of approaches to treat its content [for more details see 26]. In classical theories of life quality it is accepted to distinguish two main scientific directions: doctrines based on the objective living conditions analysis and social subjects developments; and concepts of the perceived life quality oriented to people's sensory perception of their lives and to subjective evaluation of existence parameters. However recently the offers to connect these two approaches, their rational complementarity with which it is necessary to agree sound more and more actively. In our opinion, life quality is a complex characteristic of people's vital activity level and conditions reflecting satisfaction degree of various needs and subjective life perception and its certain aspects.

Being guided by this determination, it is possible to distinguish two things: firstly, life quality combines the standard of living and living conditions; secondly, life reflects both objective and subjective characteristics of existence. The degree of material security of people allowing to satisfy their needs is offered to understand as living level. Living conditions are those circumstances which accompany a person (or society) in the course of his/her activity and promote satisfaction of various needs. Such option of life quality differentiation will remove all the disputes of scientists and practicians on a ratio of the concepts “quality of life” and “level of living”.

Distinguishing objective and subjective components in "life quality" leads not only to accounting the actual

parameters of life and personal ideas of them, but also to determination of methodical approaches to its measurement. Statistics usually studies objective life quality indicators, and sociology is engaged in objective indicators mainly. Both statistical and sociological options of life quality research have their own advantages and disadvantages, and for effective connection in a general technique require serious additional scientific, financial, organizational and managerial efforts. Therefore modern researchers, as a rule, rely either on statistics, or on sociology. The statistical analysis of food consumption of the region population (on the example of the Kemerovo region), which reflect level and living conditions and consequently, qualities of life became the purpose of this research. As for the level, conditions, and quality of life scientists use a set of indicators from the most different spheres of human existence. There is a reasonable sense to allocate those which concentrate on one of the main aspects of quality of life reflecting foremost of people's need – need for food.

OBJECTS AND METHODS OF STUDY

The objects of research were food consumption parameters in Kemerovo region and their communication with indicators of life quality. For the analysis the period from 2010 to 2014 is taken. Official statistics data became the main information source, and the main research method is the statistical analysis. At the same time the comparative analysis was kept not only in dynamics by years, but also on a territorial sign (in comparison with Russian indicators got in the Siberian Federal District (SFD) through Kemerovo region municipalities), and also on structural filling of this or that characteristic and on population categories (structure of families, profitable groups); the indicators measured both in absolute and in relative units which often are more evident for comparison of data were used.

The main methodical approach which distinguishes authors methodology from others is indicators' division into two groups: reflecting the living conditions level and characterizing it. Describing living level indicators and then its conditions it is possible to analyze quality of life in general more fully and deeply, to gain more evident and capacious impression.

As in the main part of work indicators of food consumption by Kemerovo region inhabitants are considered, and attempt of determination of their interrelation with life quality is performed, it is necessary to briefly characterize key parameters of level and living conditions of the region population which provides the traditional statistics (Table 1). It will allow understanding information about object of research better.

Table 1. Several indicators of living level of Kemerovo region population [27, 28, 29, 30]

Indicators	2010	2011	2012	2013	2014
The number of resident population (for January 1 of the year following the reporting), one thousand people	2761.3	2750.8	2742.4	2734.1	2725.0
Average per capita cash incomes (in a month), rubles	15341	16666	18511	19697	19801
The actual owned cash incomes, in % to previous year	105.4	99.4	102.3	97.6	90.9
Average monthly nominal payment of organizations employees, rubles	18028	20479	23403	25326	26809
Real accrued payment, in % to previous year	106.1	105.1	108.1	100.5	98.1
The average size of the granted pensions (for January 1 of the year following the reporting), rubles	7570	8251	9139	10008	10891
The actual size of the granted pensions, in % by the corresponding period of the previous year	112.0	104.1	102.3	103.4	97.3
The size of the living wage (LW), on average per capita, rubles a month	4648	5263	5448	6829	7455
in % to previous year	108.1	113.2	103.5	125.3	109.2
The population proportion whose cash incomes is lower than LW, in % of the total number of the population	11.0	11.6	10.6	13.9	14.5
Ratio with living wage size, %					
average per capita cash incomes	330.1	316.7	339.8	288.4	265.6
average monthly nominal accrued payment	369.9	363.5	400.4	349.1	338.7
Coefficient of funds	14.6	14.1	14.9	13.8	12.2
For information only across Russia, in % to previous year:					
the real owned cash incomes of the population	105.9	100.5	104.6	104.0	99.3
the real accrued payment	105.2	102.8	108.4	104.8	101.2
the actual size of the granted pensions (in % by the corresponding period of previous year)	134.8	101.2	104.9	102.8	100.9
Ratio with the living wage size, %					
average per capita cash incomes	333.0	326.0	357.0	355.0	345.0
average monthly nominal accrued payment	341.0	340.0	378.0	381.0	376.0
The population proportion whose cash incomes is lower than LW, in % of the total number of the population	12.5	12.7	10.7	10.8	11.2
Coefficient of funds	16.6	16.1	16.4	16.3	16.0

The population of Kemerovo region on January 1, 2015 was 2725.0 thousand people, that is 1.3% less than for January 1, 2011. The number of people in the region decreases regularly from year to year. At the beginning of the analyzed period this tendency was caused by generally natural population decline, but in the last 3 years it was caused mainly by migration [27]. Average per capita cash incomes, average monthly salary, and also average size of the granted pensions in nominal terms gradually grew, but in real measurement the situation is the following: cash incomes had multidirectional dynamics, having reduced in 2014 directly by 9% in comparison with previous year; the salary and pensions grew until 2013, though with different intensity, but in 2014 were reduced almost by 2% and 3% respectively. It should be emphasized that on average in Russia the real accrued salaries and pensions did not decrease in 5 years, even in 2014; the real owned cash incomes decreased last year, but only by 0.7%.

The minimum subsistence level grew more intensively than the population income and salary (the first indicator increased by 60.4% from 2010 to 2014, the second one – by 29.1%, the third one – by 48.7%) that is visible also on the ratio of average per capita cash incomes with the MSL which decreased considerably in the last 3 years. By the way, this tendency is not so noticeable across Russia. The difficult situation with population income in Kemerovo region caused an increase in the number of those whose cash incomes are lower than the minimum subsistence level – from 11.0% in 2010 to 14.5% in 2014. In the Russian Federation this dynamics was different, and in 2014 it was 11.2%. However, in Siberian federal district the majority of regions are characterized by a bigger population share with cash incomes below MSL (only in Omsk region the indicator was 11.9% in 2014).

One more traditional indicator characterizing living standard is coefficient of funds. It shows the level of social stratification in society and is determined as a ratio between the average cash incomes levels of 10% of the population with the highest income and 10% of the population with the lowest income. The coefficient of funds in Kemerovo region in the analyzed period decreased reaching 12.2 in 2014, though in 2012 the general tendency of the indicator fall was broken. The all-Russian coefficient of funds was always higher than in Kuznetsk coal basin (Kuzbass), but it reached the minimum in 2014 too.

The data provided in table 1 characterize living standard in general. If speaking about conditions, the indicators quantity can increase many times. Therefore we will provide only some characteristics reflecting various living conditions of Kuzbass population. The most important of them (the complex one) is life expectancy. In Kemerovo region as in Russia in general, it gradually grew over the last 5 years and in 2014 constituted 67.8 years and 70.9 years respectively [28, pp. 67, 69]. The unemployment rate in Kemerovo region (general and registered) was reduced till 2013, but in 2014 there was a small growth (by 6.2% and 2.0% respectively). In the Russian Federation the tendency of unemployment reduction has remained till 2014 (5.2% and 1.2%) [28, p. 74]. The size of total housing area in Kuzbass is about 20 sq.m, but in Russia it is a bit larger – 21.4 sq.m [28, p. 103]. The number of pensioners on 1000 of people in Kemerovo region constitutes 314.7, which is also significantly higher than in the country (287.9 people) and it is the highest in Siberian federal district [28, p. 109]. The population morbidity calculated as number of the registered patients with the diagnosis established for the first time on 1000 people of the population in Kemerovo region in all years of the analyzed period was higher, than in the Russian Federation, constituting 865.4 in 2014 [28, p. 157]. The number of doctors on 10000 people in Kemerovo region in 2014 was 45.9, and in Russia – 48.5 [28, p. 181]. It is possible to characterize the education level by the number of people on 1000 at the age of 15 and older, having higher education. According to census of 2010, in Kemerovo region this indicator was 185, and in Russia – 234 [28, p. 89]. Thus, the majority of living conditions indicators in Kemerovo region is lower than in Russian and, unfortunately, in the last couple of years they tend to deteriorate.

RESULTS AND DISCUSSION

It is reasonable to start the analysis of the living level indicators related to food consumption with the corresponding consumer spending (Table 2) which in general are understood as the part of cash expenditures directed to consumer goods and services purchase (at full price regardless the purchase purpose, with exception of works of art, jewelry, antiques, building materials purchased as capital investments and investment works).

It clear from the Table 2 that the consumer spendings in Kemerovo region including food and soft drinks in ruble expression grow from year to year, however these indicators do not consider inflation processes. Nevertheless, it is possible to count that since 2010 to 2014 consumer spendings in general have grown by 37%, and food expenses by 41%, that demonstrates the decrease of living.

Relative values show a share of product costs in general and food expense structure. Throughout the analyzed period the account food and soft drinks part was about a third of the total amount of consumer spendings and had no unambiguous dynamics (it was minimum in 2013 and maximum in 2014). A Kuzbass dweller spent about 2% of the budget for soft drinks. The biggest amount – about 10% – was spent on meat. The average dweller spends a little more than 5% of consumer expenditure to bakery products and grain. The part of costs falling on dairy products, cheese and eggs is approximately the same. Further by descending follow fruit; sweets (sugar, jam, honey, chocolate, candies); vegetables; fish and seafood. Oil, fats, and other food are at the bottom of the list. Less than 1% of family money in each case is spent on these two groups. The expenditure for everything provided in the table of products category had no certain dynamics, and fluctuated within 5 years.

