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REVIEW

REVIEW OF SCIENTIFIC RESEARCH RESULTS IN IDENTIFICATION OF PLANT RAW MATERIALS IN FOOD PRODUCTS

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Abstract: Currently, the science-based capabilities have been generated to develop and test various identification methods of food products and reveal adulteration using advanced technique and processes. This article reviews researches and developments to identify the plant raw materials in food products based on morphological, anatomic, physical and chemical test methods and the latest DNA-technologies. Review of physical, chemical, anatomic and morphological test methods to identify raw materials both as discrete and as the food content validated that these methods are useful to differ the herbal material with apparent specific peculiarities in structure and chemical content, though, in most cases, they are not adequate enough to differentiate the used raw material by species and genus. In the sphere where DNA-technologies are applied to identify the plant raw material, various methods for DNA extraction, requirements to DNA-targets, methods to optimize the polymerase chain reaction (PCR) stages have been developed; a range of developed methods are in place for species identification of plant-based additives in food products by species which is rather relevant in view of promotion in the market of cheaper substitutions for food ingredient components. The review of national and international scientific publications and intellectual property items to work with PCR-based species identification of the fruit raw material showed that this method differs in high specificity and is practically the only method of species identification available.

Keywords: food products, plant raw material, identification, DNA-technologies, PCR analysis

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INTRODUCTION

Currently, the food industry concentrates, to a greater extent, on the production of mixed food products enriched with herbal supplements that contain bioactive substances as elements wholesome for the human health. Food processing plants may often replace the content of fruit and berry supplements in various food products by the dye staff and flavoring that are cheaper for reasons of cost efficiency. Problems with identification of falsified plant raw material in food require the relevant identification methods to solve them. These methods are of high priority in the list of measures to be taken for safety and quality of food products to be sold.

The Federal Law on Technical Regulation specifies the development of new requirements to each type of food product, including vegetable derivative that should be developed as technical regulations with the main requirement to ensure chemical and biological safety to protect human health and life and to prevent actions that confuse consumers.

In connection with the above, the important task is to develop objective species identification methods of plant ingredients that make up finished products.

The purpose of this article was to review scientific developments in the sphere of herbal material identification in food products using morphological, anatomic, physical and chemical test methods and the latest DNA technologies.

OBJECTS AND METHODS OF STUDY

The work is performed at the Kemerovo Institute of Food Science and Technology (University). The study object included: scientific and methodic publications, articles in scientific periodicals, conference materials, intellectual property items, regulatory documents, Internet resources. To analyze the theoretical data, the methods used included registration, filing, grouping, classification, comparative analysis and consolidation of scientific materials.

REVIEW

The review and analysis of scientific literature indicated that researches and developments are available to identify the plant raw materials in food products, pharmaceuticals, and methods are offered of herbal material identification based on morphological,

anatomic, physical and chemical test methods and using the latest DNA technologies.

The works performed by Pchelkina V.A. [1, 2], Pchelkina V.A. and Burlakova S.S. [3] on the development of micro-structural methods of plant component identification in the meat raw stock and finished products studied morphological peculiarities of plant elements of proteic and carbohydrate origin used in the production of meat products, their modification when processed. Microstructural parameters (shape, particle size, tinctorial characteristics) and their evaluation criteria are defined for identification purposes. It was found out that the methods developed make it possible to quickly and objectively evaluate the structure of both raw meat and end products. As compared with physical, chemical and PCR methods applied, the developed methods will help to cost-effectively determine the herbal components and analyze the raw meat and finished products.

Popov A.I. et al [4] developed and proposed the morphologic and anatomic analysis of subterranean organs of spice plant rhizomes, namely ginger and turmeric that may be used to characterize the herbal material in detail in the solid, fragmented and powdered form and used to make supplementary documentation to standard documentation articles on raw material identification.

The identification method of raw stock and products of animal and plant origin based on BOB-electrophoresis is developed. By using the SDS ionic detergent electrophoresis (8B8), the method developed to analyze meat products enables species identification of the protein, quantification of the multicomponent minced meat components, including protein supplements of plant origin. Characteristic species-specific marker protein areas are determined for species identification of the protein as the component of food product. A collection of electrophoretograms is compiled for proteins of animal and plant origin [5, 6].

The technical approach is known to perform the screening gas chromatography and mass spectrometric studies of the qualitative composition of the medicinal herbal substances in BAA (Biologically Active Additives). The qualitative composition of 50 pharmacopoeial species of herbal substances is studied by the method of gas chromatography and mass spectrometry. Biochemical markers are proposed for 15 species of herbal substances that make it possible to identify them as part of multi-component herbal collections and BAA. The method of qualitative chromatographic amd mass spectrometric analysis is developed to determine the marker substance 4-vinylquaicola in garden sage leaves [7, 8, 9].

Certain works relate to the study of local herbal substance use as the source of bioactive substances for syrup and beverage production. The plant raw materials were screened as the source of bioactive substances of antioxidant nature to create basic syrup recipes; the proportion of ingredients in syrups in view of taste thresholds of sensitivity and redox dynamics (EhRed/ox) of extracts is justified; extraction parameters of vegetable raw materials by mathematical modeling are optimized; syrups recipes and techniques are worked out using Laminal and Modifilan; the trade analysis is performed for extracts and syrups made of vegetable raw materials, nutrition value, bioavailability and antioxidant activity during production and storage are indicated, methods of raw material analysis are selected [10, 11, 12].

Eller K.I. and Balusova A.S. [13] analyzed flavanoid components in herbal estracts and herbal products by the combination of HPLC and solid phase purification; authenticity of herbal extracts is established as the raw stock for BAA [14]. Indicative polyphenol compounds of the propolis are identified and measured [15].

Research and methodology concepts are developed to evaluate the quality of plant raw material and its derived products that allow revealing most evaluate (significant) criteria to detect the wheat grain falsification. Comprehensive researches are performed and the criteria selection is scientifically validated to determine the type composition of hard and soft wheat to identify, by quality and quantity, the soft wheat impurities in hard wheat sorts [16].

The major task of food product identification is the elemental analysis study against trace amounts in the plant raw materials and plant-based products. I.V. Podkolozin [17] worked out the method to define rare earth elements and uranium in the natural mineral water, tea and coffee by ICPMS - Inductively coupled plasma mass spectrometry – combined with the dispersive liquid-liquid micro-extraction; its analytical and metrlogic parameters were assessed, either. A database is created to identify food products by the content of certain elements, including rare earths elements and uranium. The study results are used by the chemical analysis laboratory of the Federal Animal Health Care Center to identify the food products.

The All-Russian Scientific Research Institute of Food Biotechnology of the Russian Academy of Agricultural Sciences developed absorption and luminescence methods for identification and evaluation of safety and quality of the rectified ethyl alcohol by the spectral analysis of organic impurity trace amounts contained in the tested item. Savelyeva V.B. [18] obtained experimental data to indicate that the rectified ethyl alcohol from various raw materials are characterized by availability of the group of luminescent and non-luminescent traces available in each of them that result in the excitation spectrum – emission – absorption individual for the given sample.

In recent years, new spectroscopy methods are used to study the quality of food and medicinal herbal stock. The Fourier transform infrared spectroscopy (FTIR) of the attenuation total reflection (ATR) is getting more popular in the study of the raw stock quality and authenticity. When using this method, the infrared light penetrates the sample to about one micrometer in depth, and the detector defines the absorption spectrum. This method has some advantages to the technique of transmission measurement. Any samples of any form and state of aggregation – solid and liquid, powders, pastes, granules, suspensions, fibers, etc. – may be studied. The entire analysis takes a minute only

including the sample arrangement, data collection and processing [19, 20, 21].

Values of characteristic IR spectrum frequencies are identified corresponding to the sample chemical composition and the authenticity of the food or herbal substances is defined as per tabulated spectral data for reference raw samples. In practice, while interpreting the spectrum, the position of absorption bands and their intensity (high, medium and weak) are determined. The IR spectrum is compared starting with the analysis of characteristic bands that are usually well seen in the spectrum, whereas the low frequency region is compared when they match each other. The match of the spectral curve of the test substance with the standard spectrum pattern indicates the identity of two substances (raw material types). The absence of bands in the spectrum of the test substance observed in the spectrum of the reference sample clearly indicates that these substances differ. The presence of the greater number of bands in the test sample spectrum as compared with the reference spectrum can be explained as the test sample contamination and dissimilarity of both substances. Thus, the test sample IR spectrum should have absorption bands fully matched with those of the reference spectrum in position and relative intensity.

E.N. Grin'ko [22] was the first to obain and descrie IR and Raman spectra for 11 kinds of herbal substances and 8 reference samples.

The infrared spectroscopy method is widely used to assess authentication and quality of herbal substances, in particular to assess the industry-related substance contamination [23–27] in studies to identify the components of herbal substances and food products of plant origin [28, 29].

The Kazan State University validated the use of electrochemical methods to evaluate the integral antioxidant capacity of medicinal herbs and food products [30]. A new approach is elaborated to evaluate the integral antioxidant capacity using electrogenerated standard solutions (bromine compounds). Stoichiometric respond factors are specified for 12 separate bioactive compounds with antioxidant properties with electrogenerated halogens and some oxidants – metal ions. A study was performed of the antioxidant activity of 46 food extracts and 42 herbal agents based on medicinal herbal substances.

Sechenov I.M. First Moscow State Medical University elaborated unique methods to identify and measure hydroxicinnamic acids in medicinal herbal substances and alimentary food stock, BAAs and food products. Most efficient chromatographic conditions are arranged to determine chlorogenic, neochlorogenic, cryptochlorogenic, caftaric, caffeic, p-coumaric, ferulic, cichoric, dicaffeoylquinic, dicaffeic, feruloylquinic acids. The assay methods of hydroxycinnamic acids in medicinal herbal substances and alimentary plant stock, food products is elaborated. The raw stock that can be the source of hydroxycinnamic acids and where hydroxycinnamic acids may act as indicator components. The raw stock is identified containing a small amount of hydroxycinnamic acids and the raw stock is extracted that does not contain hydroxycinnamic acids [31–36].

The patent search was performed in the field of physical and chemical methods developed to detect adulteration and identification of plant-based products.

The patent 2208785 [37] of the Russian Federation is in place on “Cognac identification Method” that involves the creation of sensor matrix to detect major components of the test product flavor by modification with sorbents of resonator electrodes, introduction of reference and test samples in the cell detection, registration of analytical persorption signals of the flavor main components. Dinonyl phthalate, polystyrene, polyethylene adipate, Triton X-100, bee glue, crown ether, beeswax, polyethylene glycol of PEG-2000 grade are used as sorbents. Electrodes are modified with sorbents of 10–20 mg in mass, and samples are put in detection cells at 0.01 dm^3. Analytical signals are recorded sequentially in line with individual kinetic interaction parameters of main components of cognac flavor. The spider diagram is plotted as per signals and identified by visual comparison.

Patent RF no. 2006120721 [38] offers the method for express determination of integral falsification of dry and liquid food products as per the total score of their capillary adhesion properties that ensure active extraction of discrete elements of the restored aqueous composition exposed to the complex mechanical and gravimetric power and at total application of capillary adhesion forces, pressure fixation that retains the capillary volume of the discrete extracted element of the tested aquatic food composition, followed by gravimetric determination of dynamic characteristic parameters of discrete elements.

The object identification method is available to create its characteristic electrophoretic profile [39]. The object identification is based on comparison of characteristic profiles that correspond to the certain object with "limits". The invention ensures rapidity, simplicity and informativeness, as well as improvement of efficiency and selectivity in separation of characteristic components of the object and decrease in the detection limit that allows obtaining the proper electrophoretic profiles.

Patent RF no. 2377556 [40] describes a method to determine distinct features in the chemical composition of monogenic sunflower lines. The invention relates to the sphere of the method development on identification of the natural material composition by liquid separation resulting from sample preparation by the gas chromatography. The method includes treatment of sunflower petal extract with the adsorbent followed by determination of characteristic chemical compounds by mass spectrometry contained in extracts. Extracts were prepared by extracting the plant material with the composition of the chloroform and methanol mixture. The chloroform and methanol mixture was taken in the ratio of 7/3 by volume. The technical result is in simplicity of sample preparation and high performance. Also, this technique can be used as the method to define the variety of sunflower during seasonal researches.

The patent [41] is developed at the "Lianozovo Dairy Plant" OJSC to determine the content of tartaric acid and its salts to detect falsification of juices and lactic stock and diary products. The development ensures to detect and measure the concentration in the solution of any substances containing tartaric acid anion, i.e. as the tartaric acid itself and any of its salts (tartrates). The method may be also used to detect falsification of juice and juice drinks with tartaric acid additives or its salts. Furthermore, the development can be used in the chemical industry to analyze the industrial waste and commercial samples of tartaric acid and/or its salts – tartrates.

The patent [42] is also available that describes the method to determine the citric acid and its salts in food products to control the product quality and falsification. This method involves the sampling procedure and its oxidative bromination. Thereafter, the sample is added with an excess of reducing agent. The value of at least one optical sample characteristic is measured which is used to validate availability of citric acid and its salts. The content of citric acid and its salts is determined by multiplying the measured value of the sample optical characteristic to the calibration factor of the device. The development increases the method versatility and its accuracy.

Nizharadze Eteri Shotaevna [43] developed a method to control the tangerine juice naturality, both natural and sugared. The mass concentration values are defined in the test sample of total and amino nitrogen (official number), proline, ash mass fraction, its alkalinity, and chloramine number. If at least one of the indicators above is not within the limits of variation, the act of naturality violation is reported.

Kharkiv State University of Food Technology and Trade developed the "Method to determine the beverage concentration" [44]. The method aims to improve the concentration measurement accuracy of drinks, preferably fruit and berry juices, including in case of falsification with dilution. The method includes the selection of the test and control juice samples, measurement of the sample optical density in the visible spectrum, and concentration measurement as per the control sample calibration curve, wherein prior to measure the optical density, aqueous extracts are prepared of test and control samples and the solution of diazotized para-aminobenzoic acid is added.

The State Scientific Institution "Research Institute of Children's Food of the Russian Academy of Agricultural Sciences" developed the method of express identification of integral falsification of dry, plastic or viscoelastic food products based on their total values of capillary and adhesion properties. The process involves an active extraction of discrete elements of the restored aqueous composition exposed to complex mechanic and gravimetric force and total application of capillary and adhesive forces, pressure recording that retains the capillary volume of discrete extracted element of the test aquatic food composition, followed by gravimetric determination of dynamic characteristic parameters of discrete elements [45].

A method is described for quicker detection of peanut or shrimp allergens. For this purpose, CHI400C-type sensor of electrochemical workstation and three-electrode system is designed with the platinum electrode as the reserve electrode, the electrode Ag/AgCl is used as the reference electrode, and metallic electrode is modified to the modified electrode enveloping the antibody and is used as the service electrode. When the sample antigen or antibody in combination with the antigen or antibody is transferred to the working electrode of the plate, the plate oscillating frequency respectively decreases due to the ncreased load. Allergen detector addresses shortcomings of the existing detection methods such as ELISA and PCR which run with complex operations, usually require the standard enzyme reagent and are reported to have long time operations [46].

The patent [47] offers the method to detect the falsification of concentrated fruit juice for the sugar syrup added. The method is based on the use of fruit juice concentrates and sugar syrup mass spectrometry. The components are compared y chromatograms and validate on presence of the sugar syrup in the juice.

The analysis of physical, chemical, anatomical and morphological methods of analysis used to identify the herbal substances, both individually and in complex food systems indicates that these methods distinguish between herbal substances with distinct specific features of the structure and chemical composition, but in most cases they cannot identify specificity and variety of the raw stock used.

Continuous improvement of molecular biology methods and data accumulation on the genom of fruit and berry plants resulted in elaboration of techniques to identify plant raw materials in food products based on DNA technologies using the polymerase chain reaction (PCR). The PCR method allows obtaining the required number of DNA from single cells contained in the test sample for their identification [48]. Specificity of herbal substances detected by PCR distinguishes with versatility, the deeper level of species differentiation, high reproducibility and possibility of quantitative analysis. Furthermore, the DNA is more stable in process conditions as compared with conventionally used low molecular markers [49]. Despite the fact that PCR used for species identification of tissues of plant origin is highly appreciated by foreign experts, this trend has not yet found wide practical application in our country.

Quite many works are specialized in the study of DNA extraction methods from vegetable objects. Researchers [50] have developed the DNA extraction method without the plant material homogenization and centrifugation. The main way to get rid of the cell wall was the correctly selected enzymatic mixture of different carbohydrases isolated from *Trichoderma longibrachiatum*, that hydrolyze the cell walls. Incubation time optimization for each of tested species of plants contributed to DNA exit without its fragmentation.

Methods are used to extract DNA with the solution of fine silicon oxide "silica" when extracted from the agarose gels [51, 52], microorganisms [53, 54], soil [55, 56], some eukaryotes [57, 58, 59], from green foliage [60]. These techniques showed that DNA

isolated with the use of silicon oxide solution may be used for PCR reactions [56, 61] and other DNA manipulations [59].

There are techniques that allow DNA extraction, as per researchers, suitable for restriction and amplification purposes, from bacteria, fungi and plants without the use of potentially harmful solvents, phenol and chloroform [62, 63].

The common problem in higher plants when the DNA is extracted is contaminants, the fruits and berries are reported with the higher content of polysaccharides [64, 65, 66] and polyphenols [67–70]. Further on, it affects the use of DNA in studies by inhibiting the enzyme activity of the reaction [71]. Also, the DNA of extracted samples becomes unstable for long term storage 72, 73]. Different inhibitors in the solution induce inhibition of DNA polymerase activity [74].

The publications describe results of DNA extraction and polysaccharides removal based on various sources [66, 73–85].

There are two main classical fundamental approaches to purify the target DNA: purification by organic extraction [86] followed by DNA precipitation with alcohols and dissolving it in water and TE buffer and the differential adsorption of DNA on the solid support. Currently, the methods of DNA and RNA extraction are widely used based on nucleic acids binding with sorbing carriers. DNA extraction kits are widely commercialized. Silicate [87–89] and rarely nitrocellulose [90, 91] media are often used as sorbing agents. Gel chromatography is practically out of use to isolate nucleic acids whereas the method is popular to isolate amino acids [92].

However, it should be noted that, although methods on DNA extraction from plant resources are developed, there is no unified optimal procedure for DNA extraction, and in every particular case, depending on the type of raw material and its chemical composition, it is necessary to optimize the DNA isolation procedure [93, 94].

The works are performed at the Kemerovo Institute of Food Science and Technology (University) to select commercial kits that allow obtaining high quality DNA from fruits and berries. A comparative analysis of DNA extraction methods of food stock plants is performed. Based on these data the most efficient way to DNA extraction is suggested [95]. Golubtsova A.Yu. and Shevyakova K.A. [96] studied the method of DNA extraction from gooseberry and gooseberry-based products. It is established that DNA of higher concentration and purity was obtained when using the reagent kit “Sorb-GMO-A”. Ostroumov L.A. et al [97] conducted a comparative analysis of DNA extraction methods from samples of fruits and fruit products. It is figured out that the commercial kit “PROBA-TsTAB” and “Reagents kit for DNA extraction from plant resources and food products” (developed by “NPO DNK-tekhnologia” LLC, Moscow) are the most effective. Moskvitina N.A. et al [98] showed that the commercial reagents kits “PROBA-TsTAB” and “Sorb-GMO-A” are most suitable for DNA extraction from fruit and berry processed products.

The genome nucleotide sequences is analyzed of fruit raw material used for food production [99, 100] and their phylogenies [101–103]; parameters of the polymerase chain reaction are optimized; the possibility to use oligonucleotide primers for species identification of peach and apricot in heat-treated products is shown [104]; PCR-test system is developed to identify fruit and berries in the jam (Fragaria moschata, Pyrus, Malus, Ribes nigrum, Vaccinium myrtillus, Prunus armeniana, Prunus persica) [105].

The scholars of the Russian Academy of Agricultural Sciences elaborated the modified high-performance methods of plant resources identification. A method of DNA extraction is proposed using the ionic CTAB detergent (cetyltrimethylammonium bromide), and using the Silica sorbent (Si02). By using the elaborated methods, the DNA may be obtained free of inhibitory impurities and in the amounts required from a variety of products containing components of animal and plant origin. DNA is suitable for qualitative and quantitative analysis. The techniques may be used for DNA extraction and purification of both multicomponent and single component mixtures. Monitoring researches are performed of the raw stock and food products using the techniques elaborated that showed the capacity to be used for screening and quantitative tests to detect non-declared genetically modified ingredients (GMI) in products of animal and plant origin [106–109].

Panyushkin A.I. [110] improved the method of GMO detection, based on the multiplex PCR followed by DNA-hybridization, in heat-treated and non-heat-treated products and forage. The sensitivity and specificity of the modified technique are identified.

Roshchupkina L.V. [111] proposed advanced methods to identify the product components of plant and animal origin based on the protein analysis by immunodiffusion and based on DNA by nucleic acid hybridization with specific DNA probes, and also by using the modified amplification-based procedure that helps to detect components of animal and plant origin, including components from genetically modified sources. High sensitivity and specificity of methods based on PCR are proved which help to analyze mixed minced meat, semi-finished products and heat-treated meat-vegetable sausages.

Fomina T.A. [112] elaborated the method of species identification of meat and vegetable ingredients based on real time PCR.

Intellectual property items are available in our country and abroad to determine specificity of plant resources both discretely and in DNA technology-based food products.

The patent authors [113] elaborated primers and the technique to detect specific sequences. This development can be used to select primers that may detect the specific genome sequence of kiwi, walnut, apple, and banana. The PCR method is the main method to detect and identify the specific sequence and primers comprising DNA contain 30 nucleotide sequences of kiwi, walnut, apple, banana, or soybean on 3'-terminal processing.

To detect falsification in citrus processed products, the patent [114] proposes to use trnL intron sequences to work out primers – trnL3 and trnF3. The length of DNA trnL3 and trnF3 fragments was about 424 base pair (b.p.). The method is effective to identify the orange and tangerine juices.

The patent [115] describes the fruit origin in the fruit juice and includes the following: nucleic acids amplification available in the tissue or juice by the PCR method, comparison of DNA obtained with DNA isolated from tissue of fruits of known genus, species or variety. These method identifes oranges, apples, lemons, limes, grapefruit, banana, kiwi, passion fruit, papaya, peach, pineapple and plum in juice products.

The patent [116] describes the determination method of forage herbs using ISSR-technologies of molecular markers. UBC815 and UBC835, and nucleotide sequences, respectively, UBC815-CTCTCTCTCTCTCTCTG and UBC835-AGAGAGAGAGAGAGYC, are proposed as primers. The method helps to avoid unreliable traditional morphological identification factors and can quickly and accurately distinguish seven pennisetum forage grasses, and the identification result can be used as the reliable basis to validate the category, class, strain.

The patent [117] describes the method of DNA identification in processed food products and fodders. The invention refers to DNA identification by PCR in food products. The given method is used to detect and determine proteins of plant or animal origin or fish in alimentary formula.

The patent [118] describes the method of identification of the strawberry species by the DNA test.

The patent authors [119] elaborated primers to detect fruit trees affected by ASGV (apple stem grooving capillovirus), ACLSV (apple chlorotic leafspot trichovirus), CTV (citrus tristeza virus) and CTLV (citrus tatter leaf virus).

The patent [120] describes the method to detect specific DNA or RNA fragments by the real time polymerase chain reaction. To obtain PCR or RT-PCR results, the special dye fluorescence amplifier was used. The method may be used to detect food products from genetically modified organisms, to determine the raw stock quality.

The invention [121] is used for quality control and preservation of fruits and vegetables. In the context of the melon, the region of DNA gene is identified via the genome library that is responsible for good long-term viability. This DNA region is amplified by PCR and used as a template.

The patent authors [122] elaborated the DNA molecular markers to identify falsifications in oil and fat.

The patent [123] describes the identification method of the plant resource falsification using by polymerase chain reaction (PCR). The authors proved the method reliability to detect falsified commercial products containing plant substances. This method can identify the falsification of paprika in 1% of tomato powder. This may be applied to identify falsifications with tomatoes and paprika.

The patent [124] descries the method to identify the plant specificity. It identifies the kiwi, walnut, apple, yam, banana or soya in the food product based on DNA test.

The patent authors [125] developed the method to identify the particular plant species by studying the genetic sequences of chloroplasts. The method allows identification of kiwi, walnut, apple, yam, banana or soy in alimentary systems.

The method to test soft wheat varieties for presence of hard wheat impurities and ready-made pasta by studying the DNA sequence by PCR is described in the patent [126].

The patent [127] describes the method to amplify specific nucleic acid fragments by the regular chain reaction. Developed to detect GMOs used for food production.

CONCLUSION

The scientific literature and intellectual property items in our country and abroad were reviewed and analyzed and it was figured out that there are researches and developments to identify the plant raw materials in food products, medicinal products based on morphological, anatomical, physical and chemical test methods and the latest DNA test technology.

The existing identification criteria should be expanded as new technologies for raw stock processing, innovative food production technologies are introduced, and the range of multi-component food products is expanded for accurate identification and falsification detection.

The review of scientific papers and patents in the sphere of application of physical and chemical, anatomical and morphological test methods to identify plant resources showed that these methods distinguish between plant resources with apparent specific peculiarities in structure and chemical composition, but in most cases, they do not identify specific and generic differences of the feedstock as part of the food product.

Recently the principle of specific DNA amplification (PCR) has been actively applied when developing the authentic identification of raw stock and multi-component product origin. This concept is universal since it distinguishes with the deeper level of species differentiation, high reproducibility and the capacity for the quantitative analysis.

In the PCR sphere of applications, various methods have been elaborated for DNA isolation, requirements are described set to target DNA, laboratories are equipped to synthesize gene- and species-specific primers used for PCR assay, different ways to optimize PCR stages are described, there is a range of elaborated techniques to identify plant components in food products. However, the works in this sphere are relatively limited, given the huge assortment of food produced with the variety of herbal supplements, peculiarities of creation thereof, urgent researches in the sphere of species identification of plant resources used, development and improvement of test system identification based on PCR.

Review of domestic and foreign scientific papers and intellectual property items dedicated to species identification of fruit and berry stock proved that the PCR-based identification method is of high specificity and is practically the only method of species identification.

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SCIENTIFIC AND TECHNICAL JUSTIFICATION OF CONCEPTUAL PROVISIONS OF PROTEOMICS OF DAIRY BUSINESS

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Abstract: The paradigm of formation of science about nitrogen-containing compounds (protein complex) of raw milk – Proteomics – is stated. The monitoring of fractional composition, possibility of extraction, modification and application of the whole protein complex, caseins and serum proteins, their fractions and derivatives allows to consider the traditional and innovative component of dairy business in an absolutely new light, based on nanoclusters and biotechnology. The road map of casein complex of raw milk is considered. The characteristic of the main fractions of casein, from the point of view of modern biotechnology of cheeses and cottage cheese is provided. The characteristic of serum proteins of raw milk in the native and denatured states and after the microparticulation directed into nanotubes is separately considered. The unimproved opportunities for the modernization of technologies of extraction of protein clusters with the receipt of products for import substitution with export orientation are emphasized. For the first time in the logistics of system analysis the problems of controlled proteolysis of albumins of milk – casein and serum proteins, with the receipt of products for clinical nutrition are considered.

Keywords: milk proteins, casein, whey proteins, amino acids, composition and properties of protein complex of milk, ways of receipt, ways of use

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INTRODUCTION. STATEMENT OF THE PROBLEM

According to [2] the postulates of LACTOOMICS offered [1] and adapted in print as the science about MILK, and to the principles of logistics of dairy business [3], it is advisable to put briefly some reasons down, in general, in respect of innovations and information technologies, about one of the main components of milk – PROTEINS (from Greek protey – the first) – a protein complex (nitrogen-containing compounds) as the anthem of life creation on our planet (according to F. Engels). In phenomenology logistics together with Glycoomics [4] and Lipidomics [5] the term Proteomics is used.

Proteins of milk (nitrogen-containing compounds) are present practically in all dairy products – there is no deproteinized milk (fat-free as opposed to fat and delactosylated as opposed to lactose) in nature (in practice) yet. “Pure” protein complex – milk protein concentrates, casein, its modification – caseinates and derivatives (hydrolyzates) – peptides, an amino acid pool; serum proteins as a whole and individually, and also their multiple derivatives are known in the industry in the form of industrial products. Traditional casein, except its designated purpose as food in dairy products, is known from joiner's glue to artificial caviar. I felt warm for long in a jumper of "casein wool" acquired as a memorable souvenir in Poland (1965) – the casein was Polish and the "wool" was Japanese. And all this set of irreplaceable food products, medical supplies and technical semi-finished products – proteins begins with the multicomponent composition of protein complex of raw milk, the aggregate data about the types and fractions of which, according to the information given by A. Tepel [6], with the modern interpretation by V. V. El'chaninov [7, 8, 9, 10], are stated below. At the same time, the postulates, known from biological chemistry and genetics, general in importance, composition, structure and the properties of native natural proteins do not naturally repeat, the emphasis is only on milk proteins which are part of the biosphere, in the light of Lactoomics.