Table 2. Food and soft drinks as a part of consumer spending in Kemerovo region households [29, pp. 115–116]

Indicators	2010	2011	2012	2013	2014
Total consumer spending (on average on the household member a in a month; rubles)	7796.2	8761.8	9973.3	10328.1	10689.5
Including food and soft drinks	2554.5	2939.0	3182.2	3246.0	3605.6
Total consumer spending (%)	100.0	100.0	100.0	100.0	100.0
Including food and soft drinks	32.8	33.5	31.9	31.4	33.7
Of them: food	30.7	31.5	29.9	29.6	31.7
Including: bakery products and grain	5.4	5.4	4.7	5.2	5.4
meat	10.2	9.8	10.4	9.3	10.2
fish, seafood	1.7	1.9	2.0	1.8	2.1
dairy products, cheese and eggs	5.1	5.4	5.1	5.3	5.7
oils and fats	0.7	0.8	0.6	0.6	0.5
fruit	2.4	2.4	2.3	2.4	2.6
vegetables	2.0	2.0	1.8	2.0	2.2
sugar, jam, honey, chocolate and candies	2.3	2.9	2.2	2.2	2.1
other food	0.9	0.9	0.8	0.8	0.9
soft drinks	2.1	2.0	2.0	1.9	2.0

The comparison of data on different national groups showing differences in consumer behavior of people is important for the characteristic of level of living. So, the statistics fixes consumer households spending in city and rural districts (for example, in 2014 in Kemerovo region citizens spent 3595.7 rubles a month on one person on food and soft drinks, and in villagers they spent 3663.6 rubles. At the same time the share of expenses on purchase of food from city dwellers constitutes 31.7%, and at rural 53.7% that demonstrates very strong lagging of villagers on the level of material well-being from citizens) [30, p. 12]. Food expenses comparison in households with income levels is also evident (on 10 percent national groups). In 2014 in the first group (with the smallest owned resources) 43.2% of consumer spending were on food and soft drinks, and in the tenth (with the greatest income) it is almost twice less – 23.0% [29, pp. 118–119]. In expense structure lonely people and families with 5 and more people were singled out. They spent 46.3% and 41.3% respectively on foodstuff with an average of 36.9% in 2014 [29, p. 112].

Other cut of the comparative analysis is interterritorial which allows assessing a situation in the certain territorial subject of the federation in comparison with the next subject and with the average Russian level. So, the share of expenditure for food and soft drinks in the total amount of consumer spending in Kemerovo region in 2014 was 5.2% higher than on average in Russia, and 3.3% higher than on average in Siberian Federal District. Only in two regions of Siberian federal district (Omsk region and the republic of Tyva) this indicator is higher than in Kuzbass (35.4% and 35.2% respectively). The lowest indicators of expenses on food and drinks among regions of Siberian federal district are in Krasnoyarsky Krai (26.2%) and in Tomsk region (23.9%) [28, p. 85].

In general it is necessary to emphasize that the expenses share of families on livelihood in the lump of consumer spending is one of the most evident for the characteristic of living level and it is often used in the international comparisons. So, according to LLC Rating Agency in 2014 the average expenses share of families on food in the European countries constituted 22.6%. Traditionally in this rating Luxembourg (8.6%) is the leader; the indicator in 15 countries range from 10% to 15% (The Netherlands, Denmark, Great Britain, Switzerland, Norway, Austria, Ireland, Cyprus, Finland, Germany, etc.). Russia is 28th of 40 European countries with value of 27.7%. Ukraine is the last, and it is the unique country of Europe in which a share of expenditure for food has transshipped for a half of all consumer spending of families (55.5%) [http://riara-ting.ru/infografika/20150115/610643424.html]. In our opinion, the expenses share of households on acquisition of food is the most compact, informative and adequate indicator of population life quality from all indicators connected with food consumption.

The relevant data by separate product groups [see, for example, 31] give a substantial picture of food consumption in the region and their nutrition and energy value (Table 3).

The most important of the analyzed product set is milk and dairy products – average Kuzbass dweller in the analyzed period consumes at least 250 kg of them a year. Then come grain products (not much less than 100 kg) and meat and meat products (76–85 kg). Further we can see vegetables and melon; potatoes; fruit and berries. Sugar and confectionery, fish and fish products, oil and fats are least represented in a product basket. For 4 years more or less directed increasing consumption dynamics was shown by the following groups of goods: fruit and berries; meat and meat products; eggs; fish and fish products. The opposite dynamics is with vegetables, oil and other fats. The nutrition value analysis shows that the use of proteins increases from year to year and the quantity of fats and carbohydrates, as well as of the food energy value change not so unambiguously.

Table 3. Consumption, nutrition and energy value of food in households (in average on the member of a household) [29, p. 120]

Indicators	2010	2011	2012	2013	2014
Staple food consuming, kg a year:					
Grain products	98	96	91	94	94
Potatoes	71	72	65	59	62
Vegetables and melon	73	80	84	80	79
Fruit and berries	62	64	68	72	72
Meat and meat products	76	80	83	81	85
Milk and daity products	251	271	270	261	257
Eggs, pc.	217	217	219	225	232
Fish and seafood	17	19	21	21	21
Sugar and confectionery	27	30	28	29	28
Vegetable oil and other fats	13	12	12	11	11
Nutrition value, g. in days:					
Proteins	72	74	75	75	77
Fats	107	110	108	107	107
Carbohydrates	326	332	316	324	323
Energetic value, kcal a day	2562	2627	2543	2564	2575

It is interesting that villagers eat much more bakeries, potatoes and other vegetables than citizens, but less meat, fish, dairy products, sugar and confectionery. At the beginning of the analyzed period villagers consumed fewer eggs, than citizens, but in the last two years they were ahead of residential locations inhabitants. It is also noticed that those who live in the rural zone consume more carbohydrates in comparison with those who live in the cities; in the last two years villagers' food became more high-calorie [29, pp. 120–121]. On consumption amounts direct and rather strong impact is done by a family structure, in particular, existence of children. So, the statistics shows, that the more family is and the more children under 18 it has, the less members of a household consume products practically of all analyzed groups, the nutrition value and caloric content of food is lower (for example, in 2014 the energy value of the consumed products in families with 1 child under 16 years constituted 2367 kcal a day on 1 member of a household; in families with 2 children it was 1907 kcal a day; in families with 3 children – 1813). Consumption of food is growing considerably with family income, especially on such product groups as vegetables, fruit, meat, dairy and fish products [29, p. 124; 30, pp. 24–25].

Consumed food characteristics are reasonable to compare to rational regulations which are done by many researchers [5, pp. 32–34]. A.M. Geshonkov and E.Y. Merkulova have developed a technique, which could determine the compliance degree of food consumption in Russian regions with the existing standard parameters, and also divide all territorial subjects of the federation into three groups. Kemerovo region was placed to the second (average) group on the compliance level of the actual and rational consumption [6, p. 61]. One more cut of the analysis (territorial) shows that in Kemerovo region the food value is two elements (proteins and fats) higher, than on average in Russia and in Siberian federal district, and carbohydrates and energy value is lower, especially, in comparison with indicators of Siberian Federal District [28, p. 97].

As it was already marked above, there are indices of living conditions which add parameters of a living standard and in the amount with them characterize quality of life. It is necessary to carry indices of consumer prices to the indicators of living conditions connected to consuming of food (such as a consumer price index on foodstuff and their separate groups, and also on vendors of agricultural production; average consumer prices of separate types of foodstuff; the cost of the main food consumed in households; cost of the minimum set of food); indices of retail trade turnover by foodstuff; commodity structure of retail trade turnover; objects of retail trade and public catering; turn of public catering and some other. These characteristics reflect under what circumstances a consumer behavior appears and what infrastructure, price and other conditions provide it. We will study some of them and, first of all, consumer price indexes (Table 4).

In general the consumer price index shows change in time of an overall price level and rates for the goods and services purchased by the population for non-productive consumption by fixation of the set of goods and services cost relation in a current period to its cost in a previous period. On average for the analyzed period the consumer price index on goods and services in Russia has constituted 107.9% a year [28, p. 37], and on foodstuff – 109.4%. The highest consumer price indexes on foodstuff were in 2010 and, especially, in 2014, the lowest – in 2011. Siberian Federal District and Kemerovo region keep all-Russian tendencies, but it should be noted that in Kuzbass a consumer price index on foodstuff in all years (except for 2011) is a little bit higher than on average across Siberian federal district. So, in the last two years only Altai Krai and the Republic of Buryatia exceeded Kemerovo region on this indicator.

Table 4. Consumer price indexes on foodstuff [28, p. 38; 27, p. 268]

Indicators	2010	2011	2012	2013	2014
Russian Federation	112.9	103.9	107.5	107.3	115.4
Siberian Federal ditrict	111.3	105.0	108.2	106.9	114.7
Kemerovo region, including:	111.6	104.9	108.4	107.3	115.1
Meat products	106.5	105.3	109.0	101.2	120.1
Fish products	102.7	108.4	99.3	107.1	119.1
Oil and fats	132.7	102.9	104.9	109.7	104.6
Milk and dairy products	117.5	107.4	106.1	113.3	114.0
Cheese	116.1	99.7	100.2	122.2	113.6
Eggs	117.1	108.4	97.8	133.5	104.5
Sugar	120.8	74.0	104.4	105.5	143.4
Confectionary	105.3	111.4	107.3	106.6	111.1
Coffee, tea	104.3	111.5	104.3	102.7	107.0
Salt, sauces, spices, concentrates	103.6	108.4	106.0	105.7	106.1
Wheat flour	127.8	87.8	139.7	97.3	111.5
Bread and bakery products	103.1	120.8	113.2	109.0	108.1
Grain and bean	153.2	91.2	95.8	100.0	141.4
Pasta	104.5	102.9	109.0	103.8	108.1
Potatoes	198.2	47.2	194.4	85.2	106.5
Vegetables	166.2	66.2	115.9	109.4	120.4
Fruit and citrus	113.6	100.5	108.0	99.5	118.4

Dynamics of the regional prices of specific food was not very stable. In 2010 prices on potato grew almost twice; by 66% – on vegetables, by 53% – on grain and bean; by 33% – on oil and fats; by 28% – on wheat flour. There was no prices reduction in foodstuff groups. In 2011 potato, vegetables, sugar, flour, grain and bean prices fell significantly. And the largest prices increased on bread and bakery products. In 2012 the greatest surplus of the prices was again shown by potatoes (94%) and wheat flour (40%). There was reduction of prices too, but insignificant – on fish products, eggs, grain and bean. In 2013 the maximum dynamics was shown by the prices of eggs (34%) and cheese (22%), and some decrease was fixed on flour, fruit, citrus and, especially, on potatoes. In 2014 there was no prices reduction at all, and the largest growth was on sugar (43%), grain and bean (41%), vegetables and meat products (both 20%). It is necessary to emphasize that prices instability of many food groups depend on a price situation on agricultural producers who, in turn, are caused by production volumes. For example, potato prices fluctuate, first of all, because of its productivity which differs strongly by years.

Important concept of population's living level statistics is the minimum set of food (MSF) cost. MSF is determined for a year for men of working-age and it reflects interregional differentiation of consumer prices levels. To calculate it the minimum amounts of consumption in the Russian Federation are used. The minimum set of food includes: wheat flour (20 kg), peas and haricot (7.3 kg), millet (6 kg), bread and bakery products (115 kg), vermicelli (6 kg), potatoes (150 kg), onion (20 kg), cabbage (35 kg), carrots (35 kg), cucumbers (1.8 kg), apples (18.6 kg), sugar (20 kg), cookies (0.7 kg), caramel (0.7 kg), 1st category beef (15 kg), mutton (1.8 kg), pork (4 kg), hens (14 kg), herring salty, picklings and so forth (0.7 kg), frozen fish (14 kg), milk (110 l), sour cream (1.8 kg), butter (1.8 kg), low-fat cottage cheese (10 kg), firm sorts of cheese (2.5 kg), eggs (180 pieces), margarine (6 kg), sunflower oil (7 kg), salt (3.65 kg), black leaf tea (0.5 kg), black pepper (0.73 kg) [35, p. 163]. The MSF price is calculated for a month. At the end of 2014 in Kemerovo region it constituted 3127 rubles (Table 5).

For the analyzed period MSF cost in Kemerovo region grew by 35%, but at the beginning of the period it decreased a little, and then began to grow, especially intensively in 2012 and 2014. It is interesting that the tendency of MSF cost change doesn't match the appropriate tendency of a consumer price index by years. So, in 2011 and 2013 the consumer price index advanced indices of MSF cost change and in 2012 and 2014 it lagged behind [35, p. 82]. This 10% mismatch in 2011 and 2012 was especially strong. Therefore, the consumer price index can't adequately reflect dynamics of MSF cost change. Changes of the average per capita income of the population and cost of MSF were more close indices during the period since 2011 to 2013, however in 2014 the last index strongly "shot ahead" - for 20% [35, p. 82]. It was reflected in specific weight of MSF cost in the income of the population – in 2014 it grew almost by 2% in comparison with previous years.

Table 5. Minimum set of food cost (at the end of the year) [35, pp. 80, 81, 84]

Indicators	2010	2011	2012	2013	2014
MSF cost, rub.	2322.9	2237.0	2585.4	2721.3	3127.1
MSF cost change, %	-	96.3	115.6	105.3	114.9
Specific weight of MSF cost in population income of, %	-	10.1	10.0	10.1	11.9

The territorial cutoff of the analysis shows that MSF cost in Kemerovo region at the end of 2014 was lower than on average across Russia (3297.9 rubles a month), and lower than on average across Siberian federal district (3323.7 rub). Intraregional comparing of data on the largest cities of Kuzbass (Kemerovo, Novokuznetsk, Prokopyevsk) allows to state that MSF is more available to the population in Prokopyevsk (2977.5 rub), and is less available in Novokuznetsk (3253.0 rub) [35, p. 83].

In the modern conditions the major loading on food support of population belongs to trade. The main indexes of retail foodstuff trade turnover in comparison with nonfood products are given in Table 6.

Absolute values of retail trade turnover, including drinks, and tobacco products in per capita terms grow at foodstuff from year to year, but the relative (as a percentage to previous year) have multidirectional dynamics, however in recent years, especially, in 2014, abbreviation of volumes of commodity turnover was recorded. At the same time rather stable share of retail commodity turnover by foodstuff in a total amount of commodity turnover (46–47%) in 2014 grew directly by 2%. It means that in difficult economic conditions the population is stimulated to redistribute expenditures in favor of food. So, retail commodity turnover by nonfoods in 2014 was reduced not only in the relative, but also in absolute values, including per capita.

In commodity structure of retail commodity turnover in 2014 top products were: alcoholic beverages and beer (10.1%); meat and meat products (8.1%); dairy products (4.3%); confectionery (3.4%); tobacco products (2.7%); bread and bakery products (2.4%) [29, p. 130].

The status of retail trade is also characterized by a distribution network. As of the end of 2014 Kemerovo region had: 43 hypermarkets (the area of trading floors of 442912 sq.m), 623 supermarkets (417317 sq.m), 939 specialized grocery stores (53567 sq.m), 4320 minimarkets (369635 sq.m), 1760 pavilions (47902 sq.m), 1577 tents and booths [36, p. 6].

One more factor of population food support is operation of catering establishments [37]. The statistics offers rather evident system of the indexes reflecting functioning of a public catering (Table 7).

Public catering turnover in general and it increased from year to year per capita in terms of money, but some price dynamics is ambiguous: after the growth in 2011–2012 there was some increase of indexes. At the same time in the all-Russian turn of public catering Kuzbass occupies only 1.3–1.4%. Public catering turnover in Kemerovo region per capita is much lower than in Russia, for example, in 2014 – by 43%.

Statistical information allows us to evaluate the average lunch price in a canteen, cafe, bistro (except canteens in organizations) for one person. So, in 2011 a Kuzbass dweller paid about 170.07 rubles, in 2012 – 182.36 rubles, in 2013 – 195.25 rubles, and in 2014 – 213.45 rubles. Note that the growth of lunch price was carried out by slower rates than consumer foodstuff prices, especially in 2014 – by 9.3% and 15.1% respectively [35, p. 71].

Table 6. Retail trade turnover of foodstuff, including drinks, tobacco products and nonfoods (in valid prices) [27, p. 210]

Indicators	2010	2011	2012	2013	2014
	Millions of rubles				
Foodstuff, including drinks, and tobacco products	120200	136067	148429	161135	163138
nonfoods	138777	151212	169319	183707	171954
	As a percentage to the total				
Foodstuff, including drinks, and tobacco products	46.4	47.4	46.7	46.7	48.7
nonfoods	53.6	52.6	53.3	53.3	51.3
	As a percentage to previous year				
Foodstuff, including drinks, and tobacco products	96.8	102.9	103.4	99.5	91.6
nonfoods	112.1	102.8	104.8	102.9	89.1
	Per capita, rubles				
Foodstuff, including drinks, and tobacco products	43439	49370	54040	58846	59767
nonfoods	50152	54866	61646	67089	62998

Table 7. Public catering turnover (in valid prices) [27, p. 212]

Indicators	2010	2011	2012	2013	2014
Public catering turnover:					
Million rubles	10250	11802	14385	15553	16327
Per capita, rubles	3704	4282	5237	5680	5982
As a percentage to previous year (in the comparable prices)	86.1	106.7	115.4	98.5	99.0
Specific weight in a turn of public catering across Russia, %	1.3	1.3	1.4	1.4	1.3
For information: only across Russia					
Per capita, rubles	5470	6320	7120	7885	8570
As a percentage to previous year (in the comparable prices)	103.0	106.3	106.9	104.0	101.6

Functioning indexes comparison of public catering on municipalities allows us to range the them according to their share in the regional turnover volume of public catering. The biggest cities of Kemerovo region were on top: Kemerovo (32.4%), Novokuznetsk (28.6%), Prokopyevsk (7.1%). Less than a percent in a total turnover of public catering of the region is occupied Berezovsky, Kaltansky, Krasnobrodsky, Myskovsky, Osinnikovsky, Polysayevsky and Tayginsky districts and almost all municipal regions (except for Kemerovsky, Novokuznetsky and Tashtagolsky) own less than one percent in a total turnover of the regional public catering. If we take public catering turnover indexes per capita, we can see something different: Novokuznetsky municipal region (181% of regional average level), Tashtagolsky (163%) and Kemerovsky city districts (162%), Novokuznetsky city district (142%), Mezhdurechensky city district (103%) get head. In the remaining municipalities indexes are lower than regional average value [38, p. 157].

To complete the description of the Kuzbass public catering we would like to say that at the end of 2014 Kemerovo region had 601 public bistros for 15285 people, 1128 canteens of educational institutions, organizations and industrial enterprises for 90880 people, 1386 restaurants, café and bars for 62043 people [36, p. 6].

CONCLUSION

The statistical data analysis on a living standard of the population, consumer behavior and accompanying conditions in Kemerovo region will allow revealing some facts and regularities. So, real income indexes of the Kuzbass population in 2014 were reduced in comparison with prior year, the living wage value and the poverty level grew, and more intensively than in average in Russia. However the differentiation level of the population by the income level decreased and remained lower than all-Russian. Many living conditions are less favorable than in average in the country. It is reflected in life expectancy indexes (though it grew steadily during the analyzed 5-year period), unemployment rate, general living space security, proportion of pensioners in the total number of the population, morbidity and other indicators.

Food consuming data also confirm lowering of living standard and deterioration in living conditions: the share of food-buying expenses grew by 41% from 2010 to 2014 whereas consumer expenses in general – by 37%; for the last year the foodstuff expenditures share of households increased. It is especially high among lonely people, large families, villagers and the population with the lowest income. At the same time the share of expenditure for foodstuff in Kuzbass is higher, than on average across Russia and across Siberian federal district. Dairy products, bakery and meat products prevail in the structure of the consumed food in the area. In recent years people from Kuzbass began to consume more fruit and berries, meat, eggs, fish, and less butter and other fats. An unambiguous tendency is tracked: the larger the family, the less products are eaten in households counting on one person and the lower the nutrition and energetic value of products. In general Kemerovo region is the region with the average compliance level of the actual and recommended consuming of food.

During the analyzed period food prices were growing permanently in Kemerovo region, and, as a rule, by higher rates, than on average across Siberian federal district. But prices dynamics of the specific commodity groups wasn't stable, especially, of potatoes, vegetables, sugar, flour, grain and bean. The minimum set of food cost in Kemerovo region grew by 35% in last 5 years, but it remained below the similar index in Russia and Siberian federal district. The MSF cost made one tenth of average per capita income, and in 2014 grew to 12%. However this indicator isn't proportional to a consumer price index in general, therefore it can't precisely reflect the life quality of the population.

The reduction of retail foodstuff trade turnover in the last two years (in percents to previous year) and simultaneous increase of foodstuff share in a total amount of retail trade confirm the decrease in life quality. The population is to redistribute family means in favor of food, reducing the consumption level at the same time. Trade organizations network in Kemerovo region is rather developed, what cannot be said about catering companies (except for the two largest cities Kemerovo and Novokuznetsk). Regional catering turnover per capita is much lower, than on average in Russia, and its increase cannot be provided even by lower growth rates of lunch prices in canteens and cafes in comparison with foodstuff prices dynamics in general.

Thus, the statistics connected to consumer behavior of the population concerning food is the most important characteristics of level and conditions of its existence, and it is capable to adequately show life quality, even in the absence of other data.

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Please cite this article in press as: Morozova E.A., Glushakova O.V., and Fadeikina N.V. Food consumption as an indicator of the quality of life of the population in regions. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 171–180. doi: 10.21179/2308-4057-2016-1-171-180.

BUDGETING SYSTEM IN ADMINISTRATIVE ACCOUNT OF THE MODERN ORGANIZATION

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Received April 28, 2016; Accepted in revised form June 10, 2016; Published June 27, 2016

Abstract: The ordered system of tax, measurement and information interpretation which is necessary for decision-making in the managerial account of organization, is the important constituent of a finance administration of organization. Budgeting is also a part of finance administration system, and it represents budgetary management along with planning on the centers of financial responsibility. The article gives information about the conducted research, determining interrelation between the system of budgets and managerial account in financial management. The budgeting includes the mechanism of incomes, costs, resources planning. In this case the managerial account is a data vendor. Managerial account uses the methods of system construction of plans (budgets). The authors study different approaches to budgets formation and system of the managerial account formation. The paper pays special attention to storekeeping as the basic property requiring updating and to problems of security substantiation, which many organizations give not enough attention to. The basic method used in the article is the method of scientific abstraction, and also monographic method. The authors come to the conclusions that use of budgeting principles focused on the result leads to the best finance results of the enterprise, to safe-keeping and integrity, and to the increase of staff responsibility.