OBJECTS AND METHODS OF STUDY

The total content (the initial stage of road map of raw milk) of albumins in milk is within the limits of 2.9 … 4.0% (the total content of nonprotein nitrogen is up to 0.035%), which should be considered within Proteomics in practice, especially when monitoring primary raw milk as a commercial product and when treating serum. This indicator determines, along with milk fat, the technology and economy of dairy business. Is completely determined by the level of

development of livestock production. The dairy industry correlates de facto with this indicator. Unfortunately, this indicator hasn't been formalized in this branch at the financial, technological and social levels in our country until recently. Table 1 provides the summary data on the content of albumins in a dry residual of raw milk [11].

It follows from the provided data that the protein component in raw milk is significant (at the level of 25.0%) in a solid and considerably differs by types, which naturally determines the status of received products – a complex, casein (without fractionation), whey proteins (generally, without fractionation). Table 2 provides the general characteristic about the content of protein fractions of raw (whole) milk.

The structures, main and minor components, genetic variations, polypeptide chain and amino acid pool of protein compounds of milk raw materials are diverse. They constantly replenish. The information of Professor K. K. Gorbatova [12] with additions by A. Tepel [6] is given below about the amino acid pool of main fractions of proteins of raw milk.

An exceeding information file of researches on the interpretation of primary structure of all fractions and genetic variations with the elements of poetic allegory and a practical component is at the back of each digit. For example, the polypeptide chain can give a sweet (aspartame) and a bitter taste in cheeses; it is possible to receive antibiotics (nisin) and, unfortunately, poison. And this all can be received from the protein structure of milk.

The elementary composition of milk proteins (for your reference) according to various researchers [6, 12] is accurately traced in a hierarchy, with the contents at the level of, %: carbon (C) – 53.0, oxygen (O) – 23.0, nitrogen (N) – 15.6, hydrogen (H) – 7.0, sulfur (S) – 1.5, phosphorus (P) – 0.8.

Table 1. Content of albumins in raw milk

Description of raw material	Content of		%
	Solids, g/100ml	Protein, g/100ml	
Raw milk	13.30	3.20	24.06
Cream, fat percentage 35%	41.30	2.40	5.81
Non-fat milk, fat percentage 0.05%	8.70	3.20	36.80
Butter milk	9.10	3.20	35.16
Whey	6.30	0.80	12.70

Table 2. Main groups of albumins of raw milk

Name of fractions	Content, g/100ml	%
Total content	3.27	100.0
Total of caseins	2.60	79.5
including:		
α_{S1}-casein	1.00	30.6
α_{S2}-casein	0.26	8.0
β-casein	10.01	30.8
k-casein	3.30	10.1
Total of serum proteins	0.63	19.3
including:		
α-lactalbumin	0.12	3.7
β-lactoglobulin	0.32	9.9
blood serum albumin	0.04	1.2
immunoglobulins	0.07	2.1
proteosopeptones	0.08	2.4
proteins of fat globules membranes	0.04	1.2
minor proteins	trace amounts	–

Table 3. Amino acid pool of main fractions of proteins of raw milk

Fractions of polypeptide chain	Amino acid pool, pcs.·α-AA
α_{S1}-casein	199
α_{S2}-casein	207
β-casein	209
k-casein	169
α-lactalbumin	123
β-lactoglobulin	162
blood serum albumin	582

The content of the main component of nitrogen-containing compounds of milk – casein is up to 80% of protein complex and is 2.6 … 3.2% (the more the better). Casein has a lot of fractions (it is considered that there are more than 20 now), genetic variations, fragments and groups (from 1 to 6 for each fraction), which is little considered in practice yet, for example, in cheese making and in the production of cottage cheese. A purposeful search with the use of all achievements of modern analytics of organic compounds is necessary.

The content of whey proteins – at the level of 20% (not to confuse with whey proteins) in milk is 0.4 … 0.7%. Whey proteins of milk, just as casein, are fractional – up to 19 names with 7 genetic variations. The same goes to proteins o covers of fat globules (8 fractions). It is necessary within Lacoomics to point to a special, quite a new group of nitrogen-containing compounds, – minor (a low content, an important role) proteins – up to 2% of total mass (9 fractions and the mass of genetic variations).

In general, macrocomponents (casein and whey proteins) and the minor component allow to draw a conclusion about a genetically full-weight set of clusters of nitrogen-containing compounds in milk, an irreplaceable food component during all the life cycle of mammals. From exactly this perspective, in relation to industrial processing of raw milk, this group of compounds shall be considered for all the assortment groups of products and production cycles. It is not really available in the existing study books yet!

The methodology of study of albumins of raw milk and the received products is quite enough fulfilled [13, 14]. Is based on the gnoseology of coefficient 6.38 for nitrogen and the indispensability of Kyeldal's formula. At the same time, "the floating indicator of self-deception" for nonprotein nitrogen (NPN) in milk reaches 8%, and 30% in whey. Devices like MilkoScan neutralize this problem, but they are not absolutely recognized. There are breakthroughs, especially in the field of chromatography – gas, liquid and ionic chromatography [15]. For dairy business, of special interest are HPLC with reversed phases (RP-HPLC), fast protein liquid chromatography (FPLC) and size-exclusion chromatography (SEC), and also gel filtration, applicable for casein and serum proteins.

RESULTS AND DISCUSSION

Modern classification and nomenclature of milk proteins (is constantly replenishing and changing) [6, 12]. It is necessary to emphasize within Lactoomics and Proteomics the earlier mentioned polycomponent character of all four groups of milk proteins (caseins, whey proteins, protein of fat globules membranes, minor proteins) and to reconcile it once again with the road map of practical use of components, as a whole. Separately – with a possibility of modification for the primary components – peptides and amino acids, and also microparticulation. In such a logic of knowledge and analysis the modern postulates of Proteomics are considered. They are in the dynamics of development according to the achievements of fundamental sciences and practice of researches of creative teams of the international "dairy community".

Let us consider the road map, in respect of technological monitoring, of each of the macrocomponents of milk protein complex. At the same time, we use the information file obtained from survey information [16] and an education guide [17], special researches [18, 19, 20], system publications [7–10] and the generalizing material [6].

Caseins, from the perspective of Lactoomics and Proteomics, are of special cognitive and practical interest. They draw attention of theorists and practitioners as ideal natural protein (especially in respect of polymorphism) and a source of profit for business since the time of a curious Dutch practician Mulder and the great scientist Gammersten. The depth of cognition of milk casein and the constant attention to this problematics is confirmed by the information stated above, and also by my personal observations at special IDF World Dairy Summits in the Netherlands (1973) and Austria (1975). This subject is discussed in all the events of IDF and at all Industry Summits. And the need of interest for the object of cognition can be confirmed with a simple question, which is not cleared up yet, – "why is milk white?". The trick is in casein. The confirmation follows from an observation, trivial and available to everyone, of color of milk after spontaneous or controlled (heating – cooling – souring) souring – serum is transparent with a yellowish-greenish shade, and the product is of white color.

Fig. 1 provides the modern model of casein in the system HyperChem using the example of k-casein. It is considered that it reminds figuratively a jumping horse – there is nothing to do for chemists, physicists and biotechnologists but to "bridle a racer".

Even more indicative is the computer model of casein clusters on the basis of fractal views. Fig. 2 provides the systematized information by Smykov [19] about the models of aggregation of clusters of casein micelles: diffusion limited aggregation (DLA) [21]; ballistic aggregation (BLA) [22–24]; rotation limited aggregation (RLA) [25–28]; diffusion limited cluster aggregation (DLCA) [29]; reaction limited cluster aggregation (RLCA) [30–32]; ballistic limited cluster-cluster aggregation (BLCA) [33–36].

The provided compositions of clusters characterize the alternative variants of process of structurization ("clotting") of casein micelles (daily performed by cheesemakers). They also cover the allegory of "the synthesis of the Universe" in case of the thermal denaturation of serum proteins (try it by holding your own observation). These provisions are interpreted by the outstanding author of the term and theory of fractals, American academician Benoit Mandelbrot [37], they underline the uniqueness of casein as an object of biocenosis in the Universe. And confirm once again the whey phenomenon.

The genetic polymorphism of casein is well seen in the chromatogram (Fig. 3).

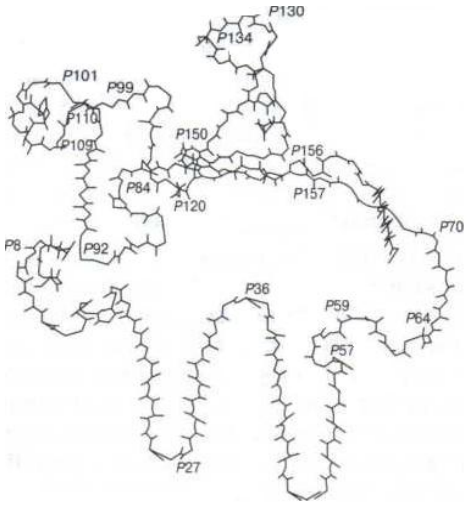


Fig. 1. Variant of visualization of model of micelle of k-casein according to HyperChem – public information.

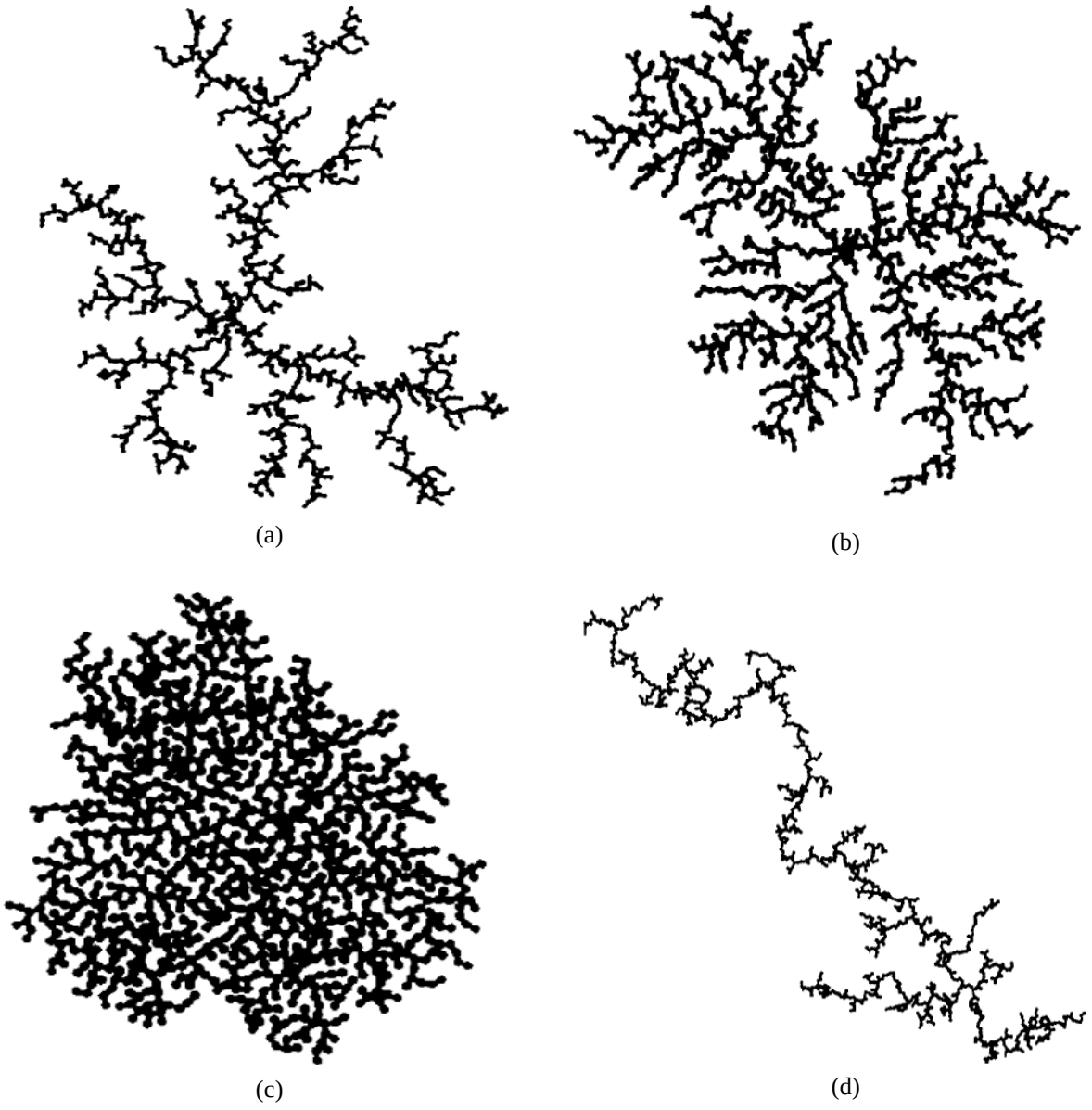
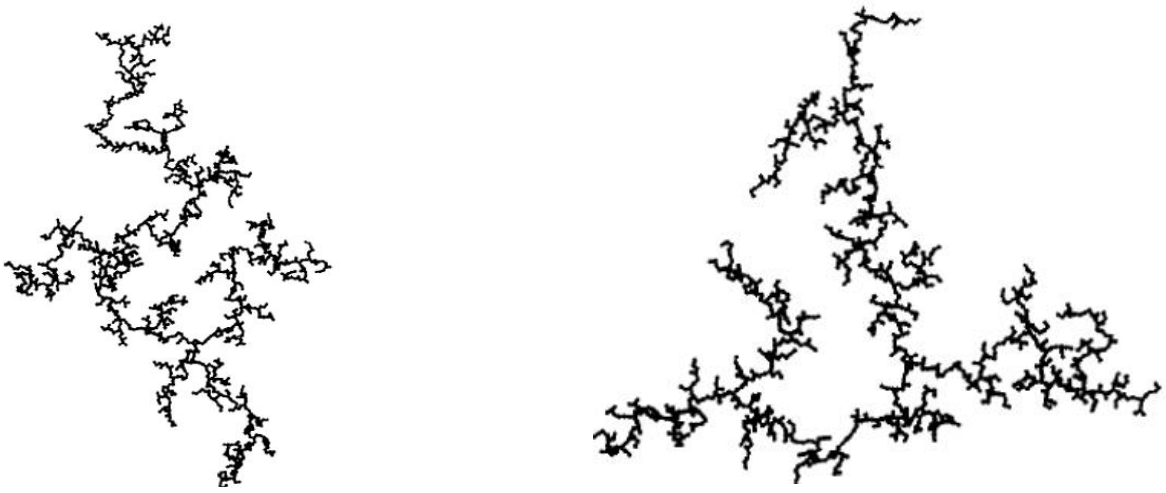


Fig. 2. *Beginning*. Computer models of formation of casein clusters: (a) DLA, (b) BLA, (c) RLA,(d) DLCA, (e) RLCA, and (f) BLCA.



(e) (f)

Fig. 2. *Ending*. Computer models of formation of casein clusters: (a) DLA, (b) BLA, (c) RLA, (d) DLCA, (e) RLCA, and (f) BLCA.

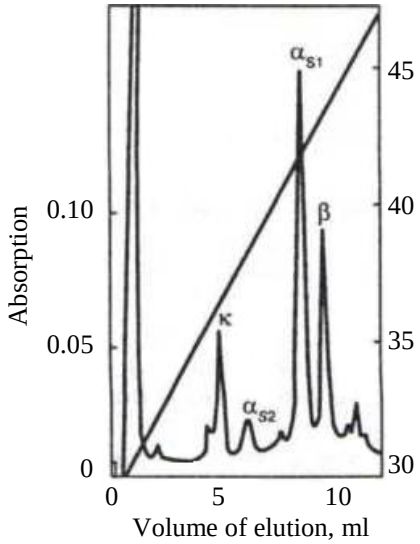


Fig. 3. Chromatogram of milk caseins.

The primary structure of casein, – a consecutive compound of amino acids, depends on a type of fraction and its genetic variation. This fine picture, worthy of "poetization", should be the cornerstone of Proteomics and demands individual consideration. The controlled hydrolysis (proteolysis) of protein compounds of casein and serum proteins turns into an independent branch which should be mastered in the dairy industry (the meat industry has earlier given complete control over it) – there were special shops by meat-processing plants. Are considered separately in respect of the controlled phenylalanine elimination.

The secondary structure – peptides, is already in technology [38] and will be considered below.

The tertiary structure of proteins is of great importance for the technological properties of milk. The three-dimensional tertiary structures can only be established in the previously crystallized proteins during the research by means of the X-ray diffraction analysis. It was not possible to perform casein crystallization till now – it is considered that it is hardly possible [6]. Is it the second problem for cognition, for example, in Skolkovo or within IDF?! The associates of micelles form a number of variants of quarternary structure of casein which is unstable and constantly demands the controlled regulation by processing methods (temperature, active acidity, mechanical effect).

95% of casein in its native state is in the type of casein micelles or associations of subunits (casein submicelles) which are the complexes of casein monomeric molecules. The behavior of milk during technological processing and industrial conversion is determined generally by the properties of casein micelles. The size of casein micelles is from 30 (nanolevel) to 300 (colloidal state), they have a spherical shape. It is they what determines the so-called yield of protein and fat products – cottage cheese and casein.

Whey proteins are polymorphic, which is well seen in the chromatogram (Fig. 4), their structure is unique (Table 4) and their rheomorphism is incomparable (Fig. 5). Their nanosize – at the level of 10 nanometers – is accurate, which forms, along with lactose, an authentically soluble system of milk (serum and ultrafiltrates). The protein complex of whey is specific, for example, due to the availability of k-casein, and demands individual consideration with the elements of repetition with Table 2.

Lactoglobulin (β-LG) – the main whey protein is non-uniform in structure. It is presented by several genetic variations A, B, C, D, E, F and G differing in amino acid structure. Their content is 50–60% of the total of serum proteins.

Lactoalbumin (α-La) is the second protein in order of importance, it is presented by the genetic variations A and B.

Immunoglobulins (IgG) is a non-uniform group of proteins – glycoproteids of monomers and polymers IgG1, IgG2, IgA and IgM.

Serum albumin (Sa) is presented by a polypeptide chain folded in four bound disulfide threads of globular segments of non-uniform proteins.

Lactoferrin, as well as transferrin, is an iron blood protein.

Osteopontin (OPN) – a multifunctional protein, is found recently, plays an important role in preserving the immune status of the newborns.

All fractions of whey proteins have small sizes and high hydrophily, which explains their high stability in solution. Unlike casein, whey proteins do not form micelles, do not coagulate under the effect of enzymes and do not precipitate in case of milk souring. A thermal effect (denaturation) is required for the realization of this process. Whey proteins have a rather low molecular weight – from 14 000 to 69 000.

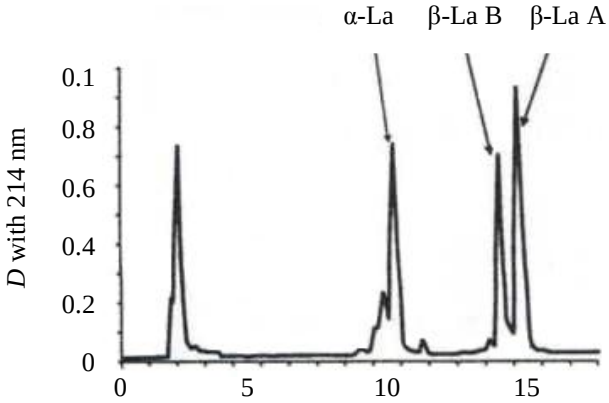
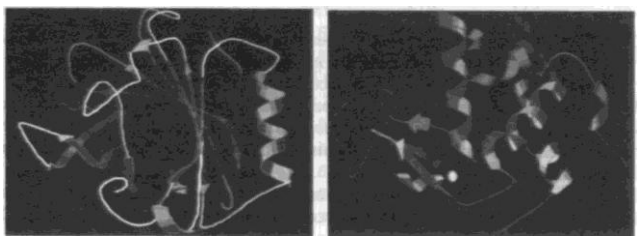


Fig. 4. Chromatogram of whey proteins.

Table 4. Characteristic of milk serum proteins

Name of fractions	Content, %	Molal weight
β-lactoglobulin	0.32	18 400
α-lactalbumin	0.12	14 000
Proteose-peptone fraction	0.12	from 10 000 to 200 000
Immunoglobulins	0.09	160 000
Serum albumin	0.03	70 000
Lactoferrin	0.02	93 000
L-Carnitine	0.03	–
Osteopontin	trace amounts	–
Total	0.73	–



β-lactoglobulinα-lactalbumin

Fig. 5. Molecular structures of some whey proteins.

Minor proteins of milk and **protein compounds of fat globules membranes** draw an increasing attention which obviously follows from the materials of seven International Whey Conferences, especially that of the fifth and sixth ones [39, 40]. For example, angiogenin was the subject of special researches [41] but the result is an original drug Milkang [42], which waits for its practical realization.

The derivation (receipt) of proteins from raw milk is an indispensable component of Proteomics and is constantly studied in respect of scientific knowledge for practical application [43].

The receipt of a **complex of milk proteins** in the form of milk protein concentrates is developed and relates to the basic researches [6, 16]. Unfortunately, this attractive technology is not widely scaled because of an inevitable loss of native properties. The problem waits for a decision. Our researches on soft cheeses [44, 45], cottage cheese products [46], the formation of the brand "LipKA" (a lipid casein albumin concentrate) [47] and the method "TermoLakt" [48], with an attempt to implement the paradigm of complex release of proteins from raw milk, confirm this provision. A search of the optimal solution within Proteomics is forthcoming. Probably, in this case the breakthrough innovations of Professor Z.S. Zobkova and her colleagues concerning enzymatic cross-linking of milk proteins are quite perspective, for example, using transglutaminase for the receipt of new structures and original functions of the derived special purpose products [49].

Coagulation of raw milk caseins – acid, rennet, acid and rennet, chlorcalcic coagulation, coagulation with the use of electrophysical methods, polysaccharides and membrane technology is quite well studied by and is applied at the level of traditions and innovations [11]. The traditions are acid and rennet coagulations. The innovations are membrane technologies (ultrafiltration). The supertechnologies are the biomembrane technology with the use of polysaccharides (membraneless reverse osmosis) and microparticulation (nanotubes).

The whey protein complex, in respect of release, is quite well studied and realized in practice [10]. The traditions are thermal denaturation; the innovations are membrane technologies. The supertechnologies are microparticulation (nanotubes).

The fundamentals of development of technology of dairy products, using the example of casein coagulation – (Laktoomics base), were worked out by A.M. Osintsev [18] in a system type. The complex extraction of milk proteins using the example of soft cheeses is thoroughly studied by I.A. Smirnova [50] and O.A. Suyunchev [48]. They are widely realized (scaled) in practice. However, the search is not finished. As an example, we will briefly consider "the membraneless technology", one of the alternative variants of extraction of casein with the implementation in the line (series) of products of the brand "Bio-TON" [51].

Historically, the cycle of development of new technology of thermodynamic fractionation of milk components (sometimes called as membraneless reverse osmosis) includes the stage of accidental observations in the thirties of spontaneous milk separation when adding polysaccharide (Patent DE 555273) for two fractions – casein concentrate and a serum fraction. It was the stage which proved a unique possibility of separation of milk into a casein and non-casein fractions. The method did not find practical application and was forgotten for more than 50 years. Then, at A.N. Nesmeyanov Institute of Organoelement Compounds (INEOS) of Academy of Sciences of the USSR, within a number of large-scale basic researches of Professor V.B. Tolstoguzov and his colleagues studying the problem of search of food reserves, a possibility of controlled separation of protein solutions by polysaccharides was theoretically proved and experimentally demonstrated. Milk was one of the ideal objects. In the State Enterprise «Scientific Research Institute of the Complex Utilization of Dairy Raw Material (formerly known as All-Union Research Institute of the Complex Utilization of Dairy Raw Material and The North Caucasian branch of All-Union Research Institute of the Cheesemaking and Buttermaking Industry) by efforts of the school of my dear colleague from RAS Professor V.V. Molochnikov complex specific researches [52, 53] on the development of non-waste technology of fractionation of milk by polysaccharides using the principle of membraneless return osmosis [54], which was named as "Bio-Ton", were performed.

Fig. 6 provides the schematic diagram of separation of milk by biopolymers (polysaccharides).

As a result of separation, natural casein concentrate is received, the characteristic of which (in respect of Proteomics) we will consider in more detail [55].

Natural casein concentrate (NCC) is a light-cream thixotropic uniform liquid with creamy consistence and with a pure milk taste and smell. It completely dissolves in water. The thermal effect up to 100°C on liquid natural casein concentrate does not change its solubility, which testifies to naturalness as it is known that milk casein has a unique structure in its native state, which specifies its resistance to the effect of denaturant agents and its high splittability by proteolytic enzymes.

Table 5 provides the average physical and chemical values of NCC in comparison with skim milk.

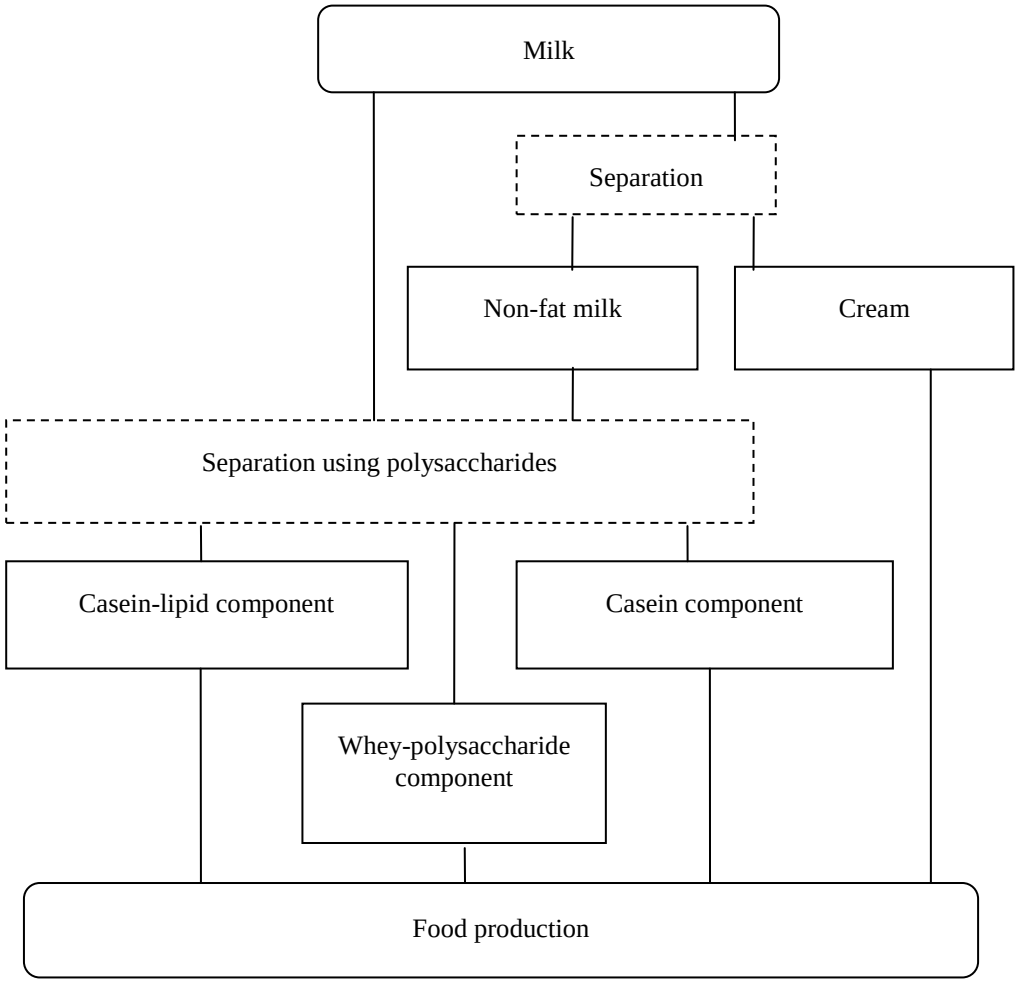


Fig. 6. Schematic diagram of separation of milk by polysaccharides.

Table 5. Average physical and chemical values of NCC in comparison with non-fat milk

Parameters	Skim milk	NCC	Ratio, %
Percentage of solids, % including: protein, %	 8.70 ± 0.20 3.15 ± 0.15	 19.45 ± 0.50 13.75 ± 0.50	 223.6 436.51
lactose, %	4.75 ± 0.05	3.60 ± 0.10	75.8
pectin, %	–	–	0.70% in the mix of FTIR
minerals, %	0.75 ± 0.05	1.80 ± 0.10	240
fat, %	0.05 ± 0.00	0.25 ± 0.05	500
Calcium, mg/100g	115 ± 5	400 ± 10	347.8
Phosphorus, mg / 100 g	95 ± 5	250 ± 10	263.2
Titrable acidity, ^{o}T	18 ± 2	50 ± 2	278
Active acidity, pH	6.7 ± 0.1	6.3 ± 0.2	< 0.40
Density, kg/m^3	1030 ± 5	1060 ± 15	> by 30

Natural casein concentrate contains the main components – proteins, carbohydrates, minerals in a soluble form. The main protein component of NCC is casein in the form of casein-calcium-phosphate complex, which allows to keep, to a wide extent, the natural properties of the main milk protein. The considerable part of calcium and phosphorus transits into NCC, which provides the preservation of mineral balance in the derived milk casein complex. The fractional composition of natural casein concentrate, in respect of electrophoretic mobility, completely coincides with the samples received in the traditional acid way. The residual fat of milk is taken away by the protein part and is concentrated in it in the course of release of casein. The high values of titrable acidity in natural casein concentrate are caused by the presence of concentrated protein and mineral salts. The increase in the percentage of proteins, carbohydrates and minerals in the concentrate increase its density up to 1060–1070 kg/m^3 in comparison with skim milk. The presence of minerals, a quantity of lactose and milk fat concentrated together with the casein-calcium-

phosphate complex of milk increases the nutrition value of products in the course of its use [56].

The main protein in NCC is casein, its content is about 98%, the content of whey proteins is insignificant and does not exceed 3%. Skim milk is separated as follows during fractionation: up to 85% of volume is a whey-polysaccharide fraction, and 15% is a casein fraction which contains about 20% of solids, including about 14% of protein. Thus, casein concentrate contains up to 70% of protein, and 30% are the components of whey-polysaccharide fraction (WPF). With such a ratio the casein complex preserves its native properties, without changing them even after drying.

"The life cycle" of Proteomics of NCC just begins.

Of special interest within Proteomics of dairy business is the receipt of **derivatives of protein complex of milk** – casein and whey proteins. This subject deserves special consideration. At the same time, separation using hydrolysis and proteolysis must be kept in mind. Casein hydrolysis is not only studied, but is also realized in practice throughout the world, including our country, for example, in drugs. Casein proteolysis in cheese – the basis of its biotechnology, determines the type and quality of a product. The hydrolysis of whey proteins has a special value in the medical-biological aspect – for baby, dietary and clinical nutrition. Let us show the significance of object, complexity of process and uniqueness of result using the example of the large-scale researches of the creative team of Professor A.Yu. Prosekov (Kemerovo Institute of Food Science and Technology) and, in particular, the works by O.O. Babich [20].

The biologically active peptides received from milk proteins are realized in the industrial technologies of developments of Kemerovo Institute of Food Science and Technology in England (according to the existing international rules). This alone, in respect of the Proteomics of dairy business, draws attention, does credit to the developers and confirms the opportunity of realization of not only import substitution of products, but also of export of innovative technologies. The informative part of researches and the obtained results are published in a special monograph [38]. Let us only consider the innovative priorities for the confirmation of postulates of Proteomics using a specific example. It is necessary to pay attention to a special role of natural polypeptide chains of amino acids (contained in raw milk) and those which are purposefully synthesized from fractions of casein and serum proteins (from tens to hundreds). For example, exomorphins are analgetics. They regulate the general hormonal background of mammals, especially that of cubs (there is no crying). And beta-casomorphins are fine immunomodulators. "Dairy peptides" increase the phagocytic ability of some bacteria of GIT, providing the resistance of the organism to infectious diseases. For example, the analog of low-molecular peptide of women's milk – lactoptin, synthesized recently in Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, has an antineoplastic and anti-metastatic activity and is absolutely safe. Angiogenin Milkang [42] plays the same role, in respect of blood vessels, wounds and burns.

Taking into account the above stated, predicting the results of exploratory researches given below, the formation of an independent section of Laktoomics with the brand of Peptidomics of dairy business is absolutely appropriate!

Table 6 provides the efficiency of hydrolysis of the basic casein fractions using various enzymatic drugs.

The analysis of the data provided in Table 6 shows that the nature of change of nitrogen of casein fractions by all the enzymes is approximately identical. Trypsin effectively hydrolyzes a k-fraction, providing a decrease in the content of nitrogen by 16.9%. Chymotrypsin and thermolysine transform the transition of some fractions of casein to other forms of nitrogen compounds. The identification of the received sequences of peptides according to the database NCBI [38] is given in Table 7.

The results of identification clearly show that the fermentation by all the tested drugs – trypsin, chymotrypsin and thermolysine provide the receipt of biologically active peptides. Table 8 specifies the optimum parameters of average and high extent of hydrolysis of casein fractions for receiving biologically active peptides with their crucial indicators.

Table 6. Total content of albumins in hydrolyzates

Sample	Percentage of nitrogen of casein fractions, mg/100g			
	α	β	γ	χ
Before fermentation	0.202	0.083	0.019	0.071
After fermentation by trypsin	0.196	0.073	0.017	0.045
After fermentation by chymotrypsin	0.195	0.080	0.018	0.055
After fermentation by thermolysin	0.198	0.081	0.018	0.064

Table 7. Peptides identified in the studied hydrolyzates

Fragment	Used enzyme	Sequence of amino acids in peptides	Name	Function
1–25	Trypsin, chymotrypsin	Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr-Arg	phosphopeptide	stimulation of digestion of mineral substances
177–183	thermolysin	Aia-Val-Pro-Tyr-Pro-Gln-Arg	P-kasokinin	the inhibitor of angiotensin converting enzyme

Table 8. Technological characteristics of the process of enzymatic hydrolysis

Parameters of process and characteristic of hydrolyzates	Casein after treatment with		
	trypsin	chymotrypsin	thermolysin
Solids in solution, %	5.18	4.98	4.80
Quantity of enzyme of the weight of substratum, %	0.01	0.01	0.01
Temperature of process, °C	50 ± 1		
pH of process	7.4÷7.6	7.4÷7.6	7.4÷7.6
Process duration, hrs	12	12	10
Temperature of enzyme inactivation, °C	93 ± 2		
Inactivation duration, min	5		
Molar weight, kD	4.5	3.1	3.1

Anti-gene activity (AG) as an objective criterion of biological safety of the received peptide drugs, in relation to pasteurized milk, is shown below.

Sample	AG (in relation to whole pasteurized cow milk)
Casein	0.79
Filtrate of tryptic hydrolyzate through a membrane of 10 kD	$7.5*10^{-6}$
Filtrate of chymotryptic hydrolyzate through a membrane of 10 kD	$3.7*10^{-6}$
Filtrate of thermolysin hydrolyzate through a membrane of 10 kD	$5.8*10^{-6}$

The given indicators of the decrease in AG in all the hydrolyzed samples, one million times in comparison with the initial substratum (milk protein) – are comparable to the characteristic of the specialized mixes proved by practice, for example, "Nutramigen" and other mixes.

In general, the purposeful researches on the currently important problematic performed by O.O. Babich, allowed to determine the main regularities of the process of hydrolysis of milk proteins with the receipt of biologically active peptides for specialized foodstuffs. Based on the same scientific paradigm, O.O. Babich performed a number of system researches [20] on the receipt of casein hydrolyzates and serum proteins with a regulated amino-acid pool, which also forms a portfolio of innovations of Laktoomics using the example of development of Proteomics, after Peptidomics.

The amino-acid pool of proteins of raw milk, with its uniqueness, can serve as an ideal for food of mammals both in a complex – "the amazing food cooked by nature" (after Academician, Nobel Laureate, our great compatriot Ivan Petrovich Pavlov) and in the form of special drugs. The peptides are mentioned above. The hydrolyzates of milk proteins – casein and serum proteins are known as medical drugs and also as baby, dietary and special foodstuffs [57].

To our great regret, these unique products are contraindicated for a part of "Homo-Sapiens" because of anomaly (allergy) and painful symptoms (to death). Therefore, the vital problem is the receipt of special foodstuff for this small but existing in the human civilization of inhabitants of our planet Earth group of newborns. It is this noble aim that the researches of O.O. Babich were devoted to [20]. Specifically, the subject was concentrated on "the poison for suffering newborns with a genetic anomaly" – phenylalanine. The diagnosis of physicians – phenylketonuria - is a trouble for the kid and a signal to food industry workers – a special product of medical appointment is necessary. The modern Proteomics of dairy business implemented abroad provides the development of such product assortment. Now the scaling of results of researches of O.O. Babich allows to hope for real import substitution with domestic drugs. And Proteomics of dairy business, as an indispensable part of Laktoomics, replenished with a new, original section in which the information available in open publications in our country [38, 58] and abroad [59, 60, 61] is concentrated.

The informative part of researches [20] included the monitoring with the choice of special yeast Aureobasidium pullulans Y863 for the biosynthesis of enzyme L-phenylalanine-ammonium-lyase - PAL (EC 4.3.1.5), its immobilization on iron oxide nanoparticles using the method of tyophen acetylation with the receipt of industrial preparation which catalyzes the reaction of reversible deamination of L-phenylalanine to the compounds safe for the organism: trans-cinnamon acid and ammonia.

Fig. 8 and Table 9 provide the results of researches.

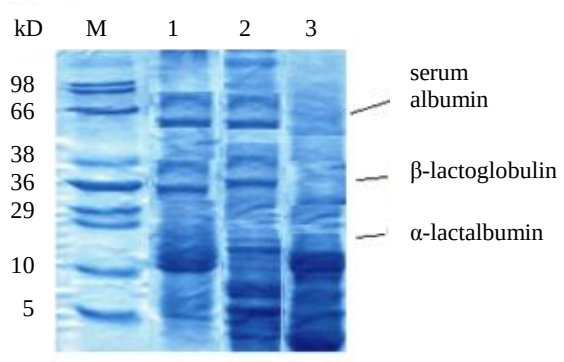


Fig. 8. Electrophoregram of enzymatic hydrolyzate of whey proteins with the different duration of hydrolysis: 1 – 30 min, 2 – 60 min, 3 – 90 min. M is a marker Roti-Mark Standard, 5–98 kD (Carl Roth, Germany).

Table 9. Characteristic of hydrolyzates of serum proteins, %

Parameter	Level of hydrolysis, with the duration of, min		
	weakly, 30	moderately, 60	deeply, 90
Percentage of split proteins in the hydrolyzate:			
α-lactalbumin	11.3 ± 0.6	45.5 ± 2.3	98.3 ± 4.9
β-lactoglobulin	5.6 ± 0.3	28.7 ± 1.4	77.1 ± 3.8
bovine serum albumin	0	13.9 ± 0.7	58.4 ± 2.9
Percentage of fraction with the molecular weight of <500 D	6.8 ± 0.3	12.4 ± 0.6	63.4 ± 4.7
Ratio of α-amino and total nitrogen	3.5 ± 0.2	8.8 ± 0.4	60.2 ± 3.0

With an increase in the depth of hydrolysis (it is regulated by the duration of process) the percentage of split proteins in a serum hydrolyzate increases, and it is the fermentolysis of α-lactalbumin that occurs most intensively. The rational parameters of controlled enzymatic hydrolysis of serum proteins using the complex of endo- and exopeptidases are the following: the temperature is 50 ± 1°C; the duration is 90 ± 1 min.; the enzyme-substratum ratio is 1 : 25.

The complex of experimental and theoretical researches served as a prerequisite for the creation of new types of specialized foodstuffs with the use of L-phenylalanine-ammonium-lyase. The technology includes the enzymatic processing of raw milk using the enzymatic system consisting of endo- and exopeptidases for the purpose of the maximum removal of phenylalanine from a polypeptide chain with the subsequent biotechnological processing of L-phenylalanine-ammonium-lyase providing the biotransformation of phenylalanine into the compounds which do not have a toxic effect on the patient's organism, and, thereby, the reduction of its content in a dairy equivalent up to 0.001%. This dairy equivalent can be the basis for the creation of a number of specialized dairy products for patients with phenylketonuria (dairy and protein ones and others). It is realized in 17 innovative developments the novelty of which is confirmed with patents for inventions. The technological developments are approved at the Institute of Molecular Biology (IMB) of the National Academy of Sciences of the Republic of Armenia (NAS RA), CJSC Khladotekhnika, Federal State-Funded Educational Institution of Higher Vocational Education "National Research Tomsk Polytechnic University" and introduced into production on a large scale in England by ClusterNanoTech LTD, in the way of transfer of technologies according to a license interstate agreement. The Russian Pentidomics of milk has come to the international level.

It means necessary now, within Proteomics, to consider briefly the original innovation which has appeared in recent years – the **MICRO-PARTICULATION** of milk proteins. In principle, this is the realization of postulates of nanobiotechnology in relation to a concrete natural object of researches. In the logic of historical development it is necessary to begin with the microparticulation of whey proteins.

The microparticulation of serum proteins began in 1984 with a US patent of the Canadian inventors N.S. Singler, Sh. Yamomoto and D. Latell on the product Simplesse [62]. The early system researches on the subject in our country were performed by Professor I.A. Smirnova in Kemerovo Institute of Food Science and Technology at the school of the master of cheese making, Professor L.A. Ostroumov. In the work by S.V. Manylov [63] the product of microparticulation of serum proteins Simplesse-100 was used in the production of cheeses and cottage cheese as the normalizer of initial mix with a positive effect. The system researches on receiving nanotubes on the basis of UV-concentrate of proteins of cheese whey are performed in Voronezh State University of Engineering Technologies for receiving the flavor (substitute) of milk by N.A. Podgornyy under the supervision of Professor E.I. Mel'nikova [64]. Fig. 9 provides the logical sequence of the technological line of receiving microparticulates of serum proteins (nanotubes), offered by the researchers, with the size of 1–3 microns.

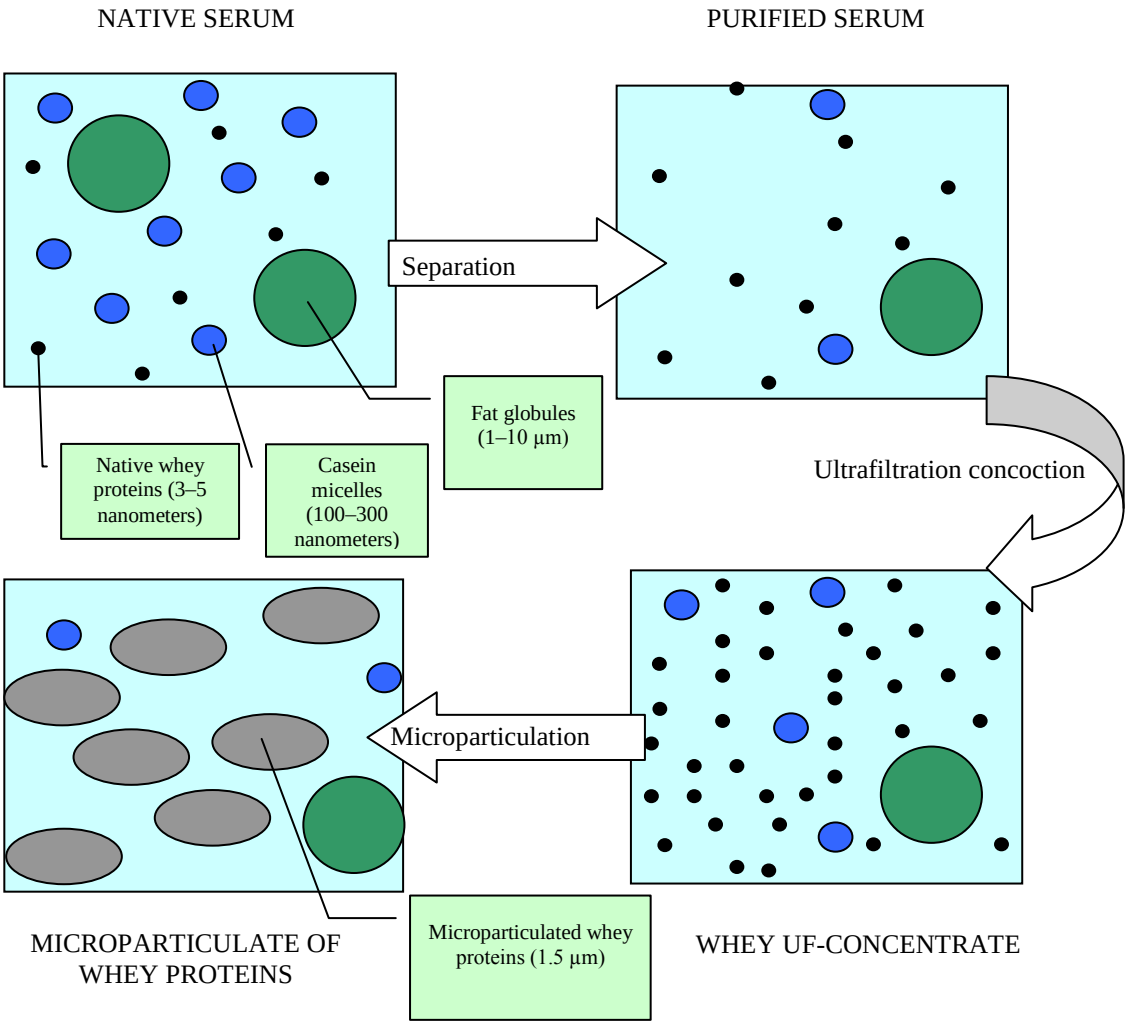


Fig. 9. Scheme of microparticulation of cheese whey proteins (according to E.I. Mel'nikova and N.A. Podgornyy).

The researches on the subject of microparticulation of synthesis of nanotubes of associates of serum proteins are actively performed by a lot of project teams, including our North Caucasus Federal University. I am sure that we expect exclusively interesting and positive results of the use of the so-called albumin milk (the former food means at distress prices) in the supertechnologies of products of functional purpose of the brand "NanoFood-NanoEda". This is a bright confirmation of the phenomenon of derivatives of components of serum at the cluster level of fractal supertechnologies.

The casein microparticulates are received in the work by V.K. Shtrigul' [65] under the supervision of Professor I.A. Smirnova (continuity of problems) in Kemerovo Institute of Food Science and Technology, which does credit to the creative team of scholar school of Professor L.A. Ostroumova and Rectror of the institute, Professor A.Yu. Prosekov.

The informative part of innovation included the search of the optimum way to "force" casein micelles to be aggregated in the nanotubes imitating the flavor of milk fat. Of the four tested variants known and used in the branch – thermal acid, acid, chlorcalcic and rennet coagulations, not the best (chlorcalcic coagulation has a bitter flavor) but a rational one was chosen – rennet coagulation. The "perk" of the process, which is schematically shown in Fig. 10, is the stage of interruption of complex formation of casein micelles at a certain stage ("know-how") – shown in the form of a road sign.

For the realization of process of interruption of complex formation of casein micelles a simple, but original operation – disoxidation up to pH 7.0 using alkaline solution of caustic sodium is used. Casein micelles, at the same time, reach the level from the initial (native) size of 40–300 nanometers to 1 micron and can imitate a flavor of milk fat after the thermal treatment combined with pasteurization, and controlled dispergating. Objectively, the jellification process (trivially – souring) is visible in case of change of viscosity of the system.

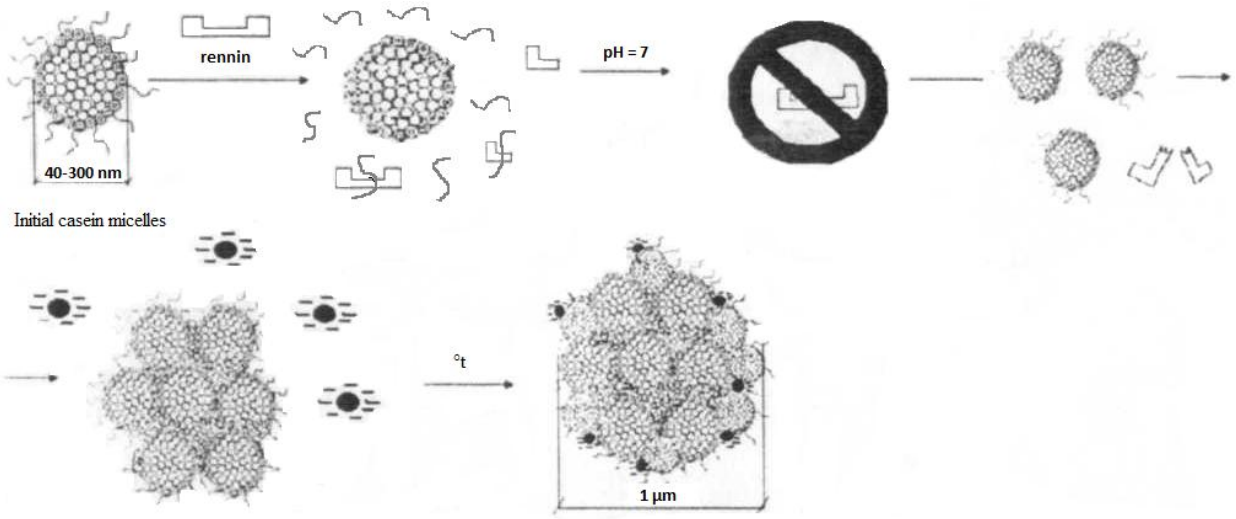


Fig. 10. Hypothetical scheme of the process of logistics of microparticulation of casein of raw milk (according to I. A. Smirnova and V.K. Shtrigul').

A line of dairy drinks of new generation is realized on the basis of casein microparticulates. The received low-fat products had a distinct creamy taste due to the flavor of milk fat of microparticulated casein (nanotubes). The stage of microparticulation organically fits in the technological process of production of traditional dairy drinks. The information about the innovation of Kemerovo Institute of Food Science and Technology is the real embodiment of high (critical) nanobiotechnologies of Laktoomics in the dairy industry, already using the example of casein Proteomics.

CONCLUSION. INNOVATIVE PRIORITIES

All the above concerns the Proteomics of dairy business and gives the grounds for the formation of paradigm of Technological Platforms of modernization of dairy industry of the agrarian and industrial complex, as a component of the food industry, at the level of the sixth technological mode – supertechnologies.

The formation of Proteomics, including that of Peptidomics and dairy business according to product line groups and individual products shall include the study of casein Genomics by cattle breeders at the stage of synthesis in the alveoli of udder of lactating female of mammals with the controlled regulation of content and ratio of fractions, and also of genetic variations. At the same time, the dairy industry with the implementation of principles of chremostatics (profit economy) shall be as the customer.

The use of the optimized model of protein complex of raw milk in products with the complete use of components, their extraction and the receipt of necessary derivatives is the prerogative of dairy industry of agrarian and industrial complex, according to the provisions of the existing food theory and the recommendations of professional nutritionists.

The critical (high) technologies of complex of milk proteins, caseins and whey proteins are well-known and correspond to the fifth technological mode – the principles of biotechnology.

The innovative supertechnologies (the sixth technological mode – nano- and pico-) are implemented in a biogenetic paradigm – complex formation, hydrolysis, proteolysis; molecular size-exclusion filtration (membrane technologies) and microparticulation. Scaling concerns dairy and milk-containing bioecoproducts of the "green basket" of functional, dietary (healthy) and clinical nutrition.

In loving memory of my dear curator, an opponent
to my Candidate's and doctoral dissertations,
Professor Pavel Fedorovich D'yachenko
who made an invaluable contribution to the proteomics of milk proteins

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FOOD PRODUCTION TECHNOLOGY

PHYTOCHEMICAL CONTENTS IN SOLID–LIQUID EXTRACTION OF AQUEOUS ALCOHOLIC EXTRACT OF CHICORY (*CICHORIUM INTYBUS* L.) LEAVES

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Abstract: The object of our current study is to study the phytochemicalc contents in solid-liquid extraction of chicory (*Cichorium intybus* L.) dry leaves grown in Bulgaria. *Cichorium intybus*, commonly known as chicory, is well known as a coffee substitute but is also discretely used as the natural product in food industry and medicine throughout its long history. Solid–liquid extraction was performed by using the 50% aqueous ethanol for 120 min which results in concentration of phytochemical contents and the findings of our present results are well consistent with those obtained in other works. The chicory leaves were analysed for the content of tannin by titrimetric method; rutin was determined spectrophotometrically by using ammonium molybdate; the total phenolics was determined by the Folin-Ciocalteu assay and the total flavonoids was identified through the colorimetric reaction with aluminum (III) chloride. The content of total phenolics and total flavonoids of chicory varied between 2.71 mg GAE/mL for 10 min and 5.65 mg GAE/mL for 120 minutes and 0.84 mg CE/mL for 10 minutes and 2.45 mg CE/mL for 120 min. The content of rutin and tannins that varied within 0.71 percent for 10 minutes and 1.39 percent for 120 min of rutin and tannins was higher than that in 50% aqueous ethanol extract of *Cichorium intybus* L. for 120 min at 1.56% and 1.08% for 10 min, respectively. Extracts obtained positively correlated with their phenolic and flavonoid contents, rutin and tannins, respectively. Therefore, the complex of phytochemical active substance in dry leaves of *Cichorium intybus* L. offers lots of opportunities for future application in herbal medicine and nutrition industry to produce healthy food.

Keywords: Total phenolics, total flavonoids, rutin, tannins, 50% aqueous ethanol extract of Bulgarian dry leaves of chicory (*Cichorium intybus* L.)

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INTRODUCTION

Since ancient times natural phytochemical compounds have been used as food and beverage, as the dye and in traditional medicine sphere. Recently, the capacity of polyphenol has generated too much interest for the large content of the compound to be identified in mere plants whereas the consumption of fruits, vegetables, spices and beverages with the high content of polyphenol may reduce the risk of several diseases due to their antioxidant power, among other factors. The conventional method of polyphenol recovery from plant is based on the solid-liquid solvent extraction. When extracting by the solid-liquid method, soluble components are removed from solids using aqueous organic solvents well mixed. Solvents should be carefully selected to minimize matrix interferences [1, 2], while experimental parameters (temperature, time, pH, solid-to-liquid ratio, particle size, stirring rate, solvent polarity) should all be optimized to obtain the quantitative extraction of molecules required [1, 3].

As a method, solid-liquid extraction is widely applied in various industries. For instance, in the production of herb-based food products; currently, it is used when the plant matrix requests extraction for further processing. Non-expensive and non-toxic solvents (*i.e.*, water) are used and tend to be used in prospective researches in combination with other mild extraction techniques [1].

Cichorium intybus L., commonly known as chicory, refers to *Asteraceae* family and widely distributed in Asia and Europe [4, 5]. All parts of this plant are of great medicinal importance due to a number of compounds of medicinally significance such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, flavonoids, saponins and tannins [4–9]. The whole plant is validated for numerous applications in food industry and medicine [10, 11]. Its dried roots are used as the substitute or adulterant in coffee powder [10, 12]. The young leaves can be added to salads and vegetable cuisine, while chicory extracts are used for production of invigorating drinks [10]. Chicory leaf is the good sources of phenols, vitamins A and C as well as potassium, calcium, and phosphorus [4, 8]. *C. intybus* has been traditionally used to treat fever, diarrhea, jaundice and gallstones [4, 13, 14]. During the past decade, the interest grew in natural plant extracts with potential antioxidant activity that contributes to the health improvement [10, 15, 16]. The expanded application is due to their properties to protect against oxidative stress disorders, as well as oxidative damage in food products [10, 17]. Polyphenols in plant extracts are well known to have strong antioxidant activity. Found in medicinal plants which are the natural source of inulin-type fructans prebiotics, polyphenols further increase the biological activity of extracts obtained [10, 18, 19]. However, the data on total phenols, total flavonoids, rutin and tannins available in leaves of medicinal plants is not complete. The common chicory (*Cichorium intybus* L.) grows in different regions of Bulgaria. Therefore, this study was intended to evaluate the phytochemical content in solid–liquid extraction of 50% aqueous alcoholic extract of chicory leaves (*Cichorium intybus* L.) grown in Bulgaria.

OBJECTS AND METHODS OF STUDY

Plant material. The chicory leaves (*Cichorium intybus* L.) were harvested from different regions of Bulgaria. All sample data is shown in the sampling report. The dried leaves were kept in a dry place for further use.

Sample preparation. A dry sample of 0.5 g was accurately weighted followed by the extraction of phenolic and flavonoid compounds with 50 mL of 50% aqueous ethanol in the ultrasonic bath for 10 to 120 min. The extract aliquot (2 mL) was ultra-centrifugated for 5 min at 14 000 rpm. The extract preparation was further used for polyphenol determination by spectrophotometric method.

Determination of total phenolics assay. The total phenolic content of *Cichorium intybus* L. was determined by the Folin-Ciocalteu assay [20].

Determination of total flavonoids assay. The total flavonoid content was measured by the aluminum chloride colorimetric assay [20].

Rutin assay. The rutin content in *Cichorium intybus* L.wasanalysed as per the The International Pharmacopoeia and AOAC method, after modified methods with 50% aqueous ethanol [21].

$$R(\%) = \frac{A_{sample} \times C \times 50 \times 100}{A_{stand} \times W \times 2},$$

where A_{sample} is the sample absorbency was determined at 360 nm, A_{stand} is the absorbancy of standard solution was determined at 360 nm, C is the concentration of the rutin standard solution (g/mL), W is the weight (g) of sample for analyses, 2 is the volume (mL) of sample for analyses, 100 is the percent, %.