Keywords: Budget, budgeting, managerial account, finance administration, company programming

DOI 10.21179/2308-4057-2016-1-181-185 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 181–185

INTRODUCTION

It is possible to increase management efficiency of enterprise by system engineering of budgets and the control over their performance which are possible at usage of materials of managerial account. Budgetary management requires construction of organizational enterprise structure which would supply formation of necessary budgetary indicators and operative control of budgetary process. The character of organizational structure in turn depends on tools of the managerial account. Therefore it is important in enterprise economic activities to generate correct organizational structure in which budgeting and managerial account supplement each other, instead of duplicate on functions. The research objective is working out of the concept of administrative account based on budgeting and storekeeping of the enterprise.

OBJECTS AND METHODS OF STUDY

One of the most famous and effective technologies of planning and management is budgeting which at many enterprises became one of the most effective tools of revealing of possibilities for increase of business concerns efficiency. All these cannot be introduced without elements of managerial account.

Russian scholars try to adapt existing western systems of budgeting to conditions of commercial relations development on domestic managing subjects. Recently there were domestic papers considering questions of the organization of budgeting with reference to the Russian enterprises.

Structural and logic method, monographic method and method of analysis of factors influencing on research productivity are applied at choosing of organizational enterprise structure directed on efficiency of finance administration.

RESULTS AND DISCUSSION

Budgeting is mostly often treated as one of management functions, process of definition of actions which should be executed in the future. A. Karpov determines budgeting as one of effective tools of management which at the competent approach to its usage allows the company to earn profit more effectively and to operate financial streams. M. Trachenko determines budgeting as technology of company management. It includes planning, analysis and control how the liability centres (TsFO) and the enterprise as a whole perform the budget [2].

Some other authors (V. Burtsev, D. Trubetskov) consider budgeting as continuous cyclic process of formation, assertion and performance of budgets. V. Burtsev considers, that budgeting is a system of short-range planning, account and control of resources

and activity results of commercial organization in the centres of responsibility and (or) in business segments, allowing to analyze the predicted and received economic indicators with the purpose of business processes management.

Some authors, speaking about budgeting, often mention financial model of the company. The financial model is a wider concept including a technique of planning, account, control and analysis of performance of budgets. Accordingly, the financial model of budgeting contains models of planning, model of account and model of analysis and budgets control.

M.K. Starovoitov defines budgeting as "special tool of management which essence can be determined as integrated system of drawing up budgets of a running control of the accepted budgets, account of deviations of actual indicators from budgetary and the analysis of reasons of essential deviations".

Yu. Brighem and L. Gapenski define budgeting as "a process of the co-ordinated scheduling and management of divisions activity by means of estimates and economic indicators".

Yu.D. Batrin, P.A. Fomin consider budgeting as "a method of resource allocation, characterized in the quantitative form, for achievement of purposes also presented quantitatively".

M.K. Samochkin, A.A. Kalyukin, O.A. Timofeeva suggest to advance budgeting as "decision-making process through which the enterprise evaluates expediency of inflow and outflow of assets".

V.E. Hrutsky, T.V. Sizova, V.V. Gamayunov treat budgeting on the one hand as "a process of drawing up accounting budgets and estimates", and, on the other hand, as administrative technology intended "for development and increase of financial validity of accepted administrative decisions" [2].

The system of managerial account should cover all divisions responsible for the account of expenses and company earnings and simultaneously to be not too difficult and bulky. In each centre there should be workers responsible for the account of incomes, costs, investments etc. They develop concrete norms of reports, forms of reports under incomes and costs of budgets of business units.

Incomes and costs should be considered in the system of managerial account in two stages. At the first stage incomes and costs are fixed irrespective of the fact of the made payment. Effected payments are reflected at the second stage. The necessity for similar system of managerial account is explained by the necessity of reflexion for items of expenses, not only executed expenses, but also what the enterprise has suffered during this period and on which it has obligations on their payment. The major factor at creation of system of managerial account is its economic efficiency. These are those benefits which the enterprise receives from availability of an accounting system at the expense of improvement of quality of accepted decisions. The operation of managerial account system will be economically sound when the positive effect received as a result of this activity will surpasses necessary expenses for creation of the given system.

Putting into practice the organization of managerial account which should cover all divisions responsible for the account of incomes, costs, investments, etc. it is necessary to exclude duplication of functions and loss of information in the absence of department awareness among them (the developed horizontal ties). The American scientist, professor D. Medouz pays attention in the work "The Alphabet of System Thinking" that "big organizations of any type, from corporations to governments, lose stability simply because mechanisms of feedback, thanks to which they receive information and react to surrounding conditions, should overcome too many consecutive delays and distortions" [10]. It is also important not to forget about the necessity of connection of financial and managerial account functions, according to a principle of the developed western companies where chief financial executive and chief accountant is one and the same person.

In the book "Corporation Reengineering. The revolution manifesto in business" M. Hammer and D. Champi have developed a substantiation of necessity of basic changes in the existing management paradigm. Authors asserted, that in new postindustrial economy the inquiries of clients receive a priority, the competitiveness amplifies, and constant changes are normal for business dealing. From the point of view of control system reorganization, the realization of reengineering principles assumes replacement of a hierarchical functional principle of management to the interfunctional one, that is the process-focused method of administrative activity organization [8].

Interaction of budgeting and system of managerial account consists that budgets allow to plan and represent necessary indicators of economic activities in that kind which is the most comprehensible for acceptance of effective administrative decisions. In this case managerial account represents the system of internal account at the enterprise, or, structural system of information transmission.

Before starting the statement of managerial account system, it is necessary to do preliminary organizational preparation. The given preparation assumes division creation which tasks will include definition of incomes and costs of managerial account system, definition of interrelation degree between data of accounting and managerial account , organization of operative control system over expenses and movement of finance, etc.

At the next stage the question on structuring of managerial account system is considered. Allocation of centers of managerial account divisions, for example, centers of income account, expenses, investments, profit should become the result of studying of organizational administrative structure of enterprise [7].

According to authors, the best variant of formation of managerial account system is creation of budget committee exercising administration of financial streams and control over them. The budget committee should systematize and classify sources of incomes and receipt of money resources of enterprise. The organization of managerial account system should detail articles of expenses, centers of consumption, production kinds in places of expenses occurrence, and budget committee should receive functions of

information generalization and its interpretation that will allow to lower information losses.

In our opinion, to divide authorities between managerial account and budgeting is possible on the basis of management organizational structures. In the competence of managerial account there are places of expenses occurrence, and for budgeting aims the centers of responsibility which are carrying out activity are used.

Thus we receive a new organizational structure of finance administration in which in places of expenses occurrence structural divisions independently form requirement for resources and bear responsibility for their use. Then information is transmitted to budget committee which executes functions of financial account and control over a cash flow and on the basis of the received information about resources movement and means it can evaluate work productivity in places of expenses occurrence and make conclusions on an overall performance of divisions. Thus we observe necessary freedom in actions on reflexion of costs and we carry out the analysis of the received results, that by comparison with allowance for cash flows, allows to make decision on the necessity of those or other places of expenses occurrence. As a result of such organizational structure the place of managerial account consists in complete formation of information necessary for acceptance of administrative decisions at level of enterprise as a whole, and budgeting becomes a planning integral part in structural division. The chief of structural division controls the fulfilment of budgets.

The first interest to management consideration as a science was noted in 1911 when American engineer F. Taylor headed the movement of scientific management (it received the name "school of scientific management" later) which was determined through the knowledge used in the course of work, of its organizations. According to the theory of a scientific direction F. Taylor proved the concept of labour division. In the work "Principles of Scientific Management" which is recognized as the beginning of science management and independent area of researches F. Taylor indicated, that "the best work organization represents the present science leaning on certain laws, rules and principles, as on its foundation". Thus, administrative knowledge produced by managers, advances the organization of executors activity, in the issue – its result [9].

The quantity of mentions in the economic literature on the budgeting focused on result increased sharply recently should attract attention.

The approach focused on results, has received enough wide circulation in activity of the state bodies of many developed countries for the purpose of perfection of mechanism controlling by the state expenditure. Such approach to distribution of public finances should be applied also in practice of the commercial organizations [3].

At introduction of the budgeting focused on result, the risk of loss of the control increases, therefore an important element of such system is growth of supervising function.

One of the main impulsive causes of budgeting introduction focused on result, is a possibility of more effective (re-) distribution of means between competing items of expenses. It is reached thanks to reception of more complete and exact information about results of realization of business processes, stage-by-stage change of control mechanisms of budget outturn.

The budgeting focused on result, does not solve a question of optimum distribution of budgetary resources between priority directions, it only creates favorable conditions for this purpose, transferring accent on achievement of end results, which these resources are allocated on.

Quality monitoring, at a wasteful method of budget formation, consists in the following: conformity between the accepted budget and performance of costs in clause-by-clause cut, and also observance of uniform specifications are checked, the check of special-purpose character of each operation on authorization of costs (it is made before realization of costs) is done.

Quality monitoring at orientation on result provides a compliance test between the planned and reached results of activity, direct and end results of activity are planned in advance, during the check which is conducted after realization of costs, correctness of the made costs is not checked, but correctness of measurement of the received results is done. The basic responsibility for legitimacy and correctness of actions are placed at managers of means at simultaneous strengthening of their responsibility for granting of services.

The budgeting focused on result, assumes preparation of reports, whether the scheduled problems are carried out, whether the planned results are reached. The answers to such questions assume engineering of indicator system which would allow to trace and evaluate results.

So managerial account forms information on the centers of responsibility and considers expenses and incomes. Managerial account is also necessary as a layer for reflexion of intracompany turnovers. The primary goal of managerial account is the answer to a question, in what condition there is an enterprise, how it is necessary to distribute present resources to increase efficiency of activity. Accordingly, managerial account requires productivity estimation on those or other parametres in time and in connection with any event. And events about which managerial account should present the information to the proprietor and are formed in structural division on the basis of budgets made by them for themselves which productivity will advance managerial account. That is managerial account does not dictate what indicators are necessary to it, indicators are formed in a place of expenses occurrence (structural division), and the aim of account is to find out efficiency of enterprise activity as a whole with allowance for the indicators (budgets) advanced in structural division.

The purpose of modular system of managerial account consists in creation of an information product for rendering of support to a management of the economic subject in acceptance of economically reasonable administrative decisions. The information formed in the account is necessary for fulfilment of the following main objectives:

(1) drawing up of the periodic (routine) internal reporting for administrative decisions;
(2) drawing up of irregular (special) reports for administrative decisions.

As any information system including its modular system, in particular managerial account, executes following functions: perceives queries entered by the user and necessary basic data, processes entered and stored in the system data according to known algorithm and forms the numerous detailed microcomplete sets of output information [12].