Tannins assay. The content of tannins in *Cichorium intybus* L.ws analysed as per The International Pharmacopoeia and AOAC method, after modified methods [22].

Calculations. Calculations are based on the averaging analysis results of duplicate samples.

Calculation of the content (%) of tannins (T) in the sample is as follows:

$$T(\%) = \frac{V - V_0 \times 0.004157 \times 250 \times 100}{g \times 25},$$

where V is the volume of 0.1 N water solution of $KMnO_4$ for sample titration, mL; V_0 is the volume of 0.1 N water solution of $KMnO_4$ for titration of blank sample, mL; 0.004157 is the tannins equivalent in 1 mL of 0.1 N water solution of $KMnO_4$; g is the sample mass for analyses, g; 250 is the volume of volumetric flask, mL; 100 is the percent, %.

Statistical analysis. All experiments were performed in triplicates. At every time point, each experiment was carried out in duplicate or triplicate. Statistical parameters are calculated in terms of reproducibility of experimental data using the general Analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The dry matter content in all experimental runs was determined and results were expressed on dry basis to provide more accurate and reliable data comparison. The 50% aqueous ethanol extract of *Cichorium intybus* L. showed the Table 1 and Table 2 in the appendixe of total phenols, total flavonoids, rutin and tannins in qualitative chemical analysis. In our works, we used the solid–liquid extraction by 50% aqueous ethanol for 10 min to 120 min, which gave concentration of polyphenols. The content for total phenolics and total flavonoids of *Cichorium intybus* L. varied within 2.71 mg GAE/mL for 10 min and 5.65 mg GAE/mL for 120 min and 0.84 mg CE/mL for 10 min and 2.45 mg CE/mL for 120 min, accordingly. This is shown in the Table 1 using the gallic acid and catechin as standards. These results indicate that 50% aqueous ethanol extract *Cichorium intybus* L. obtained for 90 min and stopped in 120 min has higher antioxidant activity than 50% aqueous ethanol extract of *Cichorium intybus* L. obtained in 10 min that may

relate to the phenolic and flavonoid content of the leaf extracts.

The flavonoids and phenolic acids are known to have antioxidant activities due to presence of structural hydroxyl groups and they significantly contribute to protect against the oxidative damage due to endogenous free radicals [4, 23]. Phenolic or polyphenols are secondary plant metabolites that ubiquitously present in plants and their products. Many of them are reported to have high levels of antioxidant activities [4, 24]. Due to their redox properties, these compounds contribute to overall antioxidant activities of plants. Usually, the antioxidant activity is to neutralize lipid free radicals and prevent decomposition of hydroperoxides into free radicals [4, 25, 26]. The chicory leaf extract used in this study was partially described with reference to total phenolic and total flavonoid compounds, rutin and tannins. *Cichorium intybus* L. is reorted to be of high medicinal importance due to phytochemical content. The results show that leaves of *Cichorium intybus* L.are a good source of phenolic compounds.

Rutin and tannins contained in 50% aqueous ethanol extract of *Cichorium intybus* L. are of significance. The level of rutin and tannins varied between 0.71 percent for 10 minutes and 1.39 percent for 120 minutes of rutin and tannins. It was found higher as compared with that in 50% aqueous ethanol extract of *Cichorium intybus* L. (120 minutes between 1.56% and 1.08% in 10 minutes, respectively). The results are shown in the Table 2 with the data obtained using rutin as the standard and the potassium permanganate as the titrant. It is important to note that is not correct to compare results for rutin and tannin contents in 50% aqueous ethanol extract of *Cichorium intybus* L. due to different methods of analysis used.

Table 1. Kinetic varieties of 50% aqueous ethanol extract of *Cichorium intybus* L. leaves in total phenols and total flavonoids

min	Total phenols, mg/mL DW	Total flavonoids, mg/mL DW
10	2.71 ± 0.03 RSD 1.3% (n = 3)	0.84 ± 0.05 RSD 6.2% (n = 3)
15	3.23 ± 0.22 RSD 6.9% (n = 3)	1.11 ± 0.01 RSD 0.9% (n = 3)
20	4.10 ± 0.17 RSD 4.2% (n = 3)	1.58 ± 0.05 RSD 3.2% (n = 3)
30	4.76 ± 0.03 RSD 0.7% (n = 3)	2.12 ± 0.03 RSD 1.6% (n = 3)
60	5.43 ± 0.05 RSD 0.9% (n = 3)	2.31 ± 0.02 RSD 3.1% (n = 3)
90	5.65 ± 0.26 RSD 4.5% (n = 3)	2.45 ± 0.08 RSD 3.5% (n = 3)
120	5.65 ± 0.26 RSD 4.5% (n = 3)	2.45 ± 0.08 RSD 3.5% (n = 3)

Table 2. Kinetic varieties of 50% aqueous ethanol extract of *Cichorium intybus* L. leaves in rutin and tannins

min	Rutin, %	Tannins, %
10	0.71 ± 0.02 RSD 2.4 (n = 3)	1.08 ± 0.02 RSD1.6 (n = 3)
15	0.82 ± 0.03 RSD 4.2 (n = 3)	1.15 ± 0.08 RSD 7.5 (n = 3)
20	0.98 ± 0.02 RSD1.7 (n = 3)	1.28 ± 0.02 RSD1.3 (n = 3)
30	1.20 ± 0.03 RSD 2.8 (n = 3)	1.37 ± 0.03 RSD 2.5 (n = 3)
60	1.28 ± 0.02 RSD 1.3 (n = 3)	1.47 ± 0.03 RSD 2.3 (n = 3)
90	1.39 ± 0.06 RSD 4.9 (n = 3)	1.56 ± 0.02 RSD 1.1 (n = 3)
120	1.39 ± 0.06 RSD 4.9 (n = 3)	1.56 ± 0.02 RSD 1.1 (n = 3)

Tannins can then generate smaller phenolic compounds (pyrogallol, catechol, and ellagic acid) with the known bactericidal activity. Tannins are polyphenolic substances with different molecular weight and a variable complexity [22, 27, 28]. Tannins, the polyphenolic compounds with high molecular weight found naturally in lots of plants proved to protect plants against micro-organisms, unfavorable climatic conditions and animal damage. On the other hand, they can form multiple hydrogen bonds with carboxylic groups of dietary proteins and proteolytic enzymes in the gastrointestinal tract which results to the reduced digestibility of proteins and finally the retardation of animal growth [4, 29]. Tannins have many biologically significant functions, such as protection against oxidative stress, and degenerative diseases [22, 27]. Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose [21, 27]. Rutin is one of bioactive flavonoid compounds found in plants in the considerable amount. The content of total phenolics, tannins, rutin and total flavonoids of the *Cichorium intybus* L. extract and its kinetics are given in Tables 1 and 2 in the annex. Phenolic compounds have such multiple biological effects as anti-atherogenic, antioxidant, anti-inflammatory, cardioprotective, antimicrobial, anticarcinogenic and neuroprotective. Secondary metabolites of plants such as phenolic compounds, terpenoids, alkaloids and lectins have an antimicrobial effect [30–32]

The kinetic study was performed by continuously measuring the absorbency of the extract by the UV–VIS spectrophotometer. The continuous measurement is faster and more accurate for kinetic studies of extraction compared to conventional discontinuous methods. In conventional methods, sampling is manual at given time intervals which is not precise, as there is always a time gap between sampling and analysis, which contributes to errors during kinetic measurements.

In all experiments, the extraction yield was significantly time-dependent and the profile of *Cichorium intybus* L. rises rapidly with time at first, getting less and less quick as the extraction progresses. This behavior can be explained by the fact that during the initial stage of extraction, when the solvent penetrates into the solid, an extremely high concentration gradients develop resulting in higher rates of mass transfer into the liquid phase. As the extraction time increases, the mass transfer of solutes from the solid to the fluid phase gets more difficult, due to the decrease in concentration driving force due to solid and liquid phases. In addition, as the extraction time proceeds, the concentration in the solid phase decreases and both the mixture solubility and the extraction rate decrease simultaneously. In all experiments, a higher extraction yield was reported, especially within 10 to 90 min, with the lower yield from 90 to 120 min.

The analysis of aqueous ethanol extract of *Cichorium intybus* L. for its phytoconstituentsshowed that dry leaves of *Cichorium intybus* L .are rich in total phenols, total flavonoids, rutin and tannins to some extent. It is well known that in general plant flavonoids and phenols act as highly effective free radical scavenging and antioxidants. The phytochemical screening and quantitative estimation of the chemical constituent percentage were evaluated in plants to prove that dry leaves of *Cichorium intybus* L. are rich in rutin and tannins. Phytochemicals, the plant-derived non-nutritive compounds, refer ещ one of different types of alimentary factors which play an important role in various functions of the human body. A great number of natural compounds found in food materials are reported to have antioxidant properties due to hydroxyl groups available in their structure. The antioxidants are the synthetic as well as natural compounds that prevent the oxidative damage to most important macromolecules such as lipids, proteins and nucleic acids present in human body as well as in food products by removal of free radicals generated through various biochemical processes [4, 33]. Free radicals generated through the reaction between oxidative stress radicals and lipids, proteins and nucleic acids cause apoptosis stimulation resulting in various neurological, cardiovascular and some other physiological disorders [4, 34].

CONCLUSION

In conclusion, the results of this research showed that total phenolic, total flavonoid, rutin and tannin contents are significant components in 50% aqueous ethanol extract of dry leaves of *Cichorium intybus* L. grown in Bulgaria. Extracts positively correlated with their phenolic contents and flavonoids contents, and rutin and tannins respectively. Therefore, this complex of phytochemical active substances in dry leaves of *Cichorium intybus* L. offers various fields of prospective applications in herbal medicine and nutrition for healthy food production.

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EVALUATION OF ANTIMUTAGENIC AND ANTIFUNGAL PROPERTIES, PARAMETERS OF ACUTE TOXICITY AND SENSITIZING ACTIVITY OF ENZYMATIC WHEY PROTEIN HYDROLYSATE

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Abstract: Biologically active peptides with antioxidative, antibacterial, immunomodulating and other properties result from the reaction between the whey proteins and proteolytic enzymes of the gastrointestinal tract or purified proteases. This work aims to determine antimutagenic and antifungal effect of the enzymatic hydrolysate of whey protein obtained, to assess its acute toxicity characteristics and sensitizing power. Antimutagenic action of native whey proteins and hydrolysates (test sample and hydrolyzate analog) was assessed by the Ames test using indicator strains of *Salmonella typhimurium* TA 98 and TA 100. When determining the antifungal activity, opportunistic strains of *Aspergillus niger* and *Candida albicans* were used. The toxicity degree of samples was defined in studies to evaluate the acute intragastric toxicity in white rats as well as in the single abdominal dose study on the white mice. Irritating influence of whey proteins and peptides on was evaluated when applied to the eye mucosa of rabbits. The sensitizing capacity of samples was evaluated using the experimental model to reproduce the delayed hyperresponsiveness in white mice. It is identified that the developed hydrolisate is classified as the safe agent and has low sensitizing ability. The sample obtained has the comparative values of antioxidant and antimutagenic activity level as compared with the analog "Vital Armor H 801 LB" (Armor Protéines, France) used to manufacture functional products. The advantages of the hydrolisate developed include the increase in the content of peptide fraction and more pronounced antifungal activity towards *A. niger*.

Keywords: whey proteins, enzymatic protein hydrolisates, peptide profile, antimutagenic activity, antifungal effect, antioxidant properties, acute toxicity, sensitizing activity

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INTRODUCTION

Milk proteins (casein and whey fractions) are the precursors of the wide range of biologically active peptides with the immunomodulating, antioxidant, antimutagenic (gene protector), hypotensive, antimicrobial, antiviral, antitumoral effect [1, 2].

Genotoxicity is identified as the general biologic feature of various factors to cause the destructing effect to the genetic structures of the body. Search of natural antimutagens is advantageous when they are able to prevent the genotoxic effect of environmental factors on the human genetic structures [3]. Antimutagenesis is the biological process that suppresses the mutation characterized in the reduction of spontaneous and induced mutation affected by natural and synthetic compounds [4]. Most data on the effect of biologically active compounds on spontaneous and induced mutagenesis are obtained from organism studies. The Ames test is widely applied in the laboratory practice to evaluate the mutagenic features of chemicals, pharmaceutical agents, environmental medium. The test is based on the count of reverse mutations to prototrophy in histidine for *Salmonella typhimurium* strain [5, 6].

It is known that alimentary proteins and peptides are of great significance for anti-infectious defense mechanism that is caused by the stimulated immune system and inhibition of the growth of pathogenic and opportunistic pathogens (antimicrobial and antifungal effects) [7]. In general, antimicrobial peptides are

positively charged; they have amphiphilic properties and specific secondary structure [8]. The micro-organism sensitivity relates to membranous components that contribute to peptide transport through the outer membrane. However, destruction of the outer membrane structure is not the major factor resulting in the death of microbial cell. Peptides penetrate the outer and cytoplasmic membranes and cause antimicrobial effect resulting from the multifactor mechanism including the impact on several intracellular ion targets [9].

To obtain enzymatic hydrolisates with preset parameters (peptide profile, biological activity), various endopeptidases and exopeptidases, of which the enzymes of microbial (alcalase, neutrase), plant (papin, ficine) and animal (pepsine, trypsin) origin may be isolated [10]. Distinctive features of enzymatic segregation of protein substrates are defined by the best efficient conditions for catalytic activity of the enzyme, its substrate and site specificity, physical and chemical parameters of proteins segregated.

Urgency of studies is associated with the demand in improvement of processes to prepare enzymatic hydrolisates of milk proteins with the required physical and chemical parameters and biologically active parameters for functional food products.

The scientific novelty lies in identification of new data on antifungal and antimutagenic effect of certain hydrolisates of serum proteins, as well as in the use of comprehensive approach to specify the correlation between the physical and chemical parameters and biologically active properties (degree of hydrolysis, antioxidant and antimutagenic activity) of native whey proteins and products of their hydrolysis.

The study was aimed to describe the biologically active properties (antimutagenic and antifungal effects) of the enzymatic hydrolisate of serum proteins obtained, define parameters of its acute toxicity and senstizing power.

OBJECTS AND METHODS OF STUDY

Production of enzymatic hydrolisate of whey proteins

The whey protein concentrate was used for the enzymatic hydrolysis obtained by ultrafiltration (WPC–UF–80, TU BY 100377914.550–2008) with protein content equal to 80 %, and the serine protease (alcalase, EC 3.4.21.62, protease from *Bacillus licheniformis*, with 2.64 U/g activity; Sigma, USA). To obtain the test hydrolisate sample, 8 % of WPC–UF–80 solution was prepared; the protein substrate was thermally treated and cooled to the temperature best suitable for hydrolysis. The enzymatic agent was added to the heat-treated solution obtained; then, the solution was hydrolised in temperature-controlled conditions. Upon the proteolysis completion, the enzyme was heat-inactivated; the liquid hydrolisate obtained was then allowed to dry (procedure as per [11]). The Table 1 shows characteristics of organoleptic and physical and chemical parameters of the hydrolisate obtained [12].

Table 1. Organoleptic and physical and chemical parameters of whey protein hydrolysate

Parameter	Parameter value
Appearance and consistency	Powder, yellow to creamy
Flavor and odor	Flavor typical to milk Poor bitter milk taste
Solubility	Water-soluble
Active acidity, pH (1 % solution)	6.7
Total protein w/w%	80.0
Peptide profile: Fragments with molecular mass > 10 kDa, %	2*
Native whey proteins	Not detected**
Residual antigenicity, 10^{-3} per unit value.	1.22 ± 0.07
Antioxidative activity, μmol of trolox / mg of protein	0.551 ± 0.035

Note. * − Parameter values are found through determination of general nitrogen in the hydrolisate and ultrafiltrate obtained by using Amicon Ultra–4 10K filters (Millipore, USA) with 10 kDa permeability. ** − As per SDS electrophoresis-data, HPLC and mass spectrometry [12].

Analysis of bioactive properties of the obtained hydrolisate

Method to determine the antimutagenic activity of enzymatic hydrolisates of whey proteins (as per requirements [13]). In the course of short-term analysis to define antimutagenic properties, indicator strains *Salmonella typhimurium* TA 98 and TA 100 (Ames test) were used. These strains are auxotroph mutants for histidine. Antimutagenic effect in the test preparation is identified by the reduction in frequency of reverse mutations from auxotrophy in histidine to prototrophy. In this analysis, positive results indicate that the test compound induces gene mutations of base pair replacement (for TA 100 strain) or reading frame shift (for TA 98 strain). Negative results indicate that the test compound has no mutagenic effect in these conditions on *S. typhimurium* strains used. Antimutagenic activity in native whey proteins (WPC) and the hydrolysate obtained was assessed. The whey protein hydrolisate "Vital Armor H 801 LB" (Armor Protéines, France) was used as the reference sample.

Test samples (WPC and hydrolysates) were assessed for antimutagenic properties when the following was added to the mutagen test-system in the volume of 10 μg/plate: etidium bromide (when exposed to TA 98 strain) and sodium azide (when exposed to

TA 100). The quantity of revertants is obtained when 1.88–30 μg of test compounds was added to the plate; 3 plates were used for each control and test samples.

Culture media components: microbiologic agar-agar (3AO "Pyat' okeanov" CJSC, RF), glucose (as per GOST 6038–79), beef-extract broth (as per GOST 20730–75), beef-extract agar (as per GOST 29112–91), trisodium citrate (as per GOST 31227–2004), $K_2HPO_4 \times 3H_2O$ (as per GOST 2493–75), KH_2PO_4 (as per GOST 4198–75), $(NH_4)_2SO_4$ (as per GOST 9027–82), $MgSO_4 \times 7H_2O$ (as per GOST 20490–75), NaC1 (as per GOST 4233–77), KCl (as per GOST 4234–77); biotin, histidine, L-tryptophan, sodium azide, etidium bromide (Merck, USA).

The standard microbiological laboratory equipment was used for the study purposes as follows: refrigerated heating circulator, electronic laboratory balance, control water bath GFL 1031 (GFL, Germany), pH–meter Hanna pH–211 (Hanna Instruments, Germany), thermal hygrometer IVA–6 (Scientific-Industrial Complex "Mikrofor", RF), Vortex mixer (IKA, Germany). Test conditions: temperature – 21–22ºC, humidity – 63–67%, pressure – 737–742 Hg mm.

Media and solutions used. All media and solutions were made using the distilled water. Upon preparation, they were autoclaved. Beef-extract agar containing 0.6%, 1.5% and 2% agar: 6, 15 and 20 g of agar, accordingly, are added to 1 L of the beef-extract broth. Aqueous agar (2 %): 20 g of agar is dissolved in 1000 ml of water. Evaporate concentrate: 2.0 g of trisodium citrate, 42.0 g of $K_2HPO_4 \times 3H_2O$, 18 g of KH_2PO_4, 4.0 g of $(NH_4)_2SO_4$ are diluted with water to make up 1 L (pH 7.2). Glucose solution (20%): 200 g of D-glucose is dissolved in 800 ml of water. The minimal medium is prepared based on the evaporate concentrate that is 4-fold diluted with water. Seletive agar to assess mutagenesis: 300 ml of aqueous agar, 100 ml of evaporate concentrate, 10 ml of glucose solution and 2 ml of 2% aqueous liquid of $MgSO_4 \times 7H_2O$ are mixed. Semiliquid minimal agar (0.7%): 7 g of agar and 6 g of NaC1 are diluted with water to make up 1 L. Histidine solution (0.5 mmol): 9.6 mg of histidine is dissolved in water to make up a final volume of 100 ml. Biotin solution (0.25 mmol): 6.1 mg of biotin is dissolved in water to make up a final volume of 100 ml. Surface semi-liquid semi-enriched agar: 80 ml of semi-liquid minimal agar (0.7%), 10 ml of histidine solution and 10 ml of biotin solution were mixed well. The mixture is poured in 2 ml tubes. Potash chloride solution (0.15 mol): 11.5 g of KCl is diluted with water to make up 1 L.

Test description (without metabolic activation). 0.1 ml of test agents (WPC solutions and hydrolysates with the protein concentration 18.8–300 μg/ml) is put in tubes with agar. Then, 0.1 ml of bacteria suspension, positive control (mutagen solution) are added, quickly mixed in the mixer and poured out over the layer of the bottom minimal agar in Petri dish. An even and thin layer of the semi-liquid agar covers the surface of the bottom agar. The Petri dish is kept at the room temperature for 30−40 min and thermostated, upon full jelling, at 37°C. The results are read in 48 h upon incubation. The relevant volume of the solvent (physiological solution) is added into the layer of the top semi-liquid agar with the bacteria suspension for the control sample. The test is proved with positive control. 3 replications are made for each control and test samples.

Decrease of mutation level (I_m, %) was assessed by the formula:

$$I_m = 100 - \frac{N_1}{N_2} \times 100,$$

where N_1 is the number of revertants in an experiment, N_2 is the number of revertants in positive control.

Statistic result analysis. To validate test results, calculations were made by the method of Dunnett's multiple comparisons [14]. The significance of revertants reduced in number is assessed in test versus control samples. The efficiency and sustainability of the method of Dunnett's multiple comparisons versus pair-wise comparison method for test and control samples are proved by the reduction in the rate of false-positive and false-negative results recorded, since the type I error probability (usually 5%) may be specified for the entire test by this method but not only for selected comparison. This approach does not consider using experiments with zero value of revertant number when testing. This is feasible in practice when handling substances with bactericidal effect, or in case of procedure error.

Method to determine antimicrobial (antifungal) activity of whey proteins and peptides. Opportunistic strains *Aspergillus niger* and *Candida albicans*, obtained from the bank of the Educational Institution “Belorusian State Medical University” as the test subject.

To prepare culture media, pancreatic casein hydrolysate (as per TU (Standard Specifications) 9385–002–00479327–94), peptone for bacteriological culture media (manufactured by the “Pharmacotherapy Research and Development Center” CJSC, Russia), glucose (as per GOST 6038–79), dry nutrient broth for microorganism cultivation (GRM-broth based on pancreatic hydrolysate of fish meal, as per TU 9398–021–78095326–2006) and microbiological agar-agar (manufactured by "Pyat' okeanov" CJSC, Russia).

The test sample of the milk whey protein hydrolysate was assessed for antimicrobial properties. Whey protein concentrate (WPC–UF–80, TU BY 100377914.550–2008; primary substrate to obtain hydrolysates) was used as the reference sample. Whey protein hydrolysate "Vital Armor H 801 LB" (Armor Protéines, France) was used as the standard.

Media and solutions used. Opportunistic fungal pathogenes (*A. niger* and *C. albicans*) were cultured on the agarized native culture media (Saburo and FMH-medium) in the course of the study to assess he antifungal effect. To obtain Saburo medium, 0.5 g of pancreatic casein hydrolysate, 0.5 g of peptone, 2 g of glucose and 1.5 g of agar-agar were dissolved in 100 ml of distilled water. To prepare the medium based on pancreatic hydrolysate of fish meal, 3.8 g of dry nutrient broth to cultivate microorganisms and 1.7 g of

agar-agar were dissolved in 100 ml of distilled water. The active acidity of solutions was made up to 7.0 pH and then, the solutions were autoclaved at 0.5 atm for 30 minutes (FMH-medium) and 60 minutes (Saburo).

WPC solutions and whey protein hydrolysates were used in the physiological solution with the protein original concentration of 50 mg/ml. To eliminate microbiological contamination, the solutions obtained were twice filtered using polypropilene filters (Rolitabo®-syringe filters, Ø 25 mm, 0.45 μm; Carl Roth, Germany).

Test description. Mycelium fragment *A. niger* or *C. albicans* was cultured in Petri dishes with the culture medium (Saburo or fish hydrolysate-based medium), containing 5.0 mg/ml of WPC and whey protein hydrolysates. Then, they were cultivated in the thermostat at 37 ºC for 4 days. Petri dishes with the medium without the tested protein component (WPC and whey protein hydrolysates) were used as the reference. Three Petri dishes were used for each control and test samples. The degree of inhibition (*DI*, %) of the mycelium growth was assessed by the formula:

$$DI = 100 - \frac{d}{d_k} \times 100,$$

where d is the arithmetic mean value of the fungal mycelium diameter of the test sample (with WPC and whey protein hydrolysates added), n = 3; d_k is the arithmetic mean value of the fungal mycelium diameter of the control sample, n = 3.

The results of quantitative test data processing are shown as the arithmetic mean value of inhibition (*I*, %), calculated for three independent experiments.

The toxicological-hygienic assessment of WPC and enzymatic whey proteins hydrolysate (EWPH). The degree of toxicity effect of native whey proteins and products of their enzymatic hydrolysis was determined in tests on the white rats and mice. The irritating effect of WPC and EWPH on eye mucosa of rabbits was evaluated. The sensitizing effect of samples was analysed using the experimental model to reproduce the delayed hyperresponsiveness in white mice. Experiments were made to comply with requirements of technological regulations and using new practices and customization of existing ones [15–17]. Toxicological and hygienic study of submitted samples was performed on laboratory animals as follow: white mice, non-linear male and female white rats (baseline body weight is 180–220 g) and albino rabbits (2500–3000 g) supplied from the vivarium of the Republican unitary enterprise "Applied Research Centre of Hygiene". The study design, scope and methods are shown in the Table 2.

Exposure groups were randomly formed in view of the body weight as the key factor (the difference in the body weight was ≤ 10%). During experiments, the animal health state was assessed on a daily basis, as well as the rate of food and water consumption. Toxicological and hygienic studies were performed as per requirements of the Instruction 1.1.11-12-35-2004 [15].

Graph plotting and mathematical treatment of study results were made via the Microsoft Office Excel 2003 (Microsoft Corporation, USA) software. The statistic data was processed using the Student t-test [14] and *X*-Van der Waerden Criteria [18].

RESULTS AND DISCUSSION

Evaluation of antimutagenic activity of enzymatic hydrolysates of whey proteins. Studies were performed to evaluate antimutagenic properties of the test sample of whey protein hydrolisates (WPH) and the analogue "Vital Armor H 801 LB". The required condition to process results of this study was the mutagenic effect available in positive control samples for all test strains (*S. typhimurium* TA 98 and TA 100). As per the results of study data processing by the method of Dunnett's multiple comparisons, the specified values in the number of revertants in the control and test samples were statistically valid.

Statistically significant reduction in the induced mutation for all study variations was reported in the test sample of the WPC hydrolysate and "Vital Armor H 801 LB". Differences in the number of revertant in the control and test samples revealed were statistically valid ($p < 0.05$) when 1.88–30 μg of hydrolysate samples were added to the test system which is shown in Tables 3–6.

Table 2. Design and scope of toxicological-hygienic studies

Name of the test and its design	Animal species	Test methods (observation period)
Determination of toxicity and hazard parameters of the medicinal product at a single intragastric dose administered by animal subjects.	Rats (*n* = 18)	Records of clinical signs of toxication and test animal survival (14 days)
Study of the drug toxicity parameters at a single dose administered by animals intraperitoneally	Mice (*n* = 24)	Records of clinical signs of toxication and test animal survival (14 days)
Single-dose evaluation of the local irritation of the drug for 4-hours at 20 mg/cm^2 (application area is 16 cm^2) on the animal back skin	Rats (*n* = 12)	Control of clinical signs of intoxication and cutaneous skin condition (4 h, 24 h, 10 days). Evaluation of the skin fitness state by the erythema intensity and edema size
The study of the drug action on the mucous membranes and the visual organ; 20% single dose administration of the drug to the lower conjunctival fornix of the tested eye of the animal	Rabbits (*n* = 6)	Visual monitoring of the eye mucosa and conjunctiva (14 days)

Table 3. Statistical evaluation of antimutagenic activity of the hydrolysate test sample by the Ames test performed on the strain *S. typhimurium* TA 98

Sample volume, μg per plate	Quantity of revertants, $x_{cp} \pm \sigma$	Decrease of mutation level, %
30	97 ± 6	49.2
15	105 ± 12	45.0
7.5	116 ± 5	39.3
3.75	147 ± 12	23.0
1.88	161 ± 33	15.7
0	21 ± 2	–
Positive control	191 ± 12	–

Note. Mutagen – ethidium bromide, 10 μg per plate. The strain response to mutagens was within normal limits.

Table 4. Statistical evaluation of antimutagenic activity of the hydrolysate test sample by the Ames test on the strain *S. typhimurium* TA 100

Sample volume, μg per plate	Quantity of revertants, $x_{cp} \pm \sigma$	Decrease of mutation level, %
30	346 ± 11	52.1
15	368 ± 25	49.0
7.5	454 ± 61	37.1
3.75	541 ± 52	25.1
1.88	586 ± 53	18.8
0	98 ± 2	–
Positive control	722 ± 75	–

Note. Mutagen– sodium azide, 10 μg per plate. The strain response to mutagens was within normal limits.