In information system necessary functional components (functional modular systems) which help to understand restrictions of various architecture of information systems are allocated. In a contour of managerial account objects are defined, information about which is interesting to managers of the company from various functional modular systems (information systems of accounting financial account, tax account, keeping of statistical records, etc.). As a result from the general functionality of system separate components which are used through a call of methods in various modular systems of information system are allocated. Functional components of modular systems mount a substantial basis of information system which bases on models, methods and algorithms of reception of the operating information.

The result of conducted research is working out by authors the concept of management perfection by industrial stocks (Fig. 1).

The given concept captures the basic directions of creation of complex support of decision-making in the field of management perfection by industrial stocks and expenses. The concept allows to combine system and situational approaches to management and it gives the chance to consider influence on managerial processes of internal and external factors, current requirements of the enterprise and its long-term objectives. The possibility to depart from storekeeping and expenses of production from a direction "plan-fact" in the sphere "efficiency – tactics – strategy" is granted the company, allowing to open new possible prospects in the given area of research.

As a result of the conducted researches the conclusions are made, that the system of administrative account should cover all divisions responsible for the account of expenses and company earnings, thus to exercise administration of financial streams and control over them. Also an important conceptual moment in this system of administrative account is a necessity of reflexion in expenses items, not only the made expenses, but also what the enterprise has suffered during this period and on which it has obligations on their payment. The statement of system of administrative account should include organizational preparation then system structuring at which in the competence of administrative account there are places of expenses occurrence, and for budgeting aims the centres of responsibility which are carrying out activity are used. Thus we receive a new organizational structure of finance administration where in places of expenses occurrence structural divisions independently form requirement for resources and bear responsibility for their use.

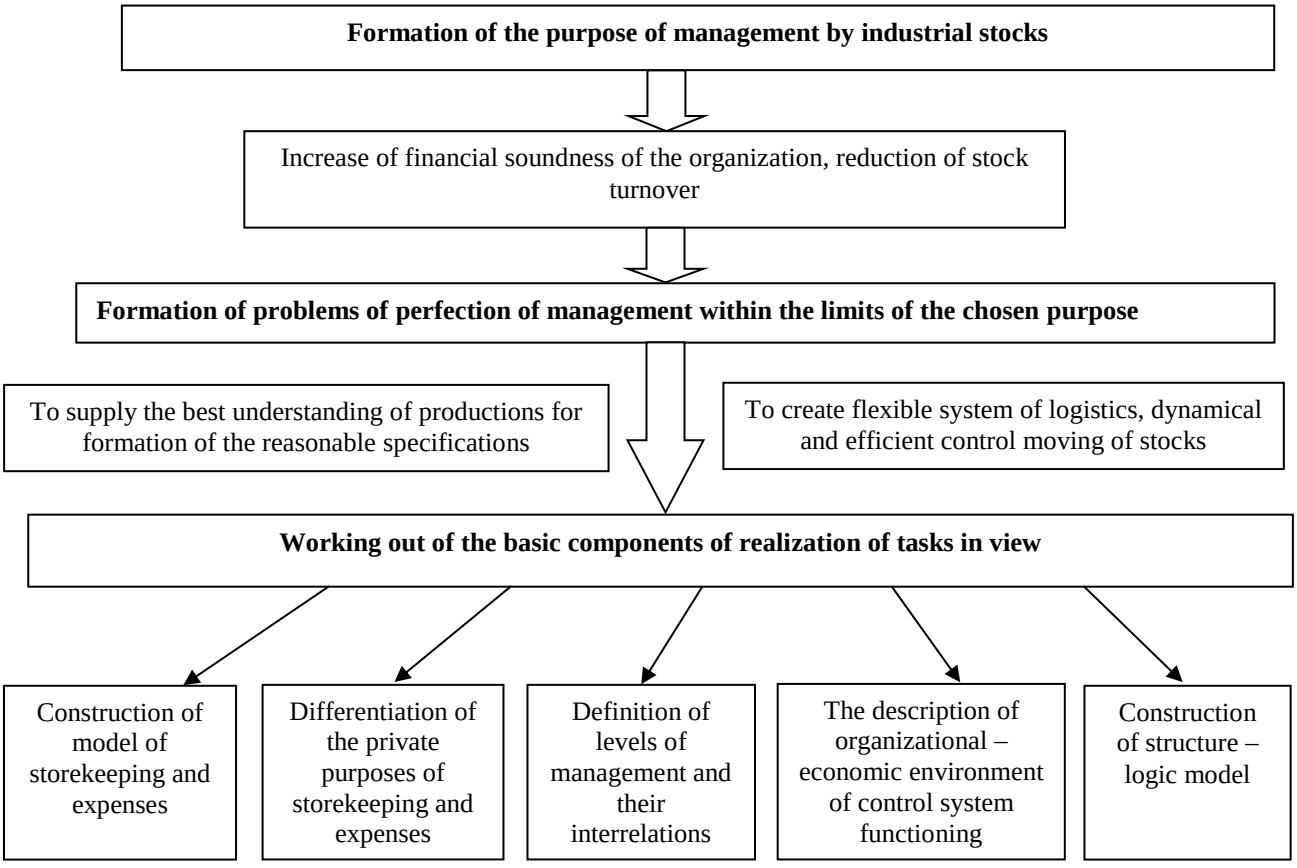

Fig. 1. Concept of perfection of management of industrial expenses.

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Please cite this article in press as: Ryabko T.V., Zhidkova E.A., and Zotov V.P. Budgeting system in administrative account of the modern organization. *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 181–185. doi: 10.21179/2308-4057-2016-1-181-185.

THE PROBLEM OF PROVIDING FOOD RESOURCES IN URBAN AGGLOMERATIONS (THE CASE STUDY OF THE KUZBASS AGGLOMERATION)

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Received February 07, 2016; Accepted in revised form March 20, 2016; Published June 27, 2016

Abstract: Urban agglomerations are the result of a process of spatial competition for resources. To analyze the functioning of specific urban centers, it is necessary to make a distinction between the process of agglomeration and the state of agglomeration in the spatial structure. This paper shows the interrelation of the process of urban agglomeration and the agglomeration of the production activity as its economic foundation. The study reveals the connection between the urban agglomeration process and the agglomeration of the production activity with the purpose to ensure food supplies. The authors analyze the background, causes, opportunities, goals and challenges of the Kuzbass urban agglomeration. The specifics of the Kuzbass agglomeration lie in a significant level of urbanization and in having two core cities in the region. This gives grounds to describe the Kuzbass agglomeration as a conurbation. The relationship between the two centers within the conurbation is an under-researched problem. The specifics of the Kuzbass agglomeration (conurbation) also lie in its in-between location and close proximity to the neighboring agglomerations. On one hand, its location exacerbates a competition for resources, but on the other hand, it is the basis for the solution of certain internal problems, such as food supply security. Usually, an urban agglomeration is accompanied by a reduction of the rural population, and thus, by a decrease of the opportunities for agriculture. The Kuzbass agglomeration's location allows for a solution to the problem of food security not only due to the development of its own agricultural sector, but also due to the agrarian sector in the neighboring agglomerations.

Keywords: agglomeration, urban agglomeration, conurbation, food market

DOI 10.21179/2308-4057-2016-1-186-196 *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 186–196.

INTRODUCTION

One of the trends of modern spatial structure is described by the term "agglomeration". An analysis of the economic phenomena can only be based on a clear understanding of the applicable categories. The variety of the definitions of "agglomeration", its content and its various aspects, are predetermined by a difference in the methodologies that describe the definition. Often, the research lacks the consideration of the dynamics, does not differentiate between the process of agglomeration and the state of agglomeration. There is no a clear differentiation between the concept of urban agglomeration and agglomeration of cities. An internal contradiction within the process of agglomeration, the contradiction between two tendencies – the concentration of economic activity and its dispersion – has not been described. Agglomeration is often not seen as a competition for the use of local resources. The purpose of this research is to identify and describe the specifics of the Kuzbass agglomeration from the point of view of the methodology of economic theory. The emphasis is placed on the analysis of the relationship between the process of cities' agglomeration and the agglomerations of production activity to ensure food supplies. The process of cities' agglomeration is based on the influx of new resources – territories, investment, human resources, infrastructure development. There is a redistribution of the population of the region in favour of the city. As a result, a reduction of the agricultural sector in the region takes place, and consequently, food dependence increases. This paper gives theoretical grounds to the actions of regional authorities to solve the food supply challenge under the conditions of internal and external agglomeration competition.

OBJECTS AND METHODS OF STUDY

The process**s** of agglomeration and the state of agglomeration characterize patterns of the functioning of the economic space, as well as representing the result of the patterns of the functioning of the economic space. International studies have described and explained the causes and conditions of the agglomeration process [1]. Any spatial structure is

based on economic practicability and is subject to certain spatial patterns [2]. The subject of this research analysis is the urban agglomeration as an economic category and the Kuzbass agglomeration as a real functioning spatial formation. As of today, scholars haven't clearly defined the relationship between the categories "the process of agglomeration" and "the agglomeration" as a state. There is a certain lack of analysis of what causes the urban agglomeration as a competition for resources. The basis of this study are the findings of such prominent international scholars as A. Marshall, P. Krugman, M. Fujita, T. Mori, H. Ogava, D. Harvi.

The paper analyzes the patterns and features of the functioning of the Kuzbass agglomeration. The analysis is based on theoretical conclusions. The research pays particular attention to the problem of food security in the following conditions: 1) the reduction of the rural population of the region; 2) the increase of competition for resources from neighboring conurbations; 3) the increase in international economic sanctions.

The analysis of the Kuzbass agglomeration has been carried out in the framework of the RHSF Grant "The development of a management system of social and economic development of the urban agglomerations in the Kemerovo region".

RESULTS AND DISCUSSION

A. Marshall emphasized a crucial role of external influences (externalities) in the formation of economic agglomerations. Once an industry finds its niche on the market, it will probably keep it for a long time: so great are the benefits from close proximity to other industries [3]. Industrial agglomeration, as well as urban agglomeration is the result of a "snowball effect", in which an increasing number of agents are going to benefit from a wide variety of activities and a high degree of specialization. M. Fujita and H. Ogava also pointed at the connection of urban agglomeration and the agglomeration of firms. The agglomeration's capacities grow from the interaction between a preference for diversity of products and transportation costs. In this model, the city can be both monocentric and polycentric [4].

According M. Fujita, to understand the causes and patterns of the spatial distribution of economic activity, in particular the formation of large economic agglomerations, as well as regional specialization and trade, it is necessary to make at least one of the following assumptions:

(1) The territory is variegated.

(2) There are external effects in production and consumption.

(3) Markets have inherent imperfect competition.

Thus, the economic basis of urban agglomerations is an industrial cluster or the agglomeration of firms. An agglomeration of small firms is possible as well.

The agglomeration process always has inner contradictions. The struggle between centripetal and centrifugal factors reflect a placement of the productive forces. Centripetal forces tend to "draw" the population and production into the agglomeration, and centrifugal, on the contrary, tend to break these agglomerations. Bulk savings on the industrial volume of production, consumption, and transportation are challenged by the dispersal forces in the form of, for example, the expansion of agricultural lands [5]. Thus, the agglomeration of the city as a process is a manifestation of the centripetal process of the concentration of population around the major cities, which due to endogenous or exogenous factors, have become the core (center) of the spatial structure. Agglomeration (concentration) of production generates self-reproduction. Companies place their production in locations with easy market access, at the same time, the market access improves in locations where production is concentrated. There is an urban effect (when the concentration takes place in one location, and there is a conurbation) and a localization effect (proximity effect).

Many countries have passed and are going through the stage of the geographic concentration of economic activity. Theoretical models of the "new" economic geography, starting from Krugman's model, show that with primitive and costly transportation technology, the economic activity will be distributed more evenly, so that each region could provide their own consumption, without depending on international or inter-regional trade. With the development of transportation, globalization, and the removal of other trade barriers, the concentration of production increases. At the same time, structural changes in the economy reduce the share of the agricultural sector and increase the share of industry, and then the share of the service sector. Manufacturers rush to use the benefits of scale – production and people move to the big cities, the periphery begins to consume the goods imported from the center. Only with a further decrease in transportation costs does the productivity, income and standard of living on the periphery start catching up with the "center" [6].

The spatial structure of the Russian Federation follows the global trend, which manifests itself in an increase in the share and role of metropolitan areas and, accordingly, the manifestation of the opposite tendency – a decrease of the share of developed territory. As of January 2016, the urban population of Russia was 73.9% of the entire population. 62.5% of the urban population lived in metropolitan areas, which comprise 45.1% of the total Russian population. Overall, there are about 52 urban agglomerations in Russia. The Kuzbass agglomeration is among the urban agglomerations with the maximum number of townships within the city boundaries. There are 8 townships there. The urban population is 84.9% of the Kemerovo region. As of today, the rural population continues to decline: it moves into the cities, and the mortality rate of the rural population is higher than its birth rate. In Russia, only 55% of the rural population work in the agricultural sector, the remaining 45% work in industry, transportation, the service sector and other "urban" sectors of the economy. 17.9% of the rural population of Russia lives in areas of urban agglomeration. As a result of the development of urban

agglomerations, a new category – the rural-urban population of urban agglomerations – has emerged.

The global experience shows that metropolitan areas are quite stable. Having emerged for objective reasons (like the attractiveness of the densely populated areas for businesses and jobs), they continue to function and adapt to changing internal and external conditions. In other words, the dynamics of the development of regions and cities depend on their previous history. If for some reason there was a major city in a specific location (eg., Novosibirsk), even if the initial reasons that led to the concentration of people in this place disappear, the city will continue to exist and develop. By creating a market potential, the city remains economically attractive. The downside of this mechanism is that, once settled, an inefficient allocation of resources within the country becomes stable over the long term [7].

Thus, the result of the agglomeration process in the region is the economic space compression, i.e. the concentration of economic activity and population in large agglomerations. Meanwhile, ten years ago, a significant part of the governments of the developing world has been concerned about the increased migration to cities. Such "mega-cities" of the Third World are dysfunctional, they hinder the economic progress. Another pitfall in the process of urban agglomeration is uneven geographical development. The authors of the book "The Siberian Curse: How Communist Planners froze Russia" (in Russian translation "The Siberian burden. Miscalculations in Soviet planning and the future of Russia") point out that the Russian authorities should subsidize those wishing to leave the uninhabitable areas of the Far North (Siberia), and should switch to the shift work mode instead of supporting artificially created megacities [8]. The problem of the necessity and the ability to manage the urban agglomerations remains essential and challenging. If this issue is recognized, then we have to address the challenge of the necessity and ability to manage the agglomerations, and to determine the subjects and spheres of the management.

According to experts, the international experience in the management of agglomerations involves two trends. The first one is the need to improve the efficiency of infrastructure management: the citywide infrastructure, transportation, utilities and business infrastructure. The second trend is the need to improve the efficiency of public administration. In other countries, a lot of attention is paid to the role of coordinating tools, to "soft" horizontal management structures. Thus, these two trends are interconnected in a constant conflict, and the attempt to optimize the first leads to solutions that reduce the efficiency of the second trend, and vice versa [9].

The creation of metropolitan areas is associated with a complex population movement within the city and between cities. It is necessary to track the population's movement, its intensity, its daily and seasonal variations, the ratio of the topography to infrastructure. But local governments are not always able to control and manage the dynamic changes taking place in the urban territory. The data and knowledge, which are currently widely used to make strategic decisions, are often cumbersome, inadequate, rapidly changing and may lead to wrong decisions. Therefore, we need modern geospatial technologies. They allow us to analyze complex information, including the risks and problems of the functioning of urban agglomerations, which in turn, helps to save time and money. New digital technologies have recently been introduced in the practice of urban planning. International research on the issues of geospace is paying more and more attention to the problems of the creation and functioning of the "smart" cities. The June 2015 issue of the journal "Geospatial World" published an article "SMART DATA FOR SMART CITIES" [10].

As for the need to improve the efficiency of infrastructure management, there are obvious tools such as the coordination of strategic plans, the programs of the development of the transportation and municipal infrastructure with the programs of territorial planning. Individual infrastructure facilities must be coordinated with each other.

International experience in the management of agglomerations gives scope for decision-making. The efficiency of public administration, both in Russia and in other countries, is based on two models. The first is a unitary one-tier model, with the management structure designed as a municipality on the entire territory of the agglomeration. Another one is a contract model, when the agglomeration area includes a number of municipalities, such as the agglomeration of New York, with over 2.000 municipalities which coordinate each other's activities. A two-tier model has a certain "above-municipality" level (either voluntary or mandatory). France is an example of a two-tier model, where the law requires 16 urban agglomerations to create another "above-municipality" management level and to delegate to it the general powers for the development of these agglomerations. Paris is another example of a two-tier model of state-municipal management, where one territory has both municipal and state jurisdictions, among which some functions are divided [11]. The US also encourages the creation of joint structures, which have general plans of development: transportation development, social and economic development. The Federal budget generously funds such inter-municipal structures, and the funds are mainly assigned to the development of inter-municipal infrastructure. In other words, the municipalities agree on joint problem solving. In Russia the problem of the management of depressed areas, including, mono-cities and economically "shrinking" territories, has not been solved.

The assessment of the current problems of Russian urban agglomerations is possible on the basis of international experience, paying special attention to the possible problem of competition amongst agglomerations. D. Harvi emphasizes that competition between cities is one of the determining factors in the evolution of capitalism. The competition of cities leads to an uneven geographic development [12]. The toughest competition takes place between global cities. Some urbanists believe that in the future cross-country competition will be reduced to a competition between

the largest cities. The theory of agglomeration of cities holds that agglomeration development tends to increase competition, and especially the competition for resources. The competition for resources means a competition for investments, for which agglomerations should create certain conditions, both institutional and social. Cities must have a certain quality level, they have to be comfortable for living.

The comparison of the neighboring agglomerations of the Siberian Federal District shows that the city-cores of agglomerations follow the global trend (Table 1).

Table 1. The key indicators of Novosibirsk, Kuzbass and Tomsk, Altai agglomerations (2009) [13]

Agglomeration	Population (thousands, persons), 2010 yr.	Percentage of urban population, %	Number of cities in the region, 2010 yr.	Population density per 1 km^2	The immigration rate per 10 000 population, persons	The immigration rate per 10 000 population. Urban population, persons	The immigration rate per 10 000 population. The rural population, persons
Novosibirsk	2 649 900	75.7	14	14.9	136.11%	128.80%	-19.1%
Kuzbass	2 820 600	84.9	20	29.5	77.82%	77.57%	78.2%
Tomsk	1 043 800	69.3	6	3.3	137.21%	125.22%	-7.1%
Altai	2 490 700	53.4	12	14.9	-4.80%	7.50%	-19.0%

The development of agglomerations and the concentration of population in them is influenced by transport and an environmental component. The transport component is more explicit, whereas the environmental component is veiled. The transport component is very significant in the functioning of agglomerations. Its development has an impact both on the size of the urban agglomeration, and the quality of its functioning. The analysis of the role of the transport component in agglomeration requires the addressing several theoretical issues:

– When and under what circumstances will the level of transport accessibility and transport development of the territory be sufficient for a successful actual agglomeration of cities;
– What should be or could be a critical share of the transport component to achieve a certain level of agglomeration;
– The transportation component of the process of urban agglomeration can be analyzed on the basis of qualitative and quantitative characteristics;
– The impact of the transportation component is, firstly, centrifugal, i.e., the more developed the transportation network in a particular area is, the greater potential for development it has. Secondly, the larger are the developed areas, the stronger the centripetal connections become, thus facilitating the process of agglomeration of cities [14].

The transportation component includes the level of transportation tariffs and the level of the development of the transport infrastructure, which in turn is divided into the level of the development of the transportation network and the accessibility of the territory for the development of the transportation network. There are some unsolved theoretical problems. Many researchers use the indicators of transportation availability and accessibility, which reflect the level of transportation services. They depend on many factors: the size of the transportation network; its throughput and carrying capacity; the street configuration; available detours. Though the indicators of the development of transportation services in the area are known: the density of the network per 100 km^2, the availability and accessibility of the transportation services per 10 thousand people, (the generalized index, Engel – Yuzuru Kato's formula), there is no generally accepted definition of the notion "transportation accessibility of the territory". We believe that the development of the transportation network on the territory must be preceded by the territory's accessibility to transportation. The determination of the subjects for which the territory's transportation accessibility is important, and the purposes of the availability of the transport services, are two important characteristics of the transportation accessibility of the territory. The second phase of the analysis is to determine the methods of the calculation of the transportation accessibility of the territory. There are several methods of calculation:

– The average value of the time spent on the movement of goods and passengers in the region, depending on the configuration and density of its transportation network;
– Cargo shipping;
– Passenger transportation.

The summarizing criteria of transportation accessibility is the accessibility of the agglomeration periphery based on time spent. This issue requires government efforts and very considerable financial expenses. The theory has not completely resolved the problem of the optimum ratio of internal and external transportation accessibility and the development of transportation services in the region.

Why is it important to distinguish between the concepts of the development of transportation services and the transportation accessibility of the territory? The impact of the transportation component is not a linear process, it is temporal in nature, i.e., it impacts at the initial stage of the formation of agglomerations and during the agglomeration's functioning (especially in the conurbation). In its turn, the agglomeration's already existing transportation network (i.e., the process of the agglomeration itself) may facilitate or impede the effect of agglomeration. It is also necessary

to understand the consequences of the transportation accessibility and the level of the cities' development in the agglomeration within the country, at the interregional level and within the region.

For the territory of the Russian Federation, increasing transportation accessibility is one of the priorities. Under current regulations in Russia, 90% of workers in large cities (agglomerations) should not spend more than 45 minutes on a one-way commute from home to work, or vice versa.