Table 5. Statistical evaluation of antimutagenic activity of the hydrolysate "Vital Armor H 801 LB" sample by the Ames test on the strain *S. typhimurium* TA 98

Sample volume, μg per plate	Quantity of revertants, $x_{cp} \pm \sigma$	Decrease of mutation level, %
30	105 ± 9	45.0
15	114 ± 12	40.3
7.5	122 ± 17	36.1
3.75	143 ± 21	25.1
1.88	155 ± 15	18.8
0	27 ± 7	–
Positive control	191 ± 12	–

Note. Mutagen – ethidium bromide, 10 μg per plate. The strain response to mutagens was within normal limits.

Table 6. Statistical evaluation of antimutagenic activity of the "Vital Armor H 801 LB" hydrolysate by the Ames test using the strain *S. typhimurium* TA 100

Sample volume, μg per plate	Quantity of revertants, $x_{cp} \pm \sigma$	Decrease of mutation level, %
30	375 ± 29	48.1
15	425 ± 41	41.1
7.5	455 ± 56	37.0
3.75	505 ± 32	30.1
1.88	570 ± 89	21.1
0	98 ± 2	–
Positive control	722 ± 75	–

Note. Mutagen – sodium azide, 10 μg per plate. The strain response to mutagens was within normal limits.

The effect of mutation level reduction is more evident in studies of the WPC hydrolysate test sample which made 15.7–49.2% for the strain *S. typhimurium* TA 98 and 18.8–52.1% for the strain TA 100. When using the "Vital Armor H 801 LB" hydrolysate, the effect of induced mutation reduction reached 18.8–45.0% when the strain *S. typhimurium* TA 98 was tested and 21.1–48.1% – when the strain TA 100 was tested.

As per experimental data, the antimutagenic activity of WPC was not revealed within the tested concentrations via the model test using strains *S. typhimurium* TA 98 and TA 100. The results obtained likely relate to physical and chemical

properties of proteic macromolecules, and in particular, to inaccessibility to penetrate the cellular membrane of auxotroph strains.

The studies were further conducted to evaluate **antifungal properties of peptides of the cow milk whey proteins** when opportunistic fungi strains *Aspergillus niger* and *Candida albicans* are exposed.

A. niger is the species of mold fungi of the genus *Aspergillus* (or black mould). The long-term exposure to antigens *A. niger* induces the allergic reaction resulting in allergic rinities, allergic-bronchopulmonary aspergillosis or bronchial asthma. The pathogenicity of *Aspergillus* spp. is related to heterotrophy and synthesis of amylolytic, proteolytic, lipolytic and other enzymes. *C. albicans* is the yeast-like fungi that produce pseudomycelium. *Candida* species are the part of the normal microflora in most healthy humans (80%). The disease is induced through intense propagation and/or intrusion of more pathogenic fungal strains. Pathogenic factors in fungi of genus *Candida* include the secretion of proteolytic enzyme and haemolysins, dermato-necrotic activity and adhesiveness [19].

The effect of whey proteins and peptides on the growth of opportunistic microorganism *A. niger* is studied when cultured on the agar culture media (Saburo and FMH-medium) containing 5.0 mg/ml of WPC and hydrolysates. Since the *C. albicans* pathogen is more demanding to the culture media components, it was cultured on the Saburo agar medium. The concentration of tested compounds (WPC and hydrolysates of whey proteins) was determined to comply with that the Saburo medium contains 5.0 mg/ml of the enzymatic casein hydrolysate and 5.0 mg/ml of the peptone as the source of nitrogen compound. FMH-medium includes the pancreatic fish meal hydrolysate as the protein component. So, the culture media used contain the protein substrate of various origin (milk protein hydrolysates, in particular, casein and animal meat, as well as the fish meal hydrolysate). Test samples added resulted in supplementary enrichment of culture media with milk whey proteins (WPC) and their hydrolysates.

Fig. 1 and 2 show results of the test to culture *A. niger* on the agarized culture media (Saburo and FMH-medium) containing WPC and whey protein hydrolysates. Suppression of the *A. niger* mycelium is reported when the experimental sample of the hydrolysate and "Vital Armor H 801 LB" is added when both culture media are used. At the same time, the *A. niger* mycelium more intensely grows when the fungi is cultivated on culture media with WPC added.

The experimental data obtained show that specific peptides with antagonist properties are available in the tested hydrolysates of whey proteins. The quantitative degree of inhibition (*DI*, %) of the *A. niger* mycelium was assessed by cultivation in Petri dishes with FMH-medium. This medium was chosen since it does not have the milk protein hydrolysates (casein) as a component and ensures lower rate of mycelium growth where the results are better reproduced. The degree of the *A. niger* mycelium inhibition, when it is added to the FMH-medium of the experimental sample of whey protein hydrolysates dosed at 5.0 mg/ml was about 25% and 11% when the "Vital Armor H 801 LB" sample is added.

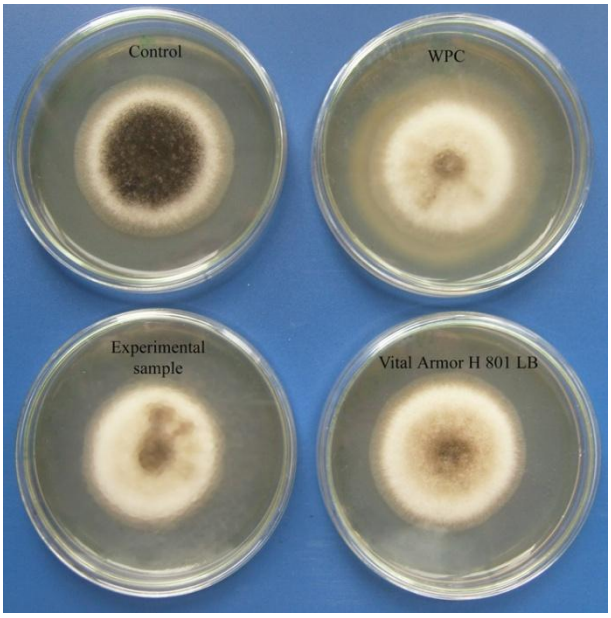

Fig. 1. Antifungal activity evaluation of whey proteins (WPC) and hydrolysates (experimental sample and "Vital Armor H 801 LB") in the volume of 5.0 mg/ml against the opportunistic fungi *A. niger* when cultivated on the agarized Saburo medium.

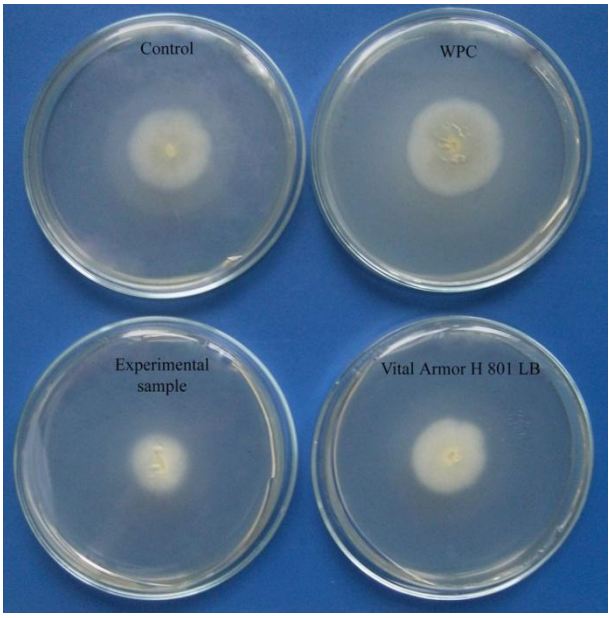

Fig. 2. Antifungal activity evaluation of whey proteins (WPC) and hydrolysates (experimental sample and "Vital Armor H 801 LB") of 5.0 mg/ml against the opportunistic fungi *A. niger* when cultivated on the agarized FMH-medium.

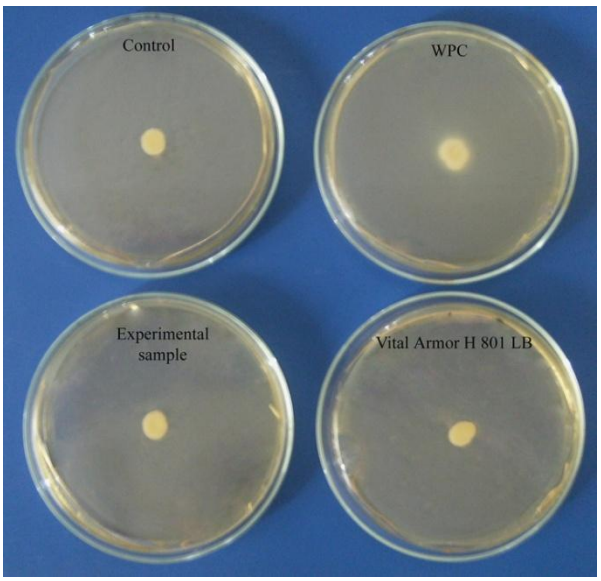

Fig. 3. Antifungal activity evaluation of whey proteins (WPC) and hydrolysates (experimental sample and "Vital Armor H 801 LB") at the level of 5.0 mg/ml against the opportunistic fungi *C. albicans* when cultivated on the agarized Saburo medium.

The Fig. 3 shows the test results of *C. albicans* cultivation on the agarized FMH-medium containing WPC and whey protein hydrolysates. Significant differences of the *C. albicans* mycelium diameter in control and test samples (when added to the test system of hydrolysate samples) are not determined. At the same time, the mycelium growth stimulation was induced by adding the native whey proteins (WPC) to the culture medium. Thus, the whey protein peptides available in the culture media did not cause any antagonistic effect on the growth of the opportunistic *C. albicans*.

The works by N. Nandhini et al. (2015) [20] with the use of whey proteins also show the varied level of antibacterial and antifungal activity when a range of opportunistic microorganisms are used. Particularly, as per the tested inhibition zone, the larger inhibition zone was reported in studies where *A. niger* was used rather than *C. albicans.*

Comparative characteristics of physical, chemical and bioactive properties of the obtained whey protein hydrolysate and "Vital Armor H 801 LB" sample.

The physical, chemical and bioactive properties of the hydrolysate test sample and its analog are given in the Table 7. Reference samples are consistent by the degree of substrate cleavage (AN/TN). In addition, the quantity of low-molecular proteic fraction (mr < 10 kDa) in the experimental hydrolysate sample is 11% greater than in the "Vital Armor H 801 LB" sample.

At the hydrolysis degree of 15.5 ± 0.6% (test sample) and 16 % ("Vital Armor H 801 LB"), the antiradical activity of proteic components tested reaches 0.551 ± 0.035 and 0.618 ± 0.001 μmol/mg, accordingly. So, the enzymatic hydrolysis of the whey proteins was responsible for 3.0−3.6 times increase in the anti-radical activity of enzymatic hydrolysates (Table 7).

At the same time, the reduction in the mutation level in experiments with the test sample of WPC hydrolysate was 15.7–49.2% for the *S. typhimurium* TA 98 strain and 18.8–52.1% for the strain TA 100. For the Vital Armor H 801 LB hydrolysate, the effect of the induced mutation decrease was less to some extent: 18.8–45.0% when tested on the *S. typhimurium* TA 98 and 21.1–48.1% – on the strain TA 100.

Both hydrolysates showed antagonistic activity against the opportunistic fungi *A. niger*. The experimental hydrolysate sample (25%) has more evident antifugal effect.

Overall, the comparable values of protein substrat hydrolysis degree, the level of antioxydant and antimutagenic activity are reported for the test sample of the partial whey protein hysrolysate and "Vital Armor H 801 LB" that is currently used for functional food production. The test hydrolysate sample has the advantage to increase the content of the peptide fraction and more evident antifungal activity against *A. niger*.

Table 7. Characteristics of partial hydrolysates of whey proteins

Name of hydrolysate	Peptide profile	Ratio of α-amino nitrogen and total nitrogen, AN/TN, %	TEAC, μmol trolox/mg of protein **	Antimutagenic activity (TA 98/ TA 100 strains), %	Antifungal activity, %
Experimental sample of hydrolysate	≤10 kDa, 98%	15.5 ± 0.6	0.551 ± 0.035	15.7–49.2 / 18.8–52.1	25
"Vital Armor H 801 LB"	<10 kDa, 87% *	16 *	0.618 ± 0.001	18.8–45.0 / 21.1–48.1	11

Note. * – parameters are shown as per manufacturer's data, ** – as per the study results as described in the article [12].

Parameters of acute toxicity and sensitizing activity of obtained hydrolysate are determined at the following study stage.

Acute toxicity parameters (median lethal dose, LD_{50}) of WPC and EWPH are determined on the white rats. Each group is of 6 animals. To evaluate the acute intragastric toxicity, the white rats received drug samples under fasting condition in 20%-aqueous solutions (by protein)- injected in the stomach with the needle probe in the maximum permissible volume (3.0 ml per 200 g of body weight). Control animals received the water in equivalent volume. The test

animals were monitored for 14 days since the date of experiment. High doses of drugs did not result in any ataxia, adynamia, clinical and tonic contractions, and paralysis in animals. The maximum dose of drugs did not result in the animal death. It is established WPC and EWPH refer to low-hazard chemical compounds (Hazard class 4 – as per GOST 12.1.007–76) by the parameters of acute intragastric toxicity.

The irritative effect of WPC and EWPH on the eye mucosa of test animals was evaluated. Two drops of 20 %-solution of WPC (by protein) and its hydrolysate instilled in the lower conjunctival fornix of the right "test" eye of animals (the distilled water was instilled in the left control eye of animals in the equivalent volume) were followed by the intense winking and lacrimation for 10–15 min, and weak mucosa irritation. The irritation effect in all animals disappeared in 1 hour upon instillation. Thus, the single exposure of tested drugs as 20% solutions to the eye mucosa - hardly resulted in the irritation.

Parameters of acute toxicity of WPC and EWPH were analyzed at a single abdominal dose administered by the white mice of both genders. Thee groups (8 animals per group) were formed for the study purpose. The control mice administered the drug in the highest possible dose of LD_{50} (3784 ± 17.0 mg (protein)/kg for EWPH, 3265 ± 34.3 mg (protein)/kg for WPC), control animals received the physiological solution. Toxication signs disappeared by the end of the first study day; the general health state of animals, their behavioral pattern, need in food and appearance did not differ from those in control animals during the 2-week observation period. Thus, as per the median lethal doses, the test drugs may be classified as relatively safe (> 3000 mg/kg at intraperitoneal administration).

The irritative effect of WPC and EWPH at a single exposure on the intact cutaneous skin of test animals was studied. A single dose of 20%- drug solutions (by protein) applied to the chipped skin of the white rack back (6 animals per group) at 20 mg/cm^2 did not result in any visible signs of intoxication of animals within 4 hours and their death within 10 days. Cutaneous skin irritations and infection were not reported in application sites.

A comparative study of the WPC and EWPH sensitizing property was performed (as per [15, 16]) on the experimental model of delayed hypersensitivity reproduction in white mice. WPC solution (1-st test group) and EWPH solution (2-nd test group) were intradermally injected to the white mice at the tail base at a single dose of 300 μg (in terms of protein) in the 1 : 1 mixture with the complete Freund's adjunct at 0.06 cm^3 per animal. Control group animals received the mixture of the physiological solution and complete Freund's adjunct (CFA). Sensibilization by the delayed hypersensitivity reproduction was defined on the 6-th day of the test by using the provocative test – paw swelling intracutaneous test. The test was performed by injection of the sample dosed at 400 μg (at the volume of 0.04 cm^3) in the pad of the hind feet (under the aponeuroses) of test and control animals. The results of paw swelling intracutaneous test were evaluated by the difference in thickness of the test paw of animals in both test and control groups using the micrometer prior to and in 24 hours upon injection on the provocative test site with the rate of accuracy up to 0.01 mm. The paw swelling intracutaneous test absolute index per each animal was expressed in 10^{-2} mm. To evaluate the sensibilization intensity, the paw swelling intracutaneous test results was scored [17].

The WPC sample showed the strong sensitizing property (antigenic activity class 1) since it caused induction in the delayed hypersensitivity reproduction in more than 75% of test animals with significant differences of relative paw swelling intracutaneous test indexes in the test and control animals a s per *X*-criterion ($p < 0.01$) (Table 8).

Table 8. Delayed hypersensitivity reproduction indexes in the white mice upon administration of the sample of native whey proteins (WPC) and its enzymatic hydrolysate (EWPH)

Parameter	Unit of measurement	Control group (n = 12)	Test group (n = 12)
Paw swelling intracutaneous test results upon intracutaneous WPC testing (test group 1):			
absolute values	10^{-2} mm	6.33 ± 1.41	18.8 ± 1.96 *
	t-criterium	–	5.15
relative values	H	1/12	12/12
	Score	0.08 ± 0.08	1.58 ± 0.19 *$^{+}$
	t-criterium	–	7.14
	X-criterium	–	8.43
Paw swelling intracutaneous test results upon intracutaneous EWPH testing (test group 2):			
absolute values	10^{-2} mm	5.75 ± 1.39	7.67 ± 1.60
	t- criterium	–	0.91
relative values	H	1/12	5/12
	Score	0.08 ± 0.08	0.42 ± 0.15 ^{0}
	t-criterium	–	1.96
	X-criterium	–	3.06

Note. Significant differences as compared with control values by t-criterion (* – $p < 0.001$; ^{0} – $p < 0.1$) and X-criterion ($^{+}$ – $p < 0.05$). Data on the number of species with positive results and total number of animals used in the study are given in slash.

A weaker induction in the delayed hypersensitivity reproduction (≤1 point) is reported in 5 of 12 animals sensitized with enzymatic WPH. The value of the provocative test integral indicator was 5.2 times higher than the control results though the difference in between statistically tended to increase in terms of *t*-criterion ($p < 0.1$) based on which the hydrolysate sample was classified as the agent with the weak sensitizing property (Class 4).

CONCLUSION

Bioactive properties (antimutagenic, antioxidant and antifungal effect) of the enzymatic whey protein hydrolysate are described. The comparable values of protein substrate hydrolysis intensity, the level of antioxidant and antimutagenic activity are given for the test hydrolysate sample and "Vital Armor H 801 LB" (Armor Protéines, France), used for functional food production. With 15–16% hydrolysis, the antiradical activity of these protein components is comparable and reaches 0.551–0.618 TEAC units. Overall, the enzymatic hydrolysis of the milk whey proteins caused an increase in antiradical properties of peptide fraction which is 3.0−3.6 times higher. When tested, a decrease in the mutation level of the test hydrolysate sample was 15.7–49.2% in the test system for *S. typhimurium* TA 98 and 18.8–52.1% for the TA 100 strain. When the foreign analog "Vital Armor H 801 LB" was tested, the reduction effect of the induced mutation reached 18.8–45.0% when the *S. typhimurium* TA 98 strain was tested and 21.1–48.1% – when the TA 100 strain was tested which is lower than values typical for the test hydrolysate sample. Antifungal property study showed that when the obtained enzymatic hydrolysate of whey proteins and the sample of "Vital Armor H 801 LB" dosed at 5.0 mg/ml were added to the culture medium, they suppressed the growth of opportunistic fungi *A. niger* mycelium for 25 and 11%, accordingly. The effect of tested hydrolysate samples on the suppression of *C. albicans* mycelium growth is not determined. At the same time, when native whey proteins (WPC) were cultured on the culture medium, they stimulated the growth of these microorganisms. The test hydrolysate sample benefits to increase the content of peptide fractions and have more evident antifungal effect against the opportunistic pathogen *A. niger*.

It is established in studies to evaluate the acute toxicity and sensitizing activity parameters on animal models that the obtained enzymatic hydrolysate of whey proteins is classified as the safe agent and has low sensitizing property. The developed partial hydrolysate is proposed to be used as the proteic component with the specified peptide structure and bioactive properties to manufacture specialized food.

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FUNCTIONAL TECHNOLOGICAL PROPERTIES AND ELECTROPHORETIC COMPOSITION OF MODIFIED WHEAT GLUTEN

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Abstract: This article provides data on correlation between functional technological properties of native and modified wheat gluten and its specific molecular weights, with an objective to develop control methods for adjustment of physical and chemical specifications of protein products. We used methods for chemical composition analysis in protein products, protein electrophoresis (PAGE), and DWG modifications. We used enzymatic preparations (EP) for DWG properties modification: endoprotease EP (Protamex®) and Flavourzyme 500 MG, which contains both endoprotease and endopeptidase simultaneously. It is shown that native DWG underperforms in its functional technological properties in comparison to sodium caseinate, soy flour, soy concentrate, and egg albumin, therefore its properties are modified by limited proteolysis with protein hydrolysis degree of 1.10–3.41%. Our findings indicate that hydrolysis duration might be used to control DWG properties: to increase solubility, foam forming capacity (FFS) up to the respective values demonstrated by egg albumin, and at the same time, to reduce water- and fat-binding capacity and fat emulsifying capacity. DWG with improved FFS contains single-chain polypeptides, both with low molecular weight (ME) (under 40 kDa), and with medium ME (40–60 kDa). Among multi-chain peptides with more pronounced foam-forming capacity, presence of single-chain peptides with low ME (12–16 kDa) seems more preferable than polypeptides with medium (27–39 kDa) and high ME (69–108 kDa). Revealed regularities in correlation of DWG functional properties and ME / composition specificity are intended to be used in DWG modification for further various applications in food industry, mostly for pastries production.

Keywords: dried wheat gluten, modification, proteolysis, electrophoresis, functional technological properties

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INTRODUCTION

Protein products of plant and animal origin are extensively used in food industry due to an ample array of functional technological properties (FTP) they possess, such as solubility, water and oil binding capacity, capacity to create stable emulsions, foams, etc. [1]. Proper FTP is a prerequisite for optimal quality indicators, as well as for enhancing nutritive and biological value of food products. Dried wheat gluten (DWG) is a byproduct of wheat starch manufacture, and it definitely requires a wider scope of utilisation. Nowadays, DWG is mostly used in flour milling and bread baking industry [2, 3]. First benefit of DWG is its natural origin, as this product is obtained from cereals by washing them with water without any chemicals involved or any demanding process requirements; the second benefit that should be mentioned is its unique technological properties, with no equivalent substitution in bread baking industry. Gluten proteins improve the strength of wheat flour dough and consumer properties of baked products. An addition of some 1–2% of DWG increases flour protein content and water absorption capacity of weak flour dough, enhances bread output, improves its texture and softness, provides an extended shelf life [4, 5]. In bread baking and flour milling industry, DWG might be used without any modification, however, its FTP do not meet the specifications of various processes in pastry production, such as solubility, foaming capacity requirements, etc. [6]. In order to achieve a wider scope of application for DWG in food industry, new efficient methods of FTP modification are being developed that deal with physical, chemical, and structural properties of proteins: surface hydrophobicity, molecular weights [7], etc.

Eduble proteins are modified by physico-chemical, chemical, and enzymatic methods. The first category

comprises protein treatment by heat [8], pressure [9], ultrasound [10], extrusion [11]; the second, protein atecylation [12], succinylation [13], phosphorylation [14], dilute acid treatment [15], the third group of methods includes hydrolysis [16], and deamination [17]. Proteins are also modified when being exposed to combinations of various factors: temperature and enzymes [18], temperature and pressure [19], proteolysis and ultrafiltration [20], among others. Protein modification results in changes in solubility, fat-emulsifying capacity (FEC) and other properties of gluten [6, 7, 16, 18, 21]. Some researchers [6, 7, 21] determined molecular weights (MW) of gluten proteins and its FTP, however, there have been no studies of a correlation between MW of proteolytically modified DWG proteins and various rheological properties and FTP that would aim to controlling the latter through the adjustment of the former. Samples of both DWG and raw native gluten might vary in their rheological properties that are known to be determined by differences in their molecule size [22]. Consequently, their functional properties might also be different. There are no reports in literature on the dependence of functional properties of DWG proteins on MW of its components (single-chain and multiple-chain polypeptides). Therefore, the objective of this study was to evaluate the FTP of DWG in comparison with properties demonstrated by other protein products, and to investigate the correlation of these properties in native and hydrolysed DWG samples that reveal different rheological parameters with MW specificity in various types of polypeptides separated by polyacrylamide gel electrophoresis (PAGE).

OBJECTS AND METHODS OF STUDY

Objects. We used the following samples: two DWG samples produced by BM company (Kazakhstan), a sample of egg albumin (manufacturer: Eurovo S.R.L., Italy), a sample of sodium caseinate (PC Milk, Belarus), a sample of soy isolate Supro 760 (Protein Technology International, US), a sample of defatted soy flour 200/80 (Cargill, Belgium), and a sample of soy concentrate **(**Technomol, Russia). Quality and safety of the protein products were confirmed by certificates of conformity issued by Rospotrebnadzor (the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing). All the protein products except soy flour were classified as concentrates and isolates (Table 1) according to their protein content, and different in their carbohydrates, ash, and fat content.

We used enzymatic preparations (EP) for protein modification: endoproteases (Protamex®) and endo- and exopeptidases (Flavourzyme 500 MG) manufactured by Novozymes (Denmark), with proteolytic activity of 125 and 85 E/g, respectively. EP activity was determined by Rukhlyaeva's modification of the classic Anson assay as per GOST 20264.2-88; this method is based on hydrolysis of the sodium caseinate protein by the EP and further sedimentation of the non hydrolyzed protein by trichloroacetic acid.

Chemical composition. Mass fraction of protein in protein products was determined by the Kjeldahl method, in Kjeltek analyzer manufactured by Tecator (Sweden) as per GOST 10846-91; humidity, as per GOST 9404-88; ash, as per GOST 27494-87. Mass fraction of fat was analyzed as per GOST 29033-9, while fiber was determined by Kürschner-Hanak method as exposed in the guidelines [23]. Mass fraction of carbohydrates was calculated by subtracting respective mass fractions of protein, fat, ash, and humidity from a total of 100 g.

Preparation of regenerated DWG and determination of its rheological properties. 4 g (±0.01 g) of DWG were placed in a porcelain cup and added by 8 cm^3 of tap water at 18–20°C. Then a dough ball was formed, covered by glass and left for 20 minutes to rest. Then gluten was washed out onto a seave, with a jet of tap water, for 15–20 minuted at the same temperature. Regenerated 4 g gluten was manually pressed out to drain water, then a required amount of 4 g (±0.01 g) was separated and molded into a smooth-surfaced ball, without discontinuities. The gluten ball was placed to rest into a cup of water at 18–20ºC for 15 minutes. Then the compressive deformation (elasticity) ($N_{def.}$) was measured in the IDK-1 apparatus, as per the test technique provided in GOST 27839-2013.

Table 1. Chemical composition of protein products

Protein products	Humidity, %	Mass fraction (% of dry weight)				
		Protein	Fat	Carbohydrates	Fibre	Ash
Egg albumin	8.0 ± 0.4	87.0 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	3.0 ± 0.5	6.0 ± 0.5
Sodium caseinate	6.0 ± 0.2	88.0 ± 0.6	2.0 ± 0.2	1.0 ± 0.5	4.0 ± 0.4	5.0 ± 0.3
Soy flour	9.0 ± 0.2	43.0 ± 0.4	14.0 ±0.1	34.0 ± 0.8	5.0 ± 0.6	4.0 ± 0.2
Soy concentrate	4.0 ± 0.5	61.0 ± 0.2	5.0 ± 0.1	26.0 ± 0.7	4.0 ± 0.2	4.0 ± 0.4
Soy isolate	4.0 ± 0.7	92.0 ± 0.5	0.5 ± 0.2	1.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.1
DWG	4.0 ± 0.1	75.0 ± 0.5	1.0 ± 0.4	22.0 ± 0.6	1.0 ± 0.1	1.0 ± 0.2

Hydrolysis degree. Hydrolysis degree of DWG proteins (Dh) was calculated according to the equation

$$Dh = Cs/Cc*100\%,$$

where C_s was total nitrogen of the amino acids within the sample (%) [29], and C_c is the amino nitrogen content in the hydrolyzed sample.

Total amino acid nitrogen was obtained after complete hydrolysis of the sample quantity (approx. 100 mg) with 10 cm^3 6 mol/dm^3 HCl solution. The sample was maintained in a thermostat at 110ºC for 24 hours, then amino nitrogen was determined by formol titration [23]. To determine amino nitrogen, the required sample quantity was mixed with certain amount of distilled water (assuming the moisture content of 63%) with a diluted proteolytic EP. Dough was made and kept in a thermostat for certain time, at a suitable temperature. Then the hydrolysate (4.5 g) was added with 65 cm^3 of distilled water, the mixture was dispersed in a homogenizer for 4–5 minutes, then centrifuged at 6000 rpm for 20 minutes. The supernatant liquid was drained, with 5 cm^3 transferred to a cuvette with distilled water (20 cm^3) for titration. The mixture was neutralized by 0.2 N of NaOH solution under pH meter control. When pH level of the solution reached 7, it was added with 0.5 cm^3 of formaldehyde with phenolphtalein, and the mixture was titrated with 0.2 N NaOH solution up to pH 9.1–9.5, which corresponded to bright red coloring of the sample. Hydrolysed DWG samples were dried in a lyophilic drying plant Continuous Freeze Dryer (US).

Electophoresis and molecular weights. In order to determine MW and polypeptide composition of the proteins, a required quantity (0.2–0.5 g) of DWG and its hydrolysates was mixed with 20 cm^3 of buffer containing 62.5 mM of Tris-HCl, 8 M of urea, 2% SDS, and 0.01% of bromphenol blue (pH 6.8). The dispersion was agitated for 1 hour at room temperature. On the next day, (in 15–17 hours) the samples were agitated once again, for 1 hour at room temperature, then centrifuged at 12 000g for 20 minutes. Protein content in solutions was determined by the Kjeldahl method in BUCHI K-424 analyzer (Switzerland).

In a reductive cleavage reaction with native and hydrolyzed proteins, sample buffer was used, with 5% content of 2-mercaptoethanol. The buffer was added to the precipitate obtained during the previous centrifugation, in the amount of 10 cm^3. The sample was incubated at 37°C for 2 hours, then centrifuged at 12 000g for 20 minutes. The centrifugate was used for the analysis of polypeptide protein composition by a one-dimensional electrophoresis; separating gel contained 10–20% of acrylamide (pH 8.8), while stacking gel, 6% (pH 6.8). Electrode buffer contained Tris-glycine (pH 8.3) and 0.1% SDS. Electrophoresis was performed at 4–6°C in 6–8 hours, with permanent electric current of 25–30 mA. Protein content in the samples was analyzed by the Bramhall [24] method with certain modifications. Standard MW markers (manufactured by Sygma, Germany) were used to calculate protein molecular weights: phosphorylase b (MW 92 kDa), bovine serum albumin (MW 69 kDa), catalase (MW 60 kDa), egg albumin (MW 46 kDa), aldolase (MW 36 kDa), carbonic anhydrase (MW 29 kDa), trypsin inhibitor (MW 20 kDa), cytochrome C (MW 12 kDa).

Functional technological properties. In order to determine solubility of the protein products, a required quantity of 2–4 g was suspended in 30 cm^3 of distilled water for 1 hour in an agitator, then the resulting dispersion was left overnight at 4ºC. Then it was agitated again, for 1 hour, then centrifuged at 16000*g* for 15 minutes, and the resulting centrifugate was drained into a volumetric flask for 100 cm^3. The solution was made up to the mark, protein content was determined by the Kjeldahl method and expressed in percentage of the total protein within the required sample amount.

In order to determine **water-binding capacity** (WBC), 1 g of the protein product was added into 25 cm^3 of distilled water, the mixture was mixed in a homogenizer at 1000 g for 1 minute and centrifuged at 8000*g* for 15 minutes. As the supernatant was drained, the centrifuge tube was turned upside down and placed onto filtering paper. In 10 minutes, the tube was weighed, and the WBC was calculated, expressed in grams of bound water per 1 g of the product. **Fat-binding capacity** (FBC) was determined by weight. 1 g of the product was added into 25 cm^3 of sunflower oil, mixed for 1 minute at 1000 g and centrifuged at 8000 g for 15 minutes. The supernatant was drained, the tube was turned upside down and placed onto filtering paper. In 10 minutes, the tube was weighed, and the FBS was calculated as a ratio of oil weight bound with the product and the original oil weight. The parameter was expressed in grams of bound fat per 1 g of the product. In order to determine fat emulsifying capacity (FAC), a required amount of 3.5 g of a protein product was placed into a mixer, then 50 cm^3 of distilled water were added. The mixture was suspended for 1 minute at 4000 g, then combined with 50 cm^3 of cornflower oil and emulsified for 5 minutes at 8000 g. The emulsion was poured into 4 tubes and centrifuged for 5 minutes at 2000 g. FAC was calculated as a ratio of emulsion volume and overall system volume expressed as a percentage. **Emulsion prepared according to the above procedure was tested for stability** (ES) after 30 minutes heating at 80°C. Then the emulsion was cooled for 15 minutes, distributed into 4 tubes, and centrifuged for 5 minutes at 2000 g. ES was determined as a ratio of emulsion volume and overall system volume expressed as a percentage. In order to determine foam forming capacity (FFC), a required amount of a protein product (0.6 g) was placed in a glass, diluted with 25 cm^3 of distilled water, and the mixture was thoroughly triturated with a glass stick. Then the resulting mass was transferred into a 100 cm^3 cylinder, the rest of the required amount was flushed with water, and total liquid volume was made up to 30 cm^3. The sample was held horizontally and agitated for 1 min, while the foam height was measured. FFC was calculated as a ratio of foam level and original liquid level, expressed as a percentage. **Foam stability** (FS) of protein products was

determined as a ratio of remaining foam level after 15 minutes of static immobility, and original foam level, expressed as a percentage.

Statistical data analysis. All results are presented as average values of 3–5 experiments with dispersion and correlation analysis and the Q-test. A multiple comparison analysis was carried out to assess significant differences among the samples. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence.

RESULTS AND DISCUSSION

Functional technological properties in different types of protein products

FTP analysis in protein products of plant and animal origin, as summarized in Table 2, showed that soy products demonstrated maximum water-binding capacity, while in DWG samples, this property was most limited. The highest FBC was demonstrated by soy isolate and soy concentrate, the lowest, in both samples of wheat gluten. Maximum FAC and FFC were found in egg albumin. In DWG, these properties are more pronounced then in sodium caseinate and in soy products, except FS. At the same time, DWG FTP did not reach the values found in egg albumin, which is most widely used in pastries production as a conventional foam forming, fat emulsifying, and water-binding angent. Therefore, we further carried out a study on modification of DWG FTP intended for application of this protein product not only in bread-baking industry, but also for pastries and other foods production.

DWG modification and determination of its functional technological properties

Functional properties of DWG were modified by limited proteolysis, with proteolytic enzymes applied as per the technique presented in [25] work. Differences in rheological properties of the regenerated DWG samples were taken into account. These properties were determined by organoleptic methods and by IDK-1 apparatus. It was found that DWG sample No. 1 had a low compressive deformation index (40 instr. units), while DWG sample No. 2, on the contrary, had a high index, 80 instr. units. The DWG sample No.1 was characterized as "short-tearing", with stretching above the ruler less than 10 cm, while the DWG sample No. 2 was "weak", stretching for 22 cm. Based on the above, hydrolysis of the DWG sample No.1 was run with endoprotease EP Protamex®, while hydrolysis of the DWG sample No. 2, with Flavozyme 500 MG, which contained both endoproteinase and exopeptidase. At the same time, we assumed the previously stated regularities: a stronger DWG sample positively relates to a more efficient application of endoproteinase, and vice versa, a weaker DWG sample is more likely to be efficiently treated with exopeptidase [25]. In both cases, EP concentration amounted to 0.3 E/g of protein, with pH 6.5 ± 0.2, and temperature 50ºC. DWG hydrolysis time with Protamex® was adjusted from 10 to 80 min., with Flavozyme 500 MG, from 40 to 160 min.

Other researchers [26, 27] have studied properties of modified proteins by using their soluble components, while we investigated the FTP of hydlolyzed DWG proteins jointly, with both soluble and insoluble parts present, assuming that protein behavior in a food system would depend on simultaneous presence of both fractions. FTP of the DWG obtained by different hydrolysis duration was evaluated in accordance with the following parameters: WBC, FBC, FAC, FFC, ES, FS, and solubility. Our findings in functional properties of hydrolysates in comparison with native DWG samples properties are summarized in Table 3. It is seen that native DWG is diluted in water by 3.2–3.6%, while hydrolyzed DWG, by 31.3–35.5%. By the end of hydrolysis, solubility of short-tearing DWG was increased by 3.6–7.2 times, of weak DWG, by 4.8–10.8 times. Maximum solubility was observed in weak gluten proteins after 2–2.5 hours of hydrolysis. Foam-forming properties of hydrolysates were also better than the ones found in native samples. As hydrolysis time was increased up to 85 minutes in DWG No.1 and up to 160 minutes in DWG No. 2, protein FFC was increased by 55 and 82%, respectively, thus exceeding absolute FFC values of eff protein (283%) (Table 2). By the end of hydrolysis process, foam stability of the samples increased by 41–51%. At the same time, hydrolysis degree of proteins in the DWG sample No. 1 amounted to 2.21–2.89%, in the DWG sample No. 2, to 3.22–3.41%.

In contrast to FCC and solubility, such protein properties as FAC, ES, WBC, and FBC were gradually reduced as hydrolysis time was increased. Thus, for example, within the hydrolysis time range of 85–160 minutes, protein FAC was decreased by 6–7 times, WBC, by 4.5–9.0 times. In hydrolyzed short-tearing DWG, FFC, FBC and solubility proved to be somewhat higher than in weak DWG, while both FAC and WBC were lower instead. Thus, we can adjust FTP of DWG by controlling hydrolysis time, as we increase its solubility and foam forming capacity, while reducing all other parameters.

Sodium dodecyl sulfate - polyacrylamide gel electrophoresis

Considering that protein hydrolysis degree reflects changes in molecular weight distribution of polypeptides [16], we further performed electrophoresis of hydrolyzed DWG proteins in comparison to native proteins. For that purpose, we used DWG No. 1 and DWG No.2 hydrolysates obtained during 80 minutes of hydrolysis, with FFC values of 310% and 243%, respectively. Hydrolysis degree was almost the same in both samples (2.86–2.89%), however, their properties were different. Table 4 and Fig. 1 provide results of polypeptides MW determination by PAGE electrophoresis with no use of mercaptoethanol (columns 1, 2, 5, 6) and with mercaptoethanol (columns 3, 4, 7, 8). Mercaptoethanol is known to reduce disulfide bonds in multi-chain proteins, which produces single-chain polypeptides with free sulfhydryl groups that migrate into gel.

Table 2. Functional technological properties of protein products

Protein products	WBC, g/g	FBC, g/g	FAC, %	ES, %	FFC, %	FS, %
Egg albumin	soluble in water	1.15 ± 0.10	70 ± 2	75 ± 3	283 ± 3	90 ± 2
Sodium caseinate	forms gel	1.64 ± 0.00	57 ± 1	47 ± 1	177 ± 2	60 ± 3
DWG, sample No. 1	1.2 ± 0.3	0.66 ± 0.30	62 ± 3	68 ± 2	200 ± 6	46 ± 0
DWG, sample No. 2	0.9 ± 0.1	0.60 ± 0.40	59 ± 1	65 ± 3	174 ± 2	45 ± 0
Soy flour	1.6 ± 0.2	1.20 ± 0.30	49 ± 1	47 ± 1	80 ± 1	60 ± 1
Soy concentrate	7.4 ± 0.5	2.20 ± 0.20	61 ± 2	48 ± 3	50 ± 3	68 ± 2
Soy isolate	7.9 ± 0.5	1.80 ± 0.60	55 ± 1	55 ± 1	55 ± 4	68 ± 1

Table 3. Functional technological properties of native and hydrolyzed DWG samples with different rheological properties

Indicators	$N_{def.}$ 40 instr. units·					$N_{def.}$ 80 instr. units.				
	Control	Hydrolysis time, minutes				Control	Hydrolysis time, minutes			
		10	40	60	80		40	80	120	160
WBC, g/g	1.20 ± 0.07	0.71 ± 0.07	0.58 ± 0.08	0.42 ± 0.05	0.13 ±0.07	0.90 ± 0.04	0.75 ± 0.70	0.61 ± 0.70	0.32 ± 0.70	0.20 ± 0.70
FBC, g/g	0.66 ± 0.07	0.64 ± 0.06	0.39 ± 0.12	0.44 ± 0.03	0.60 ± 0.11	0.60 ± 0.09	0.45 ± 0.08	0.45 ± 0.13	0.72 ± 0.02	0.43 ± 0.13
FAC, %	62.0 ± 2.0	54.0 ± 1.0	38.0 ± 2.0	14.0 ± 0.0	11.0 ± 1.0	59.0 ± 1.0	54.0 ± 0.5	30.0 ± 0.5	14.0 ± 2.0	8.0 ± 1.5
ES, %	60.0 ± 1.0	50.0 ± 2.0	39.0 ± 1.0	15.0 ± 2.0	10.0 ± 1.0	59.0 ± 0.5	52.0 ± 1.0	31.0 ± 2.0	14.0 ± 2.0	9.0 ± 0.5
FFC, %	200 ± 2	216 ± 1	250 ± 3	300 ± 3	310 ± 2	174 ± 2	216 ± 1	243 ± 3	276 ± 4	316 ± 3
FS, %	46.0 ± 1	50.0 ± 0.5	56.0 ± 0.5	60.0 ± 1.0	65.0 ± 1.0	45.0 ± 0.5	48.0 ± 1.0	57.0 ± 2.0	63.0 ± 2.0	68.0 ± 1.0
Solubility, %	3.6 ± 0.2	13.1 ± 0.4	16.2 ± 0.5	26.2 ± 0.4	31.3 ± 0.5	3.2 ± 0.5	15.3 ± 0.4	27.2 ± 0.4	35.1 ± 0.1	35.5 ± 0.2
Proteolysis degree, %	0	1.10 ± 0.05	1.72 ± 0.07	2.21 ± 0.11	2.89 ± 0.06	0	1.76 ± 0.06	2.86 ± 0.03	3.22 ± 0.08	3.41 ± 0.05

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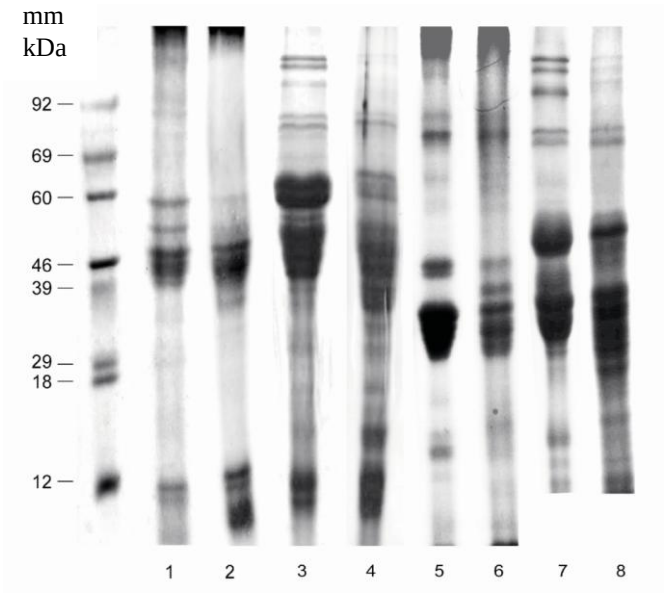

Fig. 1. Polypeptide composition of dry wheat gluten of different quality: Strips 1, 2, 3, 4 – short-tearing gluten; Samples 5, 6, 7, 8 – weak gluten; 1, 3, 5, 7 – native gluten; 2, 4, 6, 8 – hydrolyzed gluten; 1, 2, 5, 6 – without mercaptoethanol; 3, 4, 7, 8 – with mercaptoethanol.

Comparison of single-chain proteins MW distribution in native DWG samples (columns 1 and 5) shows that short-tearing gluten, which has a 26% higher FFC than weak gluten, lacks two low molecular polypeptides, with MW from 14 to 24 kDa. Comparison of hydrolysates composition (columns 2, 6) reveals that HDWG No.1, with a 67% higher FFC than HDWG No. 2 (table 2) lacks polypeptides with ME of 20, 22, 36, 39 kDa, but has proteins with ME MM 14, 54 and 60 kDa. What both HDWG samples (columns 2, 6) have in common is a reduced baseline amount of proteins with MW 120 kDa, dissociation of high molecular peptides (MW 108–110 kDa), medium molecular (MW 69, 86 kDa), and one low molecular peptide (MW 24 kDa). At the same time, HDWG sample demonstrated FFC 69–110% higher than native samples (columns 1, 5). HDWG sample No. 1 (short-tearing gluten) which had the highest FFC (column 2), even in comparison to HDWG No. 2 (weak gluten) contained no polypeptides with MW 36 and 39 kDa. Therefore, a common characteristic found in gluten protein composition in various quality samples is presence of a single-chain peptides group with MW from 40 to 60 kDa, and a peptides group with MW from 12 to 16 kDa, as well as absence of peptides with MW from 69 to 110 kDa. This characteristic ensures high FFC. Peptides with ME of 20–24 and 36–39 kDa also did not facilitate FFC increase up to 310 % of egg albumin value.

Considering that during the electrophoresis experiment a significant part of proteins remained at the baseline, we further analyzed samples of native DWG and its hydrolysates with mercaptoethanol that breaks disulfide bonds and ensures polypeptide penetration into a gel. We discovered that across the spectrum of native and hydrolyzed DWG samples of different quality with mercaptoethanol, at the baseline there were no proteins with MW > 120 kDa (columns 3, 4, 7, 8), which indicated total dissociation of all disulfide bonds. On the other hand, after disulfide bonds dissociation, a native sample of short-tearing DWG demonstrated new polypeptides with MW of 22–30 kDa and with MW of 84–110 kDa (column 3), while a weak DWG sample revealed proteins with MW from 18 to 33 and from 54 to 108 kDa (column 7).

A particularity of composition found in the DWG sample No.1 (column 4), as compared to the native DWG sample, was trace quantities of polypeptides with MW 92, 108, 110 present in multi-chain proteins, and complete absence of two polypeptides with MW 28–36, and four, with MW from 69 to 100 kDa. In weak DWG hydrolysate (column 8), multi-chain proteins contained the same high molecular peptides (MW 86–110 kDa), as the native sample did (column 7), however, peptides with medium and low MW were also present. An exception was made for peptides with MW 24 and 60 kDa. Therefore, native DWG and its hydrolysates contained both single-chain and multi-chain polypeptides, which corresponds to the data on content and structure of native raw gluten proteins [22].

Composition analysis of multi-chain proteins in HDWG No 1 hydrolysate (column 4) with the highest FFC observed (310%), revealed that its distinctive feature, as in comparison to HDWG No 2 hydrolysate (column 8), was presence of trace quantities of high molecular peptides (MW 92–110 kDa), and complete absence of six peptides with MW from18 through 39, of four peptides with MW from 69 through 102, and proteins with MW over 120 kDa. Judging by the strips intensity, this sample contained less polypeptides with medium MW (40–60 kDa) and more polypeptides with the lowest MW (12–16 kDa).

As we discuss our findings, we should note that in food industry, egg products (eggs, melange, egg powder) are conventionally used as a source of surfactants to create protein foam and emulsion systems; however, these egg product also contain fat and cholesterol. Therefore, there is an ongoing search

for alternative sources in order to reduce calories of final products and at the same time, to exclude egg albumin that some people are allergic to [28]. Along with this purpose, plant-based and animal proteins obtained from both conventional and unconventional feedstock are gradually getting more applications [14]. Just like proteins found in other cereals (rye, oats, rice, corn), gluten is poor in essential amino acids (lysine, threonine, tryptophan), however, due to its unique rheological properties it is widely used in bread baking [29]. Interacting with each other through ionic, hydrogen, disulfide, and other bonds, polypeptide chains of gliadin and glutenin form a tridimensional structure with hydrophobic and hydrophilic groups located onto its surface. Properties of this structure determine elasticity, stretching, cohesion, softness, and eventually, volume of bread, elasticity and porosity of its crumb [22]. Our findings suggest that the structure of unmodified gluten proteins does not ensure prominent functional properties for them to be used in complex pastries systems, as a foam forming or emulsifying agent (structure former) (Table 2). DWG FTPs might be achieved by controlling its proteins with proper physico-chemical, chemical, and enzymatic methods. However, due to their intrinsic safety, biotechnological methods based on use of various proteases [16], including endoproteases and exopeptidases, seem to have considerable advantages over other methods. Endoproteases hydrolyze peptide bonds from inside, as they dissociate protein molecules into smaller fragments, while exopeptidases proceed to sequential dissociation of the ends of amino acids, one by one (Fig. 2).

We used endoproteinsase EP Protamex® for proteolysis of an elastic DWG sample, as we had previously [25] established that with this EP, hydrolysis degree in time under 100 minutes is higher that with Neutrase®, which conforms with findings reported by other authors who speak of its higher efficiency [16]. However, other researchers have not considered a correlation of functional properties with proteins MW in DWG demonstrating different rheological properties. According to our data, hydrolysis of short-tearing DWG with Protamex® and of weak DWG with Flavourzym 500 MG, leads to a formation of peptide mixture with higher solubility, FFC and FS, in comparison with native gluten. This regularity is reverse to the one established for hydrolysis of rice middlings proteins with papain and alcalase [30], but it conforms with findings of the research [7] which demonstrated that improved solubility and FFC at the same protein hydrolysis degree (2.8–2.89%) might be achieved through fractioning of DWG hydrolysates by ultrafiltration. The regularity we established for WBC and FAC reduction in DWG proteins during hydrolysis do not conform with the data obtained by those authors. Therefore, properties of DWG hydrolysates are unique for each case, they depend on the rheological properties of gluten and on the enzyme type, and thus require specific case studies, which is confirmed by the findings of the present work. During protein proteolysis, new absorbed active peptides appear, consequently, additional suface activity arises, which indicates a possibility to improve protein quality and to obtain valuable nutrition ingredients. Each protein requires specific modification studies with relation to various functional properties.

According to our findings, protein hydrolysis and FTP improvement (hydrolysate FFC achieved FFC of egg albumin, about 300%) were accompanied by formation of single-chain peptides, not only with very low (below 15 kDa) [7] and low MW (below 40 kDa), as it had previously been shown [16], but also with medium MW (40–60) kDa (Table 4) – values that correspond to ω-gliadin MW, partially to γ-gliadin and low molecular glutenin subunits [31]. This is equally confirmed by comparison of our findings with data obtained in [32], where the authors showed that at 180% FFC of DWG hydrolysates with papain (which is lower than the value we obtained, 300%) polypeptides with low MW (5–15 kDa) are typical, therefore, we might conclude that polypeptides with MW of 40–60 kDa participate in ensuring enhanced foam forming capacity and solubility, along with low molecular peptides.

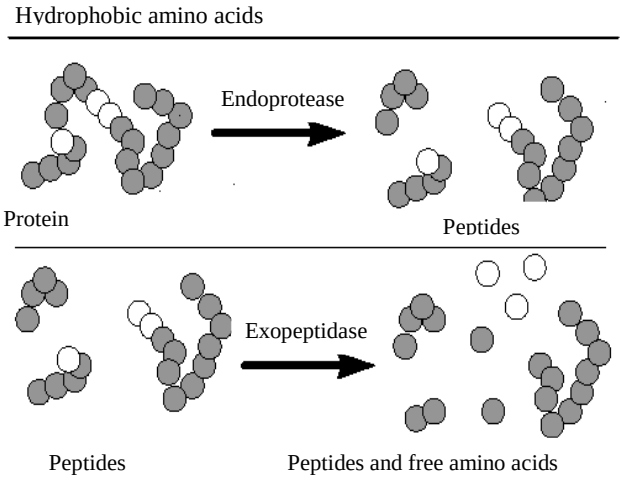

Fig. 2. Endoproteases and exoproteases action on proteins.

Table 4. Molecular weights of polypeptides present in DWG of different quality, kDa

Short-tearing gluten (DWG No. 1)				Weak gluten (DWG No. 2)			
DWG	HDWG	DWG	HDWG	DWG	HDWG	DWG	HDWG
without mercaptoethanol		with mercaptoethanol		without mercaptoethanol		with mercaptoethanol	
1	2	3	4	5	6	7	8
> 120	> 120↓	–	–	> 120	> 120↓	–	–
–	–	110	110 tr	110	–	110	110↓
108	–	108	108 tr	–	–	108	108
–	–	-	–	–	–	102	102 tr
–	–	100	–	–	–	100	100 tr
92	–	92	92 tr	92	–	92	92 tr
86	–	86	–	86	–	86	86 tr
–	–	84	–	–	–	–	–
69	–	69	–	69	–	69	69
60	60	60	60↓	60	–	60	–
54	54	54	54↓	–	–	54	54
46	46	46	46↓	46	46	46	46
44	44	44	44↓	44	44	44	44 tr
40	40	40	40↓	40	40	40	40
39	–	–	–	39	39	39	39
36	–	36	–	36	36	36	36
–	–	–	–	–	–	33	33
–	–	30	30	–	–	30	30
–	–	28	–	–	–	28	28 tr
–	–	–	–	–	–	27	27
–	–	–	–	24	–	24	–
–	–	22	22	22	22	22	22
–	–	–	–	20	20	20	20
–	–	–	–	–	–	18	18
16	16	16	16↑	16	16	–	–
14	14	14	14↑	–	–	–	–
12	12↑	12	12↑	12	12↑	–	–

Note. *DWG – control sample; HDWG – hydrolyzed sample; "tr" – trace quantities of strips, ↑ – increased intensity of strips; ↓ – decreased intensity of strips.

We have discovered a relationship between FTPs and composition not only in single-chain polypeptides present in DWG of different quality, but in multi-chain polypeptides as well. If the electrophoretic spectrum of proteins had remained the same during our experiments with mercaptoethanol, just as it had been without it, that would have meant that the proteins were composed of a single polypetide chain. However, as the proteins were composed of polypeptide chains connected with disulfide bonds, incubation with mercaptoethanol would lead to their dissociation, and separate polypertides migrated into a gel, thus changing the spectrum outlook. Multi-chain proteins of both DWG hydrolysates with higher FFC and solubility than in native samples demonstrated total absence or trace quantities of peptides with MW from 69 to over

120 kDa (Table 4, columns 4, 8). However, they contained larger amounts of polypeptides with MW from 12 to 20 kDa, that is, low molecular components in multi-chain proteins are equally important for ensuring the properties under consideration. This conclusion is confirmed by the fact that multi-chain proteins of the HDWG sample No. 2 (column 8), with lower FFC (243%) and solubility (27.2%) values in comparison to HDWG No.1 (FFC 310%, solubility 31.3%) (column 4), contained polypeptides with high MW (69–108 kDa) and medium MW (27–39 kDa), while low molecular peptides (MW 12–16 kDa) were not present. The same regularity might be observed while comparing the composition of multi-chain proteins in native DWG of different quality (column 3, 7). Short-tearing DWG with solubility of 3.6%, and FFC, 200% as compared to weak DWG with solubility of 3.2% and FFC, 174%, lacked two polypeptides with MW 84–102 kDa and four peptides with MW from 24 to 39 kDa, however, it contained low molecular peptides with MW 12–16 kDa. The above described specificity of MW in polypeptides with higher FFC, FS, and solubility, were also correlated with lower WBC and FAC values. Relationship between FBC and ME of polypeptides was not observed in this work.

Thus, a comparative study of native DWG FTPs in contrast to other protein products revealed the utility of improving these for future application in foam systems for pastries production, considering its specific rheological properties. Depth of proteolysis for weak DWG that would improve solubility, FFC, and FS, should be achieved with hydrolysis degree of 1.76 through 3.41%, under exposure to endoproteinase and exopeptidases. As for short tearing DWG, hydrolysis degree should amount to 1.1 through 2.89%, run with endoproteinase. DWG with higher FFC, FS, and solubility contained single-chain polypeptides not only with low MW (below 40 kDa), but with medium MW as well (40–60 kDa). The highest values of FFC, FS, and solubility were found in short-tearing gluten, with 80 minutes proteolysis time leading to formation of single-chain polypeptides with MW 54 and 60 kDa, and elimination of peptides with MW from 20 through 39 kDa. We revealed a dependence of FTP on MW characteristics for both single-chain and multi-chain polypeptides determined by PAGE electrophoresis run with and without mercaptoethanol. A distinctive feature of multi-chain polypeptides united by covalent disulfide bonds within DWG is presence of proteins with low MW (12–16 kDa), which are more favorable for enhancement of foam forming capacity and solubility than polypeptides with medium (27–39 kDa) and high MW (69–108 kDa**).** Multi-chain peptides found in short-tearing gluten with the highest FFC level (310%) lacked 4 polypeptides with MW from 69 to 102 kDa, and a group of peptides with MW from 27 to 33 kDa, however, they contained low molecular peptides (MW 12–16 kDa). Use of modified DWG with well researched MW characteristics of single-chain and multi-chain protein components, with hydrolysis degree of 1.10–3.41 % and higher solubility that native DWG, seems promising as a foam forming agent for pastries products containing foams and foam-emulsions.

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STUDY OF TECHNOLOGICAL PROPERTIES OF MILK-CLOTTING ENZYME FROM IRPEX LACTEUS (*Irpex lacteus* (Fr.) Fr.)