The effect of the transportation component on the agglomeration process is nonlinear. Therefore, it is important to recognize not only its possible beneficial effects, but also its possible adverse effects. In particular, the reduction in transport costs may lead to a concentration of production in large markets and around them. This can have a negative effect on the development of agriculture in the region. Currently in Russia, only 12% of the rural population have relatively easy, not more than 2-hour transport accessibility, to the developed centers, but 40% of the rural population are deprived of easy access to cities. The analysis of the development of transportation services in the agglomerations – competitors in the SFR allows us to conclude that the differences are probably due to the features of the natural landscape of the area, or due to the locations of economic activities (Table 2).

Table 2. The potential of transport services development on the territory of SFR [14]

Region	The density of the network per 100 km²:		Transportation services per 10 thousand people:	
	Automotive network	cars+railway+water networks	Automotive network	cars+railway+water networks
Novosibirsk region	8.941 km/100 km^2	9.035 km/100 km^2	580.067 km/10 thous. persons	658.616 km/10 thous. persons
Tomsk region	2.216 km/100 km^2	4.039 km/100 km^2	648.451 km/10 thous. persons	1164.155 km/10 thous. persons
Kemerovo region	14.531 km/100 km^2	16.904 km/100 km^2	507.122 km/10 thous. persons	589.951 km/10 thous. persons
Altai region	22.13 km/100 km^2	23.554 km/100 km^2	1558.911 km/10 thous. persons	1659.255 km/10 thous. persons
Krasnoyarsk region	1.118 km/100 km^2	1.569 km/100 km^2	925.226 km/10 thous. persons	1298.638 km/10 thous. persons

We should note that city competition has both positive and destructive consequences. The higher the competition is, the more unstable the society becomes; the competition is rather the way to a crisis. The government should create conditions for smoothing the competition, but without destroying it. In many countries today, the agglomerations overlap, and social and other ties between them are very strong. International experience provides many examples when the two regions were approximately equal and symmetrical, but gradually one region accumulated small initial benefits and turned into a commercial core, while the others became de-industrial peripheries. The formation of urban agglomeration reveals the presence of a core-city and an agglomeration area which includes satellite towns. The peculiarity of the agglomeration process in the Kemerovo region is the presence of the conurbation, i.e., historically formed two centers of gravity – Kemerovo and Novokuznetsk. It provides a basis for introducing a new concept of "Kuzbass agglomeration" [15] (Table 3).

Table 3. The Kuzbass conurbation [16]

Agglomeration	Population, 2015 yr.	Cities of agglomeration	Centre of agglomeration	Population 2015 yr.	Population density per 1 km^2
Kemerovo agglomeration	660 thousand	Kemerovo, Berezovsky, Topki, Kemerovo region	Kemerovo	549 thousand	1 866
Novokuznetsk agglomeration	1200 thousand	Novokuznetsk, Novokuznetsk district, Mezhdurechensk, Myski, Osinniki, Kaltan, Kiselevsk	Novokuznetsk	550 thousand	1 322

The prerequisites for the formation of the Kuzbass agglomeration are: a developed network of automotive roads; existing industrial relations; infrastructure development along the highways; the intense interaction between communities; the back-and-forth migration (related to work, education, cultural and recreational life); the land around the cities available for development; the cottage neighborhoods in the city's vicinity; the regular transportation services between communities; the so-called "hidden urban

population", permanently residing in cottages in the rural areas. The process of agglomeration of cities not only involves the city itself, but municipalities and settlements as well. As a rule, the development of agglomerations is a mutually beneficial cooperation between their actors to enhance the effectiveness of the different services.

As part of the RHSF grant "The development of a management system of social and economic development of the urban agglomerations in the Kemerovo region", we conducted a survey of the population of the agglomeration of Kemerovo. We surveyed 697 people in the Kemerovo agglomeration and 1023 people in the agglomerations of Novokuznetsk, by the method of a quota stratified sampling questionnaire. That allowed us to infer the boundaries of Novokuznetsk and the Kemerovo agglomerations, to estimate the systematic linkages and interactions between the populations of the agglomerations (the population's commutes), and to estimate the sources of the socio-economic effects of agglomeration. The boundaries of the social space of the Novokuznetsk agglomeration as a socio-territorial community have been formed, as evidenced by the high degree of homogeneity in the responses and evaluations given by the surveyed people from different cities.

The local identity is characterized by a positive attitude towards their place of residence. The residents of the agglomeration name common problems, such as: the poor state of roads, the growth of drug addiction and alcoholism, the poor lighting of backyards and streets. There is a high level of internal interactions within the Novokuznetsk city agglomeration: the people often visit neighbouring villages to receive social and economic services, or to deal with every day matters. Such socio-economic services as: education, trade, consumer services, mass cultural and sporting events are of the highest demand. The satisfaction of the social and territorial needs of the population of Novokuznetsk and the Kemerovo agglomeration is average based on their level of social well-being and quality of life. The ecology in the region is the biggest concern of the population of the Novokuznetsk agglomeration. The most important indicators of quality of life for them are: environment, transport infrastructure development and the development of recreational facilities. The material needs of the population of the Kemerovo and Novokuznetsk agglomeration, such as: food and clothing, and the need for a stable circle of close friends and relatives, are satisfied. However, such needs as: clean air (water), a good health care system, the recognition of professional and social achievements – have not been met at a satisfactory level. The residents rated their financial situation as average; and half of the population believe they can improve their well-being in the next year or two and they are confident about their future.

The residents of the Kemerovo agglomeration have a strong local identity. Satisfaction with the environmental conditions and with the transportation network contributes the most to the assessment of their quality of life, whereas the crime rate contributes the least to this assessment. The Kemerovo agglomeration has poorly formed social space boundaries. The quality of life in the center of the agglomeration – Kemerovo – is higher than in the other localities of the agglomeration. Development of the social relations of the community is at an average level which allows us to predict further development of the metropolitan area as a socio-territorial community. According to the survey, personal cars are the most popular means of transportation in the Novokuznetsk agglomeration; public buses take second place, cabs are in third place, and trains are in fourth place. The frequency of commutes varies from once a week to once a month. The most popular socio-economic services are: education, trade, and consumer services.

The analysis showed that for the development of the Kemerovo agglomeration's transportation network the most important projects are: the construction of a bypass road and the third bridge over the river Tom', the repair of the roads which connect Kemerovo, Topki, and Berezovsky; the creation of the industrial logistics parks in Topki, which will connect Topki with the federal highway 53 and with Kemerovo. In the construction sector the priority projects are: the modernization of the factory that produces construction materials, which will improve the supply of the Kemerovo agglomeration with building materials; the construction of the agricultural products processing complex.

For the development of the Novokuznetsk agglomeration's transportation network the most important projects are: the construction of a bridge across the river Kondoma, the construction of a shorter road to Mezhdurechensk, which will reduce the distance between the cities of Osinniki and Kaltan; the construction of a road linking the south of Kuzbass with Khakassia. This road will also link the Kuzbass conurbation with Central Asia and Altai. In the construction sector the priority projects are: the building of an advanced-processing complex for raw materials (coal, ore, polymetals), turning them into primary and secondary products; the creation of a cluster of the advanced processing of agricultural products.

To form a conurbation of Kuzbass, it is necessary to upgrade the road Novokuznetsk – Leninsk-Kuznetsky and to turn it into a highway.

The analysis revealed a multi-level conflict of interests between participants in urban agglomerations. First of all, there is a clash of interests within the agglomeration: different cities, competing with each other as producers and as receivers of government and private investments. Another conflict of interests is between the interests of cities and large owners of means of service production (the latter, in some cases, are not interested to locate the production and services' facilities wanted by the city; or they wish to develop industries in which the city is not interested; or they are unwilling to dismantle the existing ones that are undesired by the city). There is a competition between the cities for regional budget funds and for labour resources. In addition, there is a group of external

contradictions: between the cities (which may act as a defender of regional interests, and can have their own interests) and the regions; between the interests of the region and of each town individually.

The relationship of the two centers within the conurbation is an interesting and unexplored problem. The agglomeration processes require a new system of public administration, a multi-functional system of local government. Another peculiarity of the Kuzbass agglomeration is that it experiences the impact of both external and internal competitive forces. First, it has a central position between the agglomerations of Novosibirsk and Tomsk. Thus, for the border settlements of the Kemerovo region, the centers of the neighboring areas' agglomerations have become the center of gravity (based on transportation accessibility criteria – the maximum commute time to the center of any metropolitan area is 1.5 hours).

Kemerovo is the administrative center of the region, and the core of the Kemerovo agglomeration. This creates the conditions for competition between Kemerovo and Novokuznetsk. We consider parties to be the competitors if they perceive each other as competitors. This means that each of them have the assurance that the other party will remain their competitor in the perspective period.

Not only material resources, but also labour resources are the objects of competition between two metropolitan areas in the Kuzbass agglomeration. The competition between the municipalities takes place around the federal and regional funding, around foreign investments; around large and medium-size businesses, around various investment projects; around the quality of labour resources (they try to achieve this by better health care, education and social benefits). The competitors use official and unofficial methods (lobbying, petitions, reputation, etc.).

Such apparent and veiled competition between the two largest cities in the Kemerovo region provides a basis to put forward a number of fundamental theoretical problems, the solution of which can contribute to the effective functioning of the conurbation. The theoretical problems are as follows: is the competition of the actors in the conurbation continuous? is this competition a driving force for economic development? what is the ratio of the competition and cooperation forces, i.e., what tendency prevails at present and in the future? is it possible to measure the level of the competition? In contrast to the competition, the agglomeration processes of cities are focused on cooperation, integration, co-ordination and co-evolution (a predetermined or planned and coordinated development).

The algorithm of the formation of the social and economic policy of the Kuzbass agglomeration inclus:

– the identification of the urban agglomeration population's needs in goods and services;

– the determination of the list of goods and services for the population, which should be provided at the level of urban agglomerations, including inter-municipal cooperation;

– the determination of the goods and services produced by the enterprises within the urban agglomerations;

– the determination of the qualifications of workers in the agglomeration's enterprises;

– the identification of the structure and composition of the job market in the metropolitan area;

– the determination of the technological capabilities, moral and physical depreciation of the fixed assets of the enterprises in the urban agglomerations.

The analysis of the formation of the Kuzbass agglomeration helped to identify the prospects for the formation of the following required clusters: mechanical engineering; the advanced processing of agricultural products; the advanced processing of extracted raw materials (coal, ore, polymetals), and waste disposal. It is possible to build an environmental (ecological, environmental and economic) cluster, which may be the key to resolving the contradiction between the economic development of the territory and its environmental well-being. This is important for the Kemerovo region, in particular for the Novokuznetsk (Southern Kuzbass) agglomeration.

For the formation of the ecological cluster within the boundaries of the agglomerations we propose the following algorithm of decision-making: 1) the assessment of the conditions for the development of the ecological and economic clusters in the metropolitan area; 2) the identification of the least developed elements in the agglomeration's infrastructure for their further development; 3) an analysis of the causes of the underdevelopment of the respective elements of the agglomeration's infrastructure; 4) recommendations and specific measures for the design of an environment of ecological and economic cluster in the metropolitan area boundaries; 5) the assessments of the environmental, economic, social effects on the ecological and economic development of the clusters. The desired effect should be the improvement of the quality of the environment, and the reduction of the negative anthropogenic load, which is the result of greening, without reducing the scale of economic activity. The ecological and economic cluster should generate greater positive effects than the costs associated with its development [17].

The development of the local agriculture and food trade affects the food security, regional markets, and hence the urban agglomerations. Agriculture requires large areas of land. One of the defining characteristics of the urban agglomerations is that a built-up area (urban) in the metropolitan area must exceed the area of agricultural land. In addition, one of the industrial characteristics is a inverse correlation of the population density and proximity to the agricultural markets [18]. Then the problem of food supply comes up. If the proportion of the rural population in urban areas decreases, how should the problem of food supply be addressed? A priori, through the use of exogenous sources, thereby creating a dependence on other regions and on food imports.

The international practice shows that there is a shift in the paradigm of development towards ensuring food security. Some countries have developed urban food strategies to support a healthy and prosperous community through the reengineering of regional food and agricultural systems. For example, in Canada,

urban food strategies are developed with the participation of multidisciplinary teams (local authorities, non-profit organizations and universities) to provide a wide range of food products and rural planning. Such teams include agrologistics specialists, farmers, retailers, and even website designers [19].

Food security means a stable supply of basic foodstuffs from own national resources, regardless of force-majeur circumstances. Currently, food imports in the RF is about 40%. The share of food in total volume of imports is 12.8%. The total volume of food imports to our country is $7.5 billion. Food imports into the United States, Canada and Australia combined are $1.5 billion [20]. Agriculture as part of the Kuzbass agglomeration can be evaluated in a controversial way. Its development is affected by negative economic and institutional factors: market price fluctuations, the international sanctions, and some technological backwardness of the agricultural industries. The food supply in the region is characterized by negative features:

– a high degree of depreciation of fixed assets;
– a lack of government support for the AIC (agri-cultural-industrial complexes);
– processing and procurement companies that ignore the interests of agricultural producers;
– a large number of resellers and a consequent rise in food prices;
– a lack of investments in innovation;
– an under-development of the rural infrastructure.

In the process of agglomeration, the rural population and the number of agricultural workers decline. This is not necessarily a negative trend, as a reduction in the number of employees may be an evidence of the use of new high-performance technologies. The Kuzbass agglomeration, with its high level of urbanization, on first glance has less opportunity for food self-sufficiency compared to neighboring areas (Table 4). However, the geographical location of the Kemerovo region – its proximity to the areas with developed agriculture – gives it an opportunity to receive food products at the lowest transportation costs.

In the Kemerovo region depends on imports of the following products: canned fruit and vegetables – 90%; confectionery – 60%; cheeses – 89%; cereals – 50%; juices – 70%; mayonnaise, sauces – 100%; Beer – 90%; vodka – 30% (Table 5).

In the Kuzbass agglomeration, 14 municipal districts have the necessary resources for the growing and processing of agricultural products. The agricultural production in the Kuzbass territorial agglomeration is unevenly distributed. A large share of the profitable and efficient agricultural enterprises is located in the Yaschkinsk, Topkinsky, Prokopyevsk, Izhmorsk, Promyschlennovsk and Leninsk-Kuznetsky areas. Thus, successful agribusiness is concentrated mainly on the territory of the Kemerovo agglomeration. The least successful agricultural enterprises are in the Chebulinsk, Tyazhinsky and Tisulsky regions. It is necessary to take into account the self-supply (the amount of goods manufactured in the village for itself and the production in private farms). In the Kemerovo region in 2013, private farms produced 51% of all agricultural products including: potatoes 83.55%, vegetables 79.35%, and milk 55.8%. The level of food self-sufficiency of the region is calculated as the value of its own production per person and indicates the level of its possible ability to meet the food needs of the population. Based on this index we can analyze the external economic ties in the region, and can give a picture of the specialization of agricultural production (Table 6).

The level of self-sufficiency has shown positive results in dynamics (Table 7). However, food consumption in the region does not meet nutritional standards. Moreover, a large range of food is not produced in the region.

Table 4. The agricultural production in the agglomerations in the SFD in 2012 at current prices, mln. roubles [21]

Agglomeration	Crop production	Livestock production
Novosibirsk	21398.9	34635.6
Kuzbass	16792.8	20594.7
Tomsk	6041.8	13534.4
Altai	45333.3	48964.1

Table 5. The level of food imports in the agglomerations of SFD (% of prev. period) 2009 [16]

Agglomeration	Share of a/c in the structure of the GRP %	Food self-sufficiency	Imports of food products and agricultural raw materials for their production. In % of prev. period	Imports from the CIS countries. Food and agricultural raw materials for their production, in % to previous. period	Turnover of retail trade Foodstuffs (% of retail trade turnover), %
Novosibirsk	13	1.3	93.25%	55.45%	90.42%
Kuzbass	9	0.7	120.63%	55.25%	109.69%
Tomsk	5	0.5	81.93%	101.22%	101.12%
Altai	24	2.4	81.04%	86.25%	118.45%

Table 6. The coefficient of food self-sufficiency in the agglomerations of SFD [21]

Agglomeration	Grain products	Meat and meat products	Milk	The average value *Kc*
Novosibirsk	3.0	0.4	0.4	1.3
Kemerovo	1.5	0.2	0.2	0.7
Tomsk	1.0	0.3	0.2	0.5
Barnaul	6.1	0.5	0.6	2.4

Table 7. The dynamics of self-sufficiency of the population of the Kemerovo region in agricultural products, % [22]

Types of products	1990 yr.	2010 yr.	2014 yr.	Not produced
Grain	59.7	120.3	> 100	Sugar, salt, margarine, canned meat, canned fish, cheese dependence on imports of fishery products for processing 70%
Potatoes	104.7	110.7	> 100	
Vegetables	78.3	98.0	> 100	
Meat (cattle and poultry for slaughter)	69.4	45.2	About 85%	
Milk	72.6	57.5	About 90%	
Eggs	99.7	90.3	About 95%	

One of the priorities of the Kuzbass conurbation's functioning is the creation of high-capacity complexes for processing agricultural products. To ensure food security, a cluster of advanced processing of agricultural products will be created. In March of 2016, Novokuznetsk began a construction of the agrocomplex "Ariant-Siberia". The agrocomplex will include the largest cattle-breeding facility for 270 thousand heads and will be producing 45 thousand tons of meat per year. The agricultural complex will include: a feed milling plant with the capacity of 40 tons per hour, an elevator for grain storage of 80 thousand tons, a meat processing plant, which will produce 300 kinds of products from fresh meat to sausages and deli meats, totaling 100 tons a day, as well as the logistics center, which will deliver the products in the "Ariant" retail chain. The possibility of construction of the road to bypass the southern capital of Kuzbass is being looked into. The road will connect the Novokuznetsk and Prokopyevsk districts. By the end of 2017 an electric substation with a capacity of 16 megawatts will start operating. It will supply electric power to the agrocomplex and villages of the Prokopyevsky district. The agrocomplex will receive water supply from local artesian wells [23].

To ensure food supply to the Kuzbass agglomeration, it is necessary to align the internal transportation accessibility of the region with the external inter-regional transportation links. Based on the degree of accessibility, there are four types of areas in the Kuzbass agglomeration. The economically well-developed territories, which are the Yurga, Kemerovo, Topki, Promyshlenovsky, L-Kuznetsky, and Prokopyevsky regions, comprise a group that are mostly oriented to external interactions. And thus, the Kemerovo agglomeration has a good transportation capacity. We can't say the same about the transportation capacity of the Novokuznetsk agglomeration. Though the city of Novokuznetsk is an area open for external economic interactions, the greater Novokuznetsk area has the semi-enclosed type of economic interactions. Most of the open areas shape an elongated zone in the north of the region, stretching along the Trans-Siberian railway line and along the federal road. The semi-closed areas make up a large part of the territory of the region. The areas that are closed to external connections are located on the south of the region. The taiga takes more than half of these areas, and a significant part of the roads are rural roads which are not accessible. Overall, the areas that are actively open for external connections comprise 22.4% of the territory of the Kemerovo region, moderately open –16.5%, semi-open – 38.9%, and closed – 19.4% [24]. This shows that most of the territory consists of semi-closed areas, and the farther from the center of the Kemerovo agglomeration the areas are, the less is their level of openness to external interactions. The road network density in the Kemerovo region is lower compared to other Kuzbass regions that have the same population density, but much less economic capacities. Insufficient density of the road network and its uneven distribution in the region reduce the level of the transportation accessibility and consequently, food supply opportunities.

The inter-regional transport accessibility is crucial for the development of the food market of the Kemerovo region. The Novosibirsk region, the Tomsk region and the Altai region bordering the Kemerovo region differ in transport accessibility. Some districts of the Tomsk region and the Altai Republic are isolated. Districts of the Novosibirsk region, the Altai Territory and the Republic of Khakassia consist mainly of closed areas, except, the semi-closed ones situated on the border with the Kemerovo region. The development of the road network in the Kemerovo region is moving south, and the connection with the Novosibirsk region is limited to one railway and one road.

This analysis allows us to infer the following. The formation of the urban agglomerations is an objective process that occurs as a result of spatial competition for resources. The formation of the urban agglomerations

is a contradictory process that incurs, on one side, the concentration of economic activities and the growth of urban population in space; on the other hand, the decrease of the rural population, and hence the decrease of agricultural production. As a result, a problem of food dependence comes up. The regional authorities have to create conditions to solve the problem of food supply in terms of internal and external competition between the agglomerations. For successful functioning of the Kuzbass agglomeration, the problem of food security is extremely important given the following circumstances: the decrease of the rural population of the region; the increase of the competition for resources between neighbouring conurbations; the increase of the international economic sanctions. The problem of food supply can be resolved both by developing local resources and by taking advantage of the competition with the neighbourhing agglomerations.

ACKNOWDGMENTS

The research has been carried out as part of the Grant RHSF – Kuzbass №15-12-42003-a (P) "The development of a management system of social and economic development of the urban agglomerations in the Kemerovo region".

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Please cite this article in press as: Zobova L.L. and Shabashev V.A. The problem of providing food resources in urban agglomerations (the case study of the Kuzbass agglomeration). *Foods and Raw Materials*, 2016, vol. 4, no. 1, pp. 186–196. doi: 10.21179/2308-4057-2016-1-186-196.

INFORMATION FOR AUTHORS

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The manuscript further goes to the scientific consultant who evaluates the validity of data, authenticity, grounds of factual evidence, completeness and representativeness, composition, logic, stylistic quality, and the realization of the goal of the research.

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