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Abstract: Due to the scarcity of natural rennet, in this study we have considered an option of using in cheesemaking a milk-clotting enzyme produced by the *Irpex lacteus* fungus. We describe main properties of *I. lacteus* coagulant: milk-clotting activity (MA), overall proteolytic activity (PA), thermal stability, MA dependence on pH level and calcium ions content. Partially purified preparations of *I. lacteus* milk-clotting enzyme was obtained by salting out and gel-filtration. Technological properties of the *I. lacteus* coagulant were compared to natural calf rennet and cow pepsin (CP). MA of *I. lacteus* enzyme amounted to 29.1 ± 0.7 RU/ml, with protein content of 23 mg/ml. The coagulant was completely inactivated at 60°C. Thermal stability of *I. lacteus* milk-clotting enzyme, MA sensitivity to pH variations and Ca^{2+} content were comparable to respective parameters of calf rennet and CP. Overall PA of the *I. lacteus* coagulant exceeded CP and calf rennet activity by 33 and 220 times, respectively. As for enzymatic specificity, the following order was observed: calf rennet (100%) > CP (14.9%) > *I. lacteus* coagulant (0.5%). These findings suggest that there is a need to increase the MA of *I. lacteus* coagulant in order to be able to use it in cheesemaking. We have considered chemical, biochemical, and genetic corrective actions applicable to technological properties of microfungal milk coagulants.

Keywords: milk-clotting enzymes, rennet, rennet substitutes, microfungal coagulants, milk-clotting activity, proteolytic activity, thermal stability, cheesemaking, mucorpepsins

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INTRODUCTION

A key step in cheesemaking is milk coagulation and obtaining a milk clot. This was conventionally achieved with rennet: a complex preparation from abomasums of nursing calves and lambs. Rennet contains two milk-clotting enzymes (ME): chymosin (EC 3.4.23.4) and pepsin (EC 3.4.23.1). Chymosin is considered to be the standard enzyme for cheesemaking, due to the combination of its process-related properties: high specificity for cleavage the Phe_{105}-Met_{106} bond in κ-casein molecules, milk-clotting activity (MA) within the mild acidic pH range, low overall proteolytic activity (PA), and thermal lability. Many prokaryotic and eukaryotic proteases demonstrate MA, however, due to the high level of nonspecific proteolysis and thermal stability, that reduce product output and result in cheese taste and texture defects, these are not used in cheesemaking, or else their application is very limited.

In the second part of the XX century, cheesemaking industry faced a challenge of rennet deficit [1], as volumes of rennet-based cheese production kept growing, causing a severe shortage of calf and lamb abomasums [2–4]. This deficit initiated a search for rennet substitutes, but a valid equivalent of cow chymosin was not to be found. In 1990–2006, the problem of rennet shortage was partially resolved when recombinant chymosin (rCh) of cow [5] and camel [6] were developed, however, there are some factors that prevent their wide application. In a number of countries, use of rCh is limited in production of cheese subject to national certification of origin. Besides, a certain customer segment is opting for cheeses produced with natural rennet, not a recombinant one. In the end of the 20th century, the problem of rennet deficiency was exacerbated due to a scrapie epidemic: a prion cattle disease [7]. This epidemic devastated the feedstock of natural ME and triggered a new – and

currently ongoing – phase in the search for rennet substitutes among animal, plant and microbial milk-clotting proteases [2, 3, 8–14]. This search is aimed at the diversification of feedstock in natural milk coagulants, and the selection of enzymes that might be adapted for cheesemaking after certain chemical, biochemical, or genetic "adjustment".

Due to the rennet shortage, starting from the 1970s, modern cheesemaking uses microfungal coagulants: mucorpepsin (E.C.3.4.23.23) and endotiapepsin (EC 3.4.23.22): aspartic proteinases *Rhizomucor miehei* (Cooney & R. Emers.) Schipper (*less often Rhizomucor pusillus* (Lindt) Schipper) and *Cryphonectria parasitica* (Murrill) M.E. Barr (synonym: *Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson). Microfungal coagulants have the following advantages: low cost of production, compliance with natural origin criteria and with vegetarian requirements, conformity with kosher and halal eating principle. Their main disadvantages are: low specificity (MA/PA ratio), and high thermal stability [2].

Object of this study is a fungus *Irpex lacteus* (synonym: *Polyporus tulipiferae* (Schwein.) Overh.), which belongs to higher basidiomycetes and is known to produce milk-clotting protease [15]. Milk coagulant, produced by *I. lacteus*, is registered in the IUBMB enzyme nomenclature database as polyporopepsin (EC 3.4.23.29), which is related to the aspartic endopeptidases family (the family of chymosin and pepsin A).

Data on process-related properties of *I. lacteus* coagulant are scarce and fragmented. We should mention a series of studies by K. Murakami et al. [15–18] that presented a partial description of the enzyme and obtained its kDNA sequence, and a number of works carried out in the Donetsk National University (Ukraine) on some aspects of the *I. lacteus* strain productivity, and studies of thermal stability of crude milk-clotting preparations of this fungus [19–21]. Nowadays, *I. lacteus* coagulant is not used in dairy industry as a rennet substitute. Meanwhile, biochemical properties of polyporopepsin indicate that its derivatives might be successfully used in cheesemaking.

The objective of this work is to diversify the feedstock of natural milk-clotting enzymes (ME). The ensuing task of this study is to assess process-related properties of the milk-clotting enzyme derived from the *I. lacteus* basidial fungus, from the point of view of potential applicability in cheesemaking.

OBJECTS AND METHODS OF STUDY

A pure culture of the *I. lacteus* fungus was derived from a spore print in the Republic of Altai (Russian Federation) in 2008. The fungus strain No. 2265 is stored in the Collection of Basidiomycetes Cultures in the Botanical institute named after V.L. Komarov (Saint-Petersburg), and in the Mycology Laboratory of the State Research Center of Virology and Biotechnology VECTOR, where the fungal biomass for this study was produced. Cultivation was performed in 0.75 l flasks, with 0.15 l of tryptone glucose nutrient medium (pH 6.0), for 7 days, in temperature-controlled shaking baths at $26 \pm 2^{\circ}$C. Liquid culture used for inoculation was obtained by fungal cultivation on a liquid medium inoculated with culture blocks grown on agar medium. Fungal biomass suspension was frozen at $-30 \pm 2^{\circ}$C [22].

Samples of milk-clotting enzyme were obtained in the nucleic acids and recombinant proteins laboratory in the Medical Biotechnology Institute of the State Research Center of Virology and Biotechnology VECTOR. Frozen *I. lacteus* biomass suspension (~850 ml) was thawed in a funnel with gauze filter. The filtrate was made up with dry ammonium sulfate (AS) to the final 70% saturation, stirred, and then left to sediment at 4°C overnight. The mixture was centrifuged at 8000g and 4°C for 15 minutes. The supernatant was rejected, and the sediment dissolved in approx. 25 ml of distilled water, and then desalted by gel-filtration on Sephadex G-25 column (gel volume – 60 ml.). Six fractions of desalted protein material (15 ml each) was obtained, these were combined into two samples: A (fr. 1–3) and B (fr. 4–6). A and B samples were sedimented again with AS at 70% saturation, precipitate dissolved in 20 ml of distilled water, and dialysed for 8 hours against two 1 l charges of 20 mM Tris-HCl, 50mM NaCl (pH 7.5). Total protein level in A and B samples was determined by the Lowry method. A and B samples were poured in 1 ml microflasks and lyophilized.

Technological properties of A and B samples were assessed in the biochemical laboratory of the FSBSI Siberian Scientific Research Institute of Cheesemaking. Milk-clotting activity, overall PA, thermal stability, MA dependence on pH and Ca^{2+} concentration were assessed in accordance with the previously published techniques [14, 23]. Along with the *I. lacteus* coagulant samples, we also studied natural ME of animal origin: an industrial control sample of calf rennet (ICS CR) with approx. 80% of chymosin content, and 100% cow pepsin (CP). Standard milk substrate, ICS CR, and CP were provided as a courtesy of the "Moscow Rennet Plant" JSC.

Milk clotting activity was assessed by mixing solutions of dry standard substrate (DSS) and test ME heated up to 35°C, mix ratio 10 : 1. The result was expressed in reference units (RU), with MA-certified ICS CR used as reference.

For assessment the overall PA, we used Hammersten grade casein solution (1%) as a substrate in 20 mM sodium phosphate buffer (pH = 5.6). ICS CR and CP activities were normalized as per MA demonstrated by the *I. lacteus* coagulant. Test ME were introduced in the substrate solution at 1 : 4 mix ratio and incubated at 35°C for 6, 60, 90, 180 minutes. The reaction was interrupted by adding trichroloacetic acid (5%), then the samples were filtered, and the resulting filtrate was assessed for optical density at $\lambda = 280$ nm (D_{280}). Specificity was determined as MA/PA ratio. PA was assumed as D_{280} of samples incubated for 180 minutes.

Thermal stability. Aliquots of milk-clotting enzymes were heated up for 30 minutes within the temperature range of 30–70°C, and then assessed for

residual MA. MA values obtained in the samples heated at 30°C were assumed to be 100%.

DSS samples with pH 5.5, 6.0, 6.5, 7.0 were prepared, and their coagulum formation time was measured, in order to determine MA dependence on the pH level. Clotting time at pH 5.5 was assumed to be 100%.

MA dependence on Ca^{2+} content was determined by introducing $CaCl_2$ into the DSS samples, making up to the 1–5 mM concentration; clot formation time was then measured. Values obtained in $CaCl_2$-free samples were assumed as 100%.

RESULTS AND DISCUSSION

Protein concentration in A and B samples amounted to 23.6 mg/ml and 29.0 mg/ml, respectively.

Milk-clotting activity. Specific MA of A sample was 29.1 ± 0.7 RU/ml, which amounted to 1230 ± 30 RU/g in protein equivalent. Sample B demonstrated residual MA (< 1 RU/ml) and was removed from further assessment.

Activity of dry commercial milk-clotting enzymes used in cheesemaking amounts to 100–200x10^3 RU/g, of liquid agents, 50–100x10^3 RU/ml. Thus, MA found in sample A is about 10^3 times lower than the required value for commercial ME. Low MA values might result from low productivity of the *I. lacteus* strain 2265, suboptimal cultivation conditions, inadequate purification of the milk-clotting enzyme, and/or presence of inhibitors.

Yet another possible explanation is partial inactivation of the coagulant in the very process of production, for example, due to the pH level of the final buffer (pH 7.5), which exceeds the pH stability limits for polyporopepsin (pH 3–6). Pepsin A, belonging to the same aspartic proteinases family as polyporopepsin, is known to be irreversibly inactivated at pH > 7.0.

MA efficiency is considerably affected by *I. lacteus* strain selection and its cultivation conditions [20]. S.M. Boyko and I.N. Ivanov used a specially selected and highly productive strain of *I. lacteus* Fr., BN-3 (stored in a specialized collection of cultures at the Institute of Botany named after N.G. Kholodniy NAN of Ukraine, Kiev) and, as they modified the surface conditions for fungus cultivation, obtained a cultural filtrate with overall MA of approx. 600 RU/ml, and with specific activity of 186 RU/mg. In deep cultivation of the BN-3 strain, activity of the cultural filtrate increased up to 900 RU/ml. After partial purification of the coagulant, the authors obtained a preparation with specific MA of 1463 RU per 1 mg of protein [21], which fully complies with the activity requirements for dry commercial ME.

Overall proteolytic activity. High overall proteolytic activity of a ME is an extremely negative factor for cheesemaking. Non-specific proteolytic activity of a ME results in considerable losses of proteolysis products with whey, and cheese output is significantly reduced. Coagulants of *R. miehei, R. pusillus and C. parasitica* might result in 0.5–1.2% decrease in cheese output in comparison to calf rennet [9]. If the remaining within granular curds milk-clotting enzyme is not inactivated by heat treatment, then cheese with a long ripening process and shelf life shall develop texture and taste defects (bitterness). An active proteolytic enzyme with low specificity affects process-related properties of cheese whey, which is used as feedstock for various dairy products and derivatives [3, 13].

The *I. lacteus* milk coagulant has high PA (Fig. 1a). Overall PA of the *I. lacteus* coagulant exceeds this parameter in ICS CR by approx. 220 times, and in CP, by approx. 33 times. Proteolytic activity of the *I. lacteus* coagulant is so much above the control enzymes values that within the scale of Fig. 1a, the curves built for ICS CR and CP almost merge with the horizontal axis. In order to demonstrate differences between ICS CR and CP, we provided a bigger scale graph (Fig. 1b).

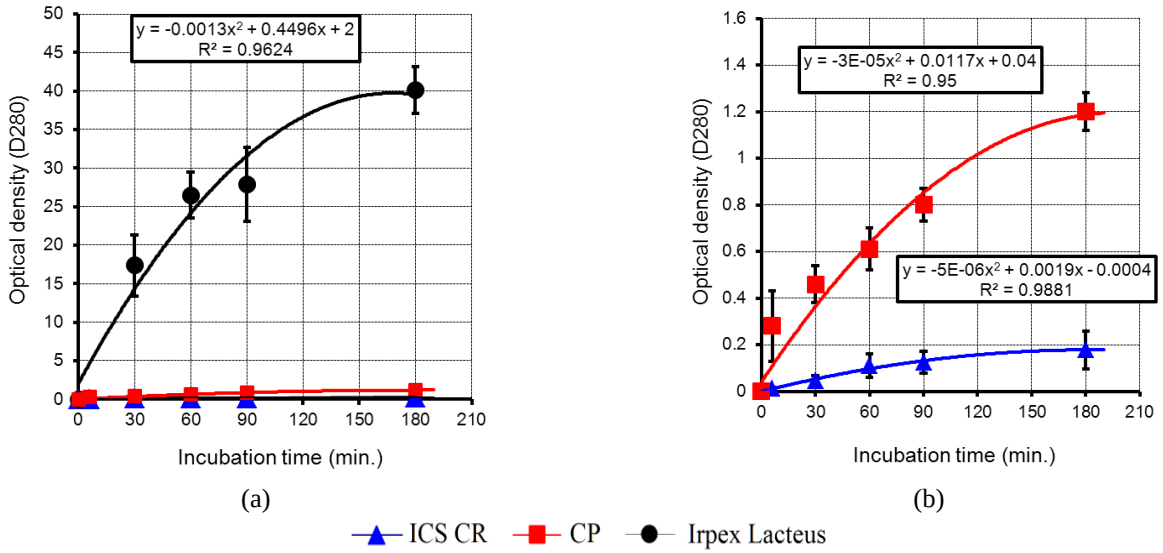

Fig. 1. Assessment of overall proteolytic activity in milk-clotting enzymes. A. Overall proteolytic activity of *I. lacteus* coagulant, ICS CR, and CP. B. Overall proteolytic activity of ICS CR and CP. (Please note: in Fig. a and b, Y-axis scales are different). Text boxes show equations for polynomial trend lines (with factor 2) and approximation probability (R^2).

Position of microfungal coagulants among ME for commercial use is determined by M. Harboe et al. [3] classification, which suggests that, along with the specificity decrease (calculated as MA/PA ratio), milk-clotting enzymes are arranges as follows: rCh (camel) > rCh (cow), calf chymosin > cow pepsin > mucorpepsin XL > mucorpepsin L > endotiapepsin. We have performed a similar arrangement, assuming the MA/PA ratio of calf rennet as 100%. We therefore obtained the following sequence aligned with specificity decrease: ICS CR (100.0%) > CP (14.9%) > *I. lacteus* coagulant (0.5%).

One of the possible causes accounting for low specificity of the *I. lacteus* coagulant might be its insufficient purification. Ammonium sulfate in 70% saturation sediments the milk-clotting enzyme together with most proteins present in the cultural liquid. "Ballast" proteolytic enzymes without MA or inhibitors of specific clotting activity are also likely to be present. Desalting allows to eliminate electrolytes and low molecular weight organics, however, it does not provide considerable purification of the resulting enzyme. MA of the *I. lacteus* might have probably been affected by a high pH level of the final buffer. We suggest that, once the purification procedure is improved and more homogeneous products are obtained, we might achieve certain increase in MA and specificity of the *I. lacteus* coagulant, which would allow for its application in cheesemaking. This hypothesis is based on data reported in literature. In 1988–1989, K. Murakami et al. reported a series of experiments on using the *I. lacteus* milk-clotting enzyme in production of soft fibrous cheese without ripening, and also such varieties as Gouda and Cheddar [16–18]. However, these works were discontinued, and the practical results of using the *I. lacteus* coagulant in cheesemaking were never confirmed by other studies.

Based on the collected data, we can conclude that cheesemaking applications for the *Irpex lacteus* milk-clotting enzyme preparations seems unlikely, unless its MA and MA/PA ratio are increased.

MA dependence on calcium ions content in the substrate. In native milk, due to exposed carboxyl and phosphate groups, the surface of casein micelles is negatively charged, which prevents them from binding and provides for aggregation stability of the colloidal system. During pasteurization process, calcium salts present in milk partially convert into insoluble state. This prolongs the duration of rennet coagulation, and results in formation of a flaccid milk clot. In order to compensate for the reduced Ca^{2+} content after pasteurization, $CaCl_2$ in concentration of 0.2–0.5 g/l is introduced into the milk mixture prepared for coagulation. Introduced $CaCl_2$ produces a two-fold effect. First, in presence of Ca^{2+}, negative charge from the surface of casein micelles is partially removed, and K-caseins, located in the "hairy" layer become more available for attacks of milk-clotting enzymes. Second, Ca^{2+} participates in formation of ion "bridges" between micelles during their aggregation phase. As a result, milk clotting time decreases.

Increased $CaCl_2$ content does not only lead to a higher MA, but to a higher overall PA of the enzyme as well. Excessive addition of $CaCl_2$ to the milk mixture might develop in cheese certain texture and taste defects. Therefore, cheesemakers try to stick to the minimum $CaCl_2$ concentration. Low sensitivity to Ca^{2+} content in the milk mixture is a valuable factor, because it provides an opportunity to vary the introduced $CaCl_2$ concentration, without worrying too much about significant MA and PA changes. Whenever preparations with high sensitivity to Ca^{2+} are used, the effect of MA and PA increase should be accounted for.

In comparison to natural ME of animal origin, clotting activity of the *I. lacteus* preparation is the least dependent on the Ca^{2+} content in the DSS (Fig. 2).

At $CaCl_2$ 3 mM (most common concentration in cheesemaking), clotting activity of the *I. lacteus* preparation is increased by 27%, and respective activities of ICS CR and CP, by 55% and 70%, respectively. The biggest differences in clotting time decrease are observed when $CaCl_2$ is increased from 0 to 3 mM. Within the range of $CaCl_2$ 3-5 mM, MA dynamics (inclination angles of all curves) is similar in all tested enzymes.

It should be remembered that ICS CR sensitivity to Ca^{2+} content is partially determined by 20% pepsin admixture. In modern high-quality natural rennet preparations for commercial use, chymosin content reaches up to 96%. Along with the pepsin percentage decrease, rennet sensitivity to Ca^{2+} shall diminish as well and approach the respective values of the *I. lacteus* coagulant.

These findings allow us to conclude that the *I. lacteus* coagulant sensitivity to Ca^{2+} ions content is in conformity with the cheesemaking requirements.

Thermal stability. In cheesemaking, thermal stability of the milk-clotting enzyme is one of the regulating factors for proteolysis intensity and specificity during cheese ripening. The substrate for ME remaining in cheese mass are α- and β-caseins. Degree of enzymatic hydrolysis of these proteins determines the level of non-specific proteolysis which, in turn, influences the ripening time, physical, chemical and organoleptic properties of cheese. All this determines the tactics for practical use of ME with different thermal stability.

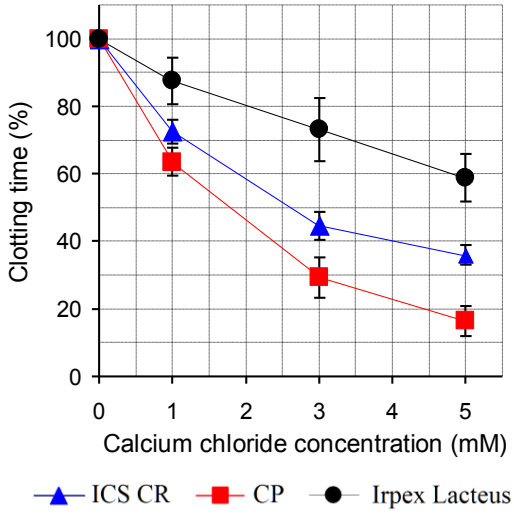

Fig. 2. Dependence of clotting time on $CaCl_2$ content.

Thermolabile enzymes are preferably used in production of hard and semi-hard cheese varieties, with high second heating temperature (52–58°C), and lengthy ripening duration and extended storage time. According to a widely applied cheesemaking practice, cheese is produced in summer, in presence of a thermolabile ME which is completely inactivated by high temperature during the second heating. This leads to a reduced proteolysis rate during cheese ripening and storage. Products made in summer with the help of a thermolabile ME are set for a lengthy storage and distributed in autumn and in winter, when cheese output drops.

Thermostable milk-clotting enzymes that remain proteolytically active at high temperatures (60–70°C) are used for production of soft cheese varieties with short ripening and storage time, or for cheese varieties that do not require ripening. In complex MEAs that consist of milk-clotting enzymes with high and low thermal stability, milk coagulation (at T~32°C) occurs through the joint activity of participating enzymes, while after heat treatment (72–74°C), the required proteolysis level in ripening cheese is ensured by thermostable component only.

Information on polyporopepsin thermal stability presented on the BRENDA enzyme portal [brenda-enzymes.org/enzyme.php?ecno=3.4.23.29] is contradictory: some data suggest that the coagulant is stable at 30°C (pH 4.6) and is completely inactivated at 45°C (pH 4.6), while according to other data, the enzyme can resist heating for 15 minutes at 50°C (pH 4.5), and all activity stops altogether at 60°C (pH 4.5).

E. Kikuchi et al. [16] conducted a comparative study of thermal stability in calf rennet ("Chr. Hansen"), mucorpepsin *R. pusillus* ("Meito"), and *I. lacteus* ME. It was shown that the *I. lacteus* milk-clotting enzyme lost approx. 40% of the baseline MA at 45°C already, while at approx. 55°C it became completely inactive. Control enzymes, calf rennet and mucorpepsin, were inactivated at approx. 60°C and approx. 70°C, respectively. Yu.P. Zagnitko [19] studied thermal stability of milk clotting preparations obtained by SA sedimentation from cultural liquid of *I. lacteus* (strain B-02), and noticed that the coagulant retained its high MA upon incubation at 25–40°C, with maximum MA (230 RU/ml) at 30°C. However, at heating temperatures above 45°C, inactivation of enzyme was observed. In the range of 45–60○C the inactivation degree of *I. lacteus* preparations (B-02 strain) was directly proportional to the exposure.

Results of comparative thermal stability study contrasting *I. lacteus* coagulant, ICS CR, and CP are presented in Fig. 3. At 40°C, all the test enzymes retained their MA around 100%. *I. lacteus* coagulant and ICS CR were almost completely inactivated at 60°C, residual MA amounting to 0.8% and 5.1% of the baseline value. However, the dynamics of inactivation for these enzymes within the range of 40–60°C is different. At 50°C, the *I. lacteus* coagulant was inactivated by 55%, while ICS CR retained almost 90% of the baseline MA. The highest thermal stability was demonstrated by CP, as its activity was reduced by 96% only after heating at 70°C.

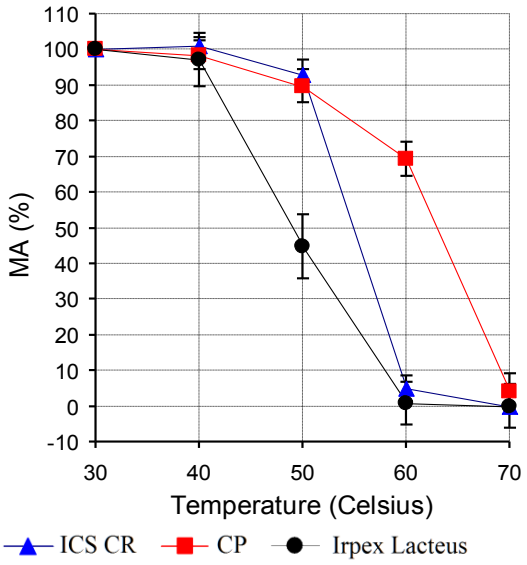

Fig. 3. Milk-clotting activity dependence on enzyme heating temperature (thermal stability).

According to the classification elaborated by "Chr. Hansen" company, microfungal milk coagulants used in cheesemaking are subdivided into several types: L type, natural (thermostable) enzymes; XL type, thermolabile coagulants obtained by oxidation; XP type and XLG type, these are chromatographically purified XL preparations, free from amylase/lipase/cellulase admixtures [2]. In accordance with this classification, the *I. lacteus* coagulant should be classified as XL type, albeit its thermal lability is a natural property of this enzyme, and not a result of chemical modification.

Thus, as far as thermal lability is concerned, the *I. lacteus* milk-clotting enzyme is comparable to ICS CR, and, consequently, complies with the requirements of the cheesemaking industry.

MA dependence on pH level of the substrate. An enzyme used for production of most varieties of cheese shall prove efficient in clotting milk within the pH range of 6.4–6.7.

Variations of pH level of the milk produce certain changes in electrostatic and hydrophobic properties of the milk casein micelle. As pH level grows and is distanced from the casein pI, their cumulative negative charges increase. As a result, forces of intermicellar electrostatic repulsion are also increased. At the same time, casein-casein hydrophobic interactions are diminished, which increases clot formation time during rennet coagulation of the milk. Therefore, whenever pH level of the milk mixture increases within the range of 5.0–7.0, rennet clotting time becomes slower.

Yet another factor affecting the MA of an enzyme is the optimum pH which depends on the substrate nature. Polyporopepsin, chymosin, and pepsin are acid proteinases. That is why, as the substrate is alkalized and its pH is distanced from the pH optimum of the test enzymes, clotting duration should increase.

I. lacteus coagulant and ICS CR are similarly dependant on pH changes in their MA (Fig. 4).

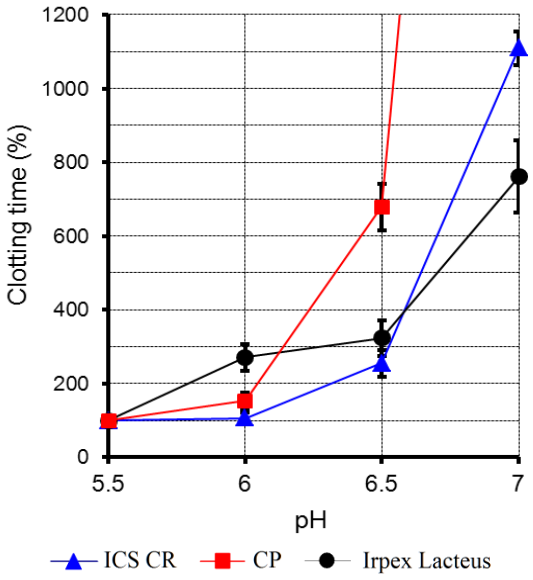

Fig. 4. Dependence of clotting time on pH.

Both preparations demonstrate high clotting activity within the pH range of 6.0–6.5, and gradually become inactivated at pH 6.5–7.0. Such results seem coherent with the BRENDA [brenda-enzymes.org/enzyme.php?ecno=3.4.23.29] data, suggesting that the optimum pH of polyporopepsin lies within the range of 2.5–4.0, and partially coincides with the chymosin optimum of 3.7–4.9. The optimum of pepsin enzymatic activity lies within a more acid range of pH 1.8–2.2. Consequently, CP preparation starts losing its activity earlier: at pH 6.5, activity loss is considerable, while at pH 7.0 the enzyme is almost completely inactivated (at pH 7.0, clotting duration is increased by 46 times).

Thus, MA of *I. lacteus* coagulant dependence on pH within the range of 5.5–7.0 is similar to that of the reference milk milk-clotting enzyme, - calf rennet.

Methods for correcting the technological properties of microfungal milk-clotting enzymes. There are several methods for correcting MA, specificity, and thermal stability of microfungal enzymes in cheesemaking. An attempt to influence one technological parameter usually results in modification of the others. Thus, although thermal stability of *I. lacteus* coagulant does not require any adjustment, we shall consider main methods and examples of changing the technological parameters of a microfungal milk-clotting enzymes in a complex.

Thermal stability of mucorpepsins is known to be positively related to the degree of glycosylation. In order to reduce the glycolysation of coagulants belonging to the *Rhizomucor* genus, several methods of chemical modification were suggested: oxidation, nitrification, acylation, carbamylation, periodate-induced and enzymatic deglycolysation [2]. D.A. Cornelius [24] introduced a method for reducing thermal stability of *R. miehei* and *R. pusillus* proteinases by oxidation run with hydrogen peroxide or photosensibilized oxidation in presence of a colorant. Oxidation does not lead to considerable loss of MA, and is currently widely used in order to reduce thermal stability of *R. miehei* coagulants [2].

Acylation by acid anhydrides significantly enhances MA of mucorpepsin produced by *R. pusillus* [24]. Maximum effect was observed during exposure of *R. pusillus* coagulant to maleic anhydride, however, that agent also inhibited MA in *R. miehei* mucorpepsin. T. Higashi et al. [26] treated *R.* pusillus coagulant with succinic anhydride, and achieved an increase in its specificity due to increased MA and MA/PA ratio. Oxidation by hydrogen peroxide run in presence of maleic anhydrid is used in order to reduced thermal stability and to prevent MA loss in *R. pusillus* mucorpepsin [27].

Methods for enzymic modification of microfungal coagulants have also been reported. Endoglycosidase H treatment for native mucorpepsin *R. miehei* ("Hannilase"), its partially oxydized version ("Modilase S") and *R. miehei* coagulant preparation ("Novoren XL"), derived from a heterologous producer (*Aspergillus oryzae* (Ahlb.) Cohn), allowed to obtain deglycosylated proteases with enhanced cheesemaking properties. Milk-clotting activity of Hannilase and Modilase S would increase by over 30%, and in case of recombinant mucorpepsin *R. miehei* produced by *A. oryzae* ("Novoren XL"), increase in specific activity amounted to approx. 45%. Besides, overall PA would decrease by ~10%, specificity (MA/PA) would increase, thermal stability would go down, pH range for MA would be more extensive, and cohesion of coagulants with milk clot would improve [28, 29].

Another potential method for influencing technological properties of microfungal coagulants is induced mutagenesis. Several focal substitutions can be singled out that might positively influence the technological properties of mucorpepsins. A101T or G186D replacement in a molecule of *R. pusillus* mucorpesin and mutant expression in *S. cerevisiae* allows to enhance significantly thermal stability of the coagulant. Curiously, the same result is ensured by any amino acid substitution in G186 position. Mutant enzyme with two simultaneous substitutions, A101T and G186D, proved to be even less thermostable than mucorpepsins with single replacements, and at the same time, did not show any decrease in MA/PA ratio. Focal substitutions of E19V and Q266E in a mucorpepsin derived from a wild strain of *R. miehei* (CBS 182-67) result in a 4.6 times specificity increase, and a 1.51% growth in cheese output, which is a very significant achievement in cheesemaking [2]. Site-targeted mutagenesis method allowed to obtain a mucorpepsin (the producer being *M. pusillus*) with two simultaneous amino acids substitutions, G186D and E13D, which led to an increased enzymatic specificity due to a higher MA [13].

One of the above listed methods for reducing high non-specific PA in mucorpepsin might lead to a higher specificity (MA/PA) of the *I. lacteus* coagulant, which in future might allow for its application in cheesemaking.

Results. We carried out a complex study of technological properties of a milk coagulant produced by a higher basidial fungus, Irpex lacteus (*I. lacteus)*, and evaluated each parameter in accordance with its suitability for cheesemaking industry application.

I. lacteus coagulant is shown to be on par with natural ME of animal origin used in dairy industry in a series of technological parameters, such as thermal stability, MA dependence on pH level, and Ca^{2+} ions content. Practical application of the milk-clotting enzyme produced by *I. lacteus* is considerably challenged by its low MA and high overall PA. We need to achieve a considerable increase in MA of the coagulant in order to remove this obstacle. There are a number of strategies to be used in order to obtain preparations with a high specific activity: selecting highly productive strains, perfecting cultivation methods, achieving optimal purification, and/or using methods of chemical, biochemical, and genetic modification.

The data we obtained might be used in further work aimed at studying and improving technological properties of a milk-clotting enzyme produced by the *I. lacteus* fungus.

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METHODOLOGICAL ASPECTS AND OPERATIONAL EXPERIENCE OF THE NEW BAA WITH TARGETED FUNCTIONAL PROPERTIES

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Abstract: Specialized food products development, including biologically active additives (BAA), is one of the priority areas in the realization of the healthy eating program and the food and pharmaceutical industries development in the Russian Federation. A lot of attention is paid to the proof of effectiveness and functional orientation of the concerned foodstuff. Dietetic therapy with the use of BAA is considered to be the most available way to improve the modern man's nutrition and health. The aim of this work was to develop and confirm specialized food products' practical application through clinical trials and mass production organization. Specialized products for dietetic nutrition and protective diet such as BAA ("Energopan", "Cleopanta", "Green Star") and the "Light mood" bar were developed. Methodological aspects of the new types of the specialized products' creation were described. Prescription formulas with account taken of pharmacological characteristics of their active principles were scientifically proven. Regulated indices of nutrition value including recommended amount of consumption of the developed product and safety criteria that allowed establishing duration of realization were identified. Clinical trials of the specialized products were conducted: "Light mood" bar and the BAA "Green Star" were included in study groups' ration and their biomedical measurements characterizing metabolic care were analyzed. Observational results allowed to recommend the "Light mood" bar for prophylaxis and treatment of the constipation in combination with other somatic diseases and the BAA "Green Star" for digestion health improvement in the function of energy absorbent and improvement of the overall level of metabolism. Developed products' formulation and technology were tested under production conditions in the SPA "Yug" and the "Art-Life" company (Russia).

Keywords: prescription ingredients, biologically active additives, functional properties, effectiveness, clinical trials

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INTRODUCTION

Nutrition is one of the most important factors determining the health of the population. Healthy eating helps to prevent diseases, to prolong life, to improve working capacity, to enable adaption to environmental conditions. At the same time there are negative trends in the health status of the population. The majority has nutrition disorders due to both poor nutrient materials intake, especially vitamins, macro- and micronutrients, and complete proteins, as well as their irrational ratio. There is every reason to believe that the most rapid, economical and scientifically grounded way to solve the nutrition rationalization problem is the widespread use of specialized products, including BAA [12, 14].

The RAND Corporation presented the results of the evaluation of the world community science and technology and social and economic development prospects for the period until 2020, including "challenge-response" mechanism. The driving forces of the modern innovation process and obstacles on its way and various scientific and technological development priorities were analyzed. Among the latter the main attention is given to healthy lifestyle and ultimate nutrition ensuring and prevention of common conditions of the infectious and nutritional (non-infectious) nature. In relation to this trend the great importance have development issues of specialized products, including BAA, study of the composition, pharmacologic orientation and functional properties of starting materials. They are determined by science and technology priorities of the modern nutritiology. Also the importance is given to the efficiency evidence and functional orientation of the concerned food products by conducting clinical trials. In such case dietetic therapy is considered to be the most available way to improve the modern man's nutrition and health [13, 15, 20].

OBJECTS AND METHODS OF STUDY

The objects of research were starting materials used in development of prescription formulae for the new BAA types such as “Energopan”, ‘Cleopanta”, “Green Star”, “Light mood” bar, and their laboratory and industrial samples.

Standard and special methods of quality and safety research of the developed products were used with the help of spectophotometery, stripping voltammetry, and liquid chromatography. Tests were carried out on the basis of accredited laboratories of the SPA “Yug” (Biysk, Russia) and “Art-life” company (Tomsk, Russia).

For the “Cleopanta” identification the panto-hematogen authenticity was conducted according to the developed technique. 10 ml of Feling reagent are added to a solution of 0.2 g of the capsule contents in 5 ml of the water and brought to boil. Glucose flocculates in russet. The resulting solution is put to test according to GOST (All Union State Standars) 30418-96 to isolate triglycerides’ fatty acid composition that occurs in plasma lipoproteins.

The content of pantohematogen is determined spectrophotometrically by the level of hemoglobin at a wavelength of 540 nm. About 0.8 g of the capsule contents is placed in a mortar, then 5 ml of 0.5% aqueous ammonia solution are added and everything is pestled until dissolved. The solution is quantitatively transferred to a 50 ml volumetric flask with wash of 0.5% ammonia solution (10 ml three times). The resulting solution is kept at room temperature in a shadowed place with intermittent mixing for an hour and filtered through cheesecloth or cotton plug, the filter is washed with 0.5% ammonia solution to bring the total filtrate volume to 50 ml. The optical density of the solution is measured on a spectrophotometer at a wavelength of 540 nm in a cuvette with 10 mm layer thickness by using the 0.5% ammonia solution as a blank solution. The optical density should be at least 0.4.

The data obtained from examination and monitoring of 30 patients with functional constipation of the 1-2 type was used when clinical trials of the “Light mood” bar were conducted. The diagnosis of functional constipation was made by a gastroenterologist after patients’ cohort study on the basis of historical studies, results of physical examination, laboratory and instrumental methods of research as well as test results of diagnosing the quality of life over time during treatment. Patients with FC were given 1-2 “Light mood” bars for 15 days before bedtime. Differences between parameters of comparison were considered statistically dissimilar at $P<0.05$. The study was conducted in accordance with the principles of the World Medical Association's Declaration of Helsinki (as revised in 2000 with the explanations given at the WMA’s General Assembly in Tokyo, 2004), the rules of International Conference on Harmonisation Good Clinical Practice (ICHGCP), the ethical principles stated in the EU directive 2001/20/EU and with the requirements of the Russian Federation legislation. Each patient signed the "Informed consent" to participate in the trials. The research was carried out at the central research laboratory of the Kemerovo State Medical Academy and at the “Medical practice” clinic (Kemerovo, Russia).

The average age of patients was 38.5 ± 8.3 years. The initial state analysis of the patients with FC was carried out according to objective research, on the basis of subjective complaints registration and gastrointestinal status in accordance with the Rome III criteria, 2006. The diagnosis of FC was made based on the exclusion of organic pathology of the abdominal cavity organs after laboratory, endoscopic, radiological and morphological diagnostic. All patients were offered the Nottingham Health Profile. Full-scale tests on effectiveness and functional orientation of the BAA “Green Star” were conducted on 25 volunteers. The study was carried out at the Siberian State Medical University’s Department of Internal Medicine, Faculty of Professional Development and Retraining.

The main group was made up of 25 volunteers without symptoms whose professional life related with vigorous physical activities as well as neuropsychic activities wasn’t interrupted: 11 drivers of public transport at the age from 36 to 47 and 14 managers at the age from 28 to 44 (4 men and 10 women). The control group consisted of 15 healthy volunteers of similar occupation, randomized by gender and age. The active treatment group took the “Green Star” bioactive complex outpatiently and the control group took multivitamin preparations.

RESULTS AND DISCUSSION

A series of specialized products of different functional orientation was developed. Regulated quality parameters and shelf life and storage conditions were developed; their efficiency and functional orientation evidences were proven.

Observance of the following methodological principles is required for specialized products (such as BAA) development:

– Prescription formula (its qualitative and quantitative composition) must be designed with consideration for macro- and micronutrients of certain groups and minor food items needs, as well as their synergistic effect on the metabolism of healthy and diseased organisms;

– Used ingredients and the product itself must meet the requirements in terms of safety and consumer preferences;

– Regulated nutritional value indicators must provide at least 10% of the daily nutrient requirement, providing the application of the recommended product amount;

– Developed products should be affordable for different groups of the population.

The prescription formula of the specialized products with account of active principles influence on healthy and sick person’s metabolic processes was scientifically grounded. In this context there is a good reason to consider the basic prescription ingredients which functional properties are presented in the existing literature and the results of their own research [2–10, 17, 18].

The description of the tested products’ basic prescription ingredients is given below.

The BAA "Cleopanta": glucose, licorice root (*Radices Glycyrrhizae*), dry pantohematogen (red deer, Manchurian deer or dappled deer blood dried up with a method of deep vacuum dehydration) and ascorbic acid.

The licorice roots (*Radices Glycyrrhizae*) have antioxidant properties, protect the body from free radicals, contain flavonoids – liquiritin, liquiritoside, isoliquiritin, neolikviritin, ramnolikviritin, uralozid, ramnoizolikviritin, which slow down body aging processes, prevent the development of atherosclerosis and cardiovascular disease. Glucose and ascorbic acid have synergistic properties in relation to the described metabolic processes.

Pantohematogen is a red dear's blood, taken in the final period of antlers growth (before ossification). Pantohematogen contains: essential and nonessential amino acids (lysine, arginine, histidine, 4-hydroxyproline, threonine, tryptophan, glutamic acid, proline, serine, glycine, alanine, valine, cystine, isoleucine), peptides, nucleic acid bases, lipids (phospholipids, triglycerides, sphingomyelin, lecithin), hormones, vitamins A and E, and a complex of various macro- and micronutrients (potassium, sodium, magnesium, ferrum etc.). Pantohematogen greatly contributes to the organism resistance to negative effects of various adverse environmental factors, improves metabolic processes, slows body aging processes, improves immunity, and increases the body's resistance to bacterial and viral infections. It also reduces fatigability, improves mental capacity and central nervous function.

The BAA "Energopan": pantohematogen, rhizomes and roots of *Rhizoma et radices Rhodiolae roseae*, glucose and ascorbic acid.

Rhodiola rosea is a perennial flowering plant in the family Crassulaceae. It grows in cold regions of the world, including mountainous areas in Eastern Siberia and Altai. Rhodiola roots contain phenols, aromatic compounds, carbohydrates (glucose, fructose), organic acids (malic, oxalic, citric, succinic), essential oil, terpenoids, and flavonoids (total of about 86 components). Rhodiola rosea has anti-viral, tonic, antitumor, antibacterial, antimicrobial, antipyretic, anti-inflammatory and antitoxic effect.

The BAA "Green Star": spirulina, chlorella, apple pectin, kelp thallus, wheatgrass, farina, catalase, rosehips, Echinacea Purpurea, lactobacterium complex, tocopheryl acetate (vitamin E), ginkgo biloba extract, blueberry, green tea, superoxide dismutase, gelee royale and coenzyme Q10.

The basis of the complex is a balanced combination of spirulina and chlorella. Spirulina is a complete protein and it contains essential amino acids which constitute up to 65–75% of its weight. Spirulina's mineral composition distinctive feature is that it contains digestible chelated microelememts. Chlorella is rich in chlorophyll which is an effective disinfectant of natural origin; it strengthens cell membranes, stimulates the connective tissues formation, and accelerates the healing of erosions, ulcers and open wounds. Seaweed cell walls contain polysaccharides which together with wheat fiber and pectin improve the functioning of the gastrointestinal tract and intestinal peristalsis. Active substances in Echinacea have immunostimulatory effects and ginkgo biloba extract has beneficial effects on blood flow. The "Green Star" complex has antioxidant activity due to the active substances of rosehips, blueberry, green tea, enzymes and vitamin E.

The specialized product "Light mood": prunes, raisins, apple, fructose, senna extract, oat flakes (milled), gum-arabic, wheat bran, caraway (milled seed), potassium sorbate, citric acid.

Prunes contain fructose, glucose, saccharose, malic acid, oxalic acid, citric acid, salicylic acid, pectin, polyphenolic and nitrogenous matters, vitamins A, C, B_1, B_2, P, significant amount of significant amounts of potassium and phosphorus, less sodium, calcium, magnesium and ferrum.

Prunes have a beneficial effect on the gastrointestinal tract, helps to get rid of constipation and to normalize of the digestive system; it is useful for heart problems and high blood pressure's solutions, kidney diseases, rheumatism, liver diseases and atherosclerosis. It improves eyesight due to the high concentration of vitamin A.

Raisins contain beneficial carbohydrates, organic acids, dietary fibers, proteins, some fat, vitamins A, C, E, H, B-group, beta carotene, trace elements – zinc, selenium, iron, manganese, copper. They are especially rich in potassium, necessary for kidneys and the heart muscle, metabolism in the skin and neurotransmission, and maintenance of normal blood composition. Because of nicotinic acid they have a calming effect and regulate the nervous system. They help to eliminate toxins and fluid due to diuretic action. Organic acids contained in raisins are characterized by antioxidant and antibacterial properties.

Apples. Their nutrient composition explains their useful properties. Apples contain vitamins C, B_1, B_2, B_6, P, E, carotene, potassium, iron, magnesium, calcium, pectins, sugar, and organic acids. They also contain the following trace elements: phosphorus, manganese, sodium, sulfur, aluminum, boron, vanadium, iodine, copper, molybdenum, nickel, fluorine, chromium and zinc. They contribute to the normalization of the gastrointestinal tract and the digestive system, and they are used to prevent constipation and to increase appetite. Apples contain chlorogenic acid which contributes to the oxalic acid elimination and normal liver functioning.

Apple pectin plays a pivotal role in the formation of dejection. This circumstance, as well as evident irritant effect on the mechanoreceptors of the intestinal mucosa, plays a key role in the stimulation of peristalsis and the regulation of the motor function of entodermal canal. The lack of pectin in nutrition leads to gallbladder and colon dyskinesia.

Pectin substances adsorb exo- and endotoxins, salts of heavy-metal and radionuclides. Pectins, reacting with bile acids, reduce fat absorption and cholesterol. They suspend gastric emptying and thus slow down the absorption of sugar by coating the mucosa of the gastrointestinal tract. They have a cidal effect on opportunistic pathogens and the causative agents of acute intestinal infections without disrupting the balance of microflora. Pectins help to improve parietal

digestion and to normalize intestinal microbiocenosis with positive effect on skin condition.

Senna extract (*Cassia acutifolia Del*.). It contains anthraglycosides (sennosides A and B, rhein, aloe-emodin), flavonoid glycosides (isorhamnetin, kaempferol, kaempferide), organic acids (stearic acid, palmic acid, etc.), and phytosterol. Senna extract has laxative properties, improves motor function of the colon, and contains no bitter or tanning principles, that is why it doesn't increase appetite and doesn't cause constipation after laxative action.

The main active substances are anthraglycosides which under the influence of digestive enzymes and bacterial processes cleave into sugar and aglycone. The latter irritate receptors of the mucosa of the gastrointestinal tract increasing motion activity but not the secretions.

Field caraway (*Carum carvi*). The substances contained in the caraway fruits have beneficial effects on various body systems. They can relieve spasms of the smooth stomach muscles, biliary ducts, and gastrointestinal tract reduce the activity of enzymes in pathological processes in the stomach and intestine, which is beneficial for health because it reduces the processes of fermentation and putrefaction.

Cummin seeds can exert diuretic and expectorant action and have galactogogue effect. Their bactericidal action is proven. Caraway fruits improve the separation of bile and similarly affect the secretion of gastric juice and pancreatic enzymes; they increase appetite and have a calming effect.

Oat (*Avéna satíva*). It contains mucus and has a coating effect on gastrointestinal tract. Oat is the source of soluble and insoluble dietary fiber, which is able to absorb and remove toxins and to exert a prebiotic effect. Oat is rich in substances that are necessary to cleanse the blood vessels from atherosclerotic plaques, maintain normal cholesterol and blood sugar, and help to normalize weight (zinc, chromium, vitamins B and F).

Oat is made up of 60% of starch, 14% of protein, enzymes, vitamins A, B, E, choline, tyrosine, silicium, copper, trigonelline, sugar, calcium and phosphorus mineral salts. The amino acid composition is the closest to the animal protein, and a unique set of organic compounds is necessary for treatment of various hepatic diseases. Oat contains an enzyme which (like the pancreatic enzyme - amylase) helps in the absorption of carbohydrates.

Gum-arabic is an air-dried exudate from the trunks and branches of the Acacia Senegal Acacia Seyal. It is a polysaccharide with prebiotic properties. Natural water-soluble fiber which is not absorbed by the upper gastrointestinal tract, in the colon is completely fermented by bacteria to the formation of carbon dioxide and organic acids. The latter, lowering the pH of the intestinal environment, prevents the development of harmful microorganisms and contributes to their removal. Gum-arabic is a substrate for probiotic microorganisms that helps to develop their own colonization resistance of microflora.

Dietary wheat bran contains fiber helps to cleanse the gastrointestinal tract and improves its activity. Fiber is recycled by intestinal bacteria into substances that prevent colon cancer, displays the body of carcinogens and other toxic substances contained in food, and helps to control sugar and cholesterol in the blood by reducing their absorption in the intestine.

The present analysis of the functional properties of the prescription ingredients and their synergistic effects on metabolic processes led to the development of specialized products' prescription formula.

According to the results of the organoleptic and physico-chemical studies in the process of production and storage, specialized products' regulated indicators of nutritional value were defined on the basis of the recommended amount of their consumption.

Hygienic well-being, terms and modes of implementation of the developed products were established through the study of sanitary-hygienic and sanitary-toxicological parameters.

Examples are the studies on the safety of the BAA "Energopan" (Table 1) which meet the requirements [16].

Table 1. BAA "Energopan" safety analysis

Definable parameter	Permissible levels in normative documents	Results	Error
Microbiological attributes			
Coliforms in 0.1 g	Not allowed	Not found	–
E.Coli in 1.0 g	Not allowed	Not found	–
Pathogens including salmonella in 10 g	Not allowed	Not found	–
QMA&OAMO	Up to 10 000 CFU/gm	< 100	–
Staphylococcus aureus	Not allowed	Not found	–
Pesticides			
Aldrin	≤ 0.002 mg/kg	< 0.002	–
Heptachlor	≤ 0.002 mg/kg	< 0.002	–
HCH (sum of isomers)	≤ 0.1 mg/kg	< 0.005	–
DDT (sum of isomers)	≤ 0.1 mg/kg	< 0.005	–
Toxic metals			
Cadmium	≤ 1.0 mg/kg	0.031	0.012
Lead	≤ 1.0 mg/kg	0.32	0.13
Arsenicum	≤ 1.5 mg/kg	< 0.02	–
Mercury	≤ 0.2 mg/kg	< 0.02	–
Physical and chemical parameters			
Iron, in 100 g	40–60 mg	57.9	5.8

Encapsulated formulation of the BAA "Green Star" (mass 0.6 g) contains (mg in 1 capsule): spirulina – 150, chlorella – 50, apple pectin – 50, kelp thallus – 50, wheatgrass – 50, farina – 20, catalase – 20, rosehips – 15, Echinacea Purpurea – 10, lactobacterium comp-lex – 10, tocopheryl acetate (vitamin E) – 5, ginkgo biloba extract – 4, blueberry – 3, green tea – 3, superoxide dismutase – 3, gelee royale – 2 and coenzyme Q10 – 1.

Take 2 capsules 2 times per day with food to get regulated ingredients (Table 2).

BAA have following competitive advantages:
– They contain protein-bound iodine. This iodine is much better absorbed by the body, and the protein serves as an additional source of amino acids;
– Minerals included in the BAA are in chelate state, i.e. in conjunction with organic molecules that help to improve the absorption of the micronutrients;
– The complex provides soft enterosorption without causing the acceleration of intestinal peristalsis.

According to the expert's opinion the bioactive complex "Green Star" is recommended when there is/are:
– Intoxication of any origin;
– Infectious and parasitic diseases;
– Diseases of the digestive system;
– Allergies, immune system dysfunction;
– Prevention and correction of dysbacteriosis;
– Chronic non-communicable inflammatory diseases;
– The prevention and treatment of thyroid diseases related to iodine deficiency;
– Preventing hypoxia at high altitudes, low temperatures, heavy physical activities, extreme sports [1].

Period of validity is 3 years at temperature not above 18 ± 0.3°C and relative humidity not more than 75 % (with a margin of "strength" – 3 months).

The specialized product "Light mood" contains, mg/1 bar (15 mg): prunes – 8259.5, raisins – 3300, apple – 2700, fructose – 1600, senna extract – 900, oat flakes (milled) – 500, gum-arabic – 400, wheat bran – 300, caraway (milled seed) – 35, potassium sorbate – 4, citric acid – 1.5.

Take 1 bar per day before sleep (Table 3).

The product is marketed as dietetic (therapeutic and preventive) nutrition; it is a source of Senna anthraquinones and dietary fiber. The bar helps in purgation, the normalization of microflora and vermicular movement of the gastrointestinal tract. It has a relaxing effect, mild laxative, coating, anti-inflammatory and antiseptic effect, helps to eliminate toxins, toxic metals and radionuclides from the body [1].

Period of validity is 12 months at temperature not above 18 ± 0.3°C and relative humidity not more than 75% (with a margin of "strength" – 3 months).

The BAA "Cleopanta" formula: dry pantohe-matogen, licorice root (Radices Glycyrrhizae), glucose and ascorbic acid in proportion, g/1 capsule: 0.025, 0.060 and 0.005. Adults take 3 capsules daily with food (1.8 g) 3 times a day that provides intake of the regulated amount of micronutrients (Table 4).

Table 2. Indicators of the nutritional value of the recommended amount of the BAA "Green Star"

Parameter name	Content	Daily value		Satisfaction of the daily requirement in Russia/abroad
		In Russia (men and women, 18–59 y.o.), [11]	Abroad (men and women, 19–50 y.o.), [19]	
Vitamin E, mg	20	15	15	133/133
Iodine, mg	0.115	0.150	0.150	77/77
Coenzyme Q10, mg	4	30	no data	13/-
Dietary fiber, g	1.04	20	20	5/5
Lactic bacteria	$2*10^5$ CFU	–	–	–

Table 3. Indicators of the nutritional value of the recommended amount of the specialized product "Light mood"

Parameter name	1 bar (15 g) content	Daily value		Satisfaction of the daily requirement in Russia/abroad
		In Russia (men and women, 18–59 y.o.), [11]	Abroad (men and women, 19–50 y.o.), [19]	
Soluble dietary fiber, g	1.8	20	20	9/9
The content of total aglycones of anthracene series, in terms of methyl chrysazin, mg	9.0	10	No data	90/-

Table 4. Indicators of the nutritional value of the recommended amount of the BAA "Cleopamta"

Parameter name	Content in the recommended product's amount	Daily value (averaage)		Satisfaction of the daily requirement in Russia/abroad
		In Russia (men and women, 18–59 y.o.), [11]	Abroad (men and women, 19–50 y.o.), [19]	
Glycyrrhizic acid, mg	49	no data	no data	–
Iron, mg	2.7	10 (men) 18 (women)	8 (men) 18 (women)	27/34 15/15
Ascorbic acid, mg	135	90	82	150/165

The BAA is used as a source of flavonoids, vitamin C and iron. The pharmacological focus of the active principles of the biologically active components defines the functional orientation of the specialized product. Its use in the correction of metabolic disorders:

– Restores the hormonal balance in woman's body;
– Prevents premature menopause, slows the aging process;
– Increases the body's resistance in diseases of the female reproductive organs;
– Helps to normalize the process of puberty for young girls;
– Improves the functional state of the female reproductive system;
– Normalizes the metabolism of estrogen;
– Reduces the risk of hormone-dependent diseases of the female reproductive system;
– Helps to restore the menstrual function of the body [5].

The test results should contain the following data with acceptable deviations of each indicator ±10% when compared with samples obtained from the blood of cattle (Table 5).

Period of validity is 2 years in a dry shadowed place or household refrigerator (with a margin of "strength" – 3 months).

The BAA "Energopan" contains, g/1 capsule (0.8 g): rhizomes and roots of *Rhizoma et radices Rhodiolae* roseae – 0.150, pantohematogen – 0.025, glucose – 0.020, ascorbic acid – 0.005 (Table 6).

Take 3 capsules 2 times daily.

The pharmacological focus of the active principles of the biologically active components defines the functional orientation of the specialized product and its use in the correction of metabolic disorders:

– Performance incoordination;
– Contributes to a comprehensive correction of chronic fatigue syndrome;
– Helps to prevent fatigue;
– Normalizes the immune system activity;
– Reduces the level of anxiety;
– Improves cerebral circulation
– Improves concentration and memory;
– Normalizes and improves sexual function;
– Has a normalising effect in the treatment of sexual neurosis and functional impotence;
– Optimizes the central nervous system [5].

Period of validity is 2 years in a dry shadowed place or household refrigerator (with a margin of "strength" - 3 months).

The studies to confirm the effectiveness and functional orientation of the developed products were carried out on representative population.

Clinical trials of the specialized product, "Light mood" bar, were carried out.

Positive dynamics of patients' objective and subjective state was shown as a result of diet therapy. Good acceptability of the product was proven; the side effects of the internal organs, nervous and cardiovascular systems, and the skin were not revealed.

The table 7 shows that a positive dynamics of FC clinical implications was noted as a result of the specialized product taking.

General well-being of the majority of patients (87%) improved; defecation became more frequent (85% of patients had it daily in the morning). Others had defecation 1 time in 2–3 days, and only one patient had to add lactose.

Table 5. The authenticity of pantohematogen on comparative fatty acid composition of plasma lipoprotein triglycerides

Parameter name, % from FA sum	Original composition	The composition, obtained from the cattle blood
Lauric acid (12:0)	< 0.1	< 0.1
Myristic acid (14:0)	1.0	1.1
Palmitoleic acid (16:1)	2.6	1.1
Palmic acid (16:0)	20.6	18.5
Stearic acid (18:0)	17.5	20.0
Oleic acid (18:1)	28.6	21.3
Linoleic acid (18:2)	15.8	24.9
Linolenoic acid (18:3)	2.7	6.4
Arachidonic acid	6.6	3.3

Table 6. Indicators of the nutritional value of the recommended amount of the BAA "Energopan"

Parameter name	Content in the recommended product's amount	Daily value		Satisfaction of the daily requirement in Russia/abroad
		In Russia (men and women, 18–59 y.o.), [11]	Abroad (men and women, 19–50 y.o.), [19]	
Ascorbic acid, mg	120	90	82	133/146
Polyphenol (salidroside), mg	36	no data	no data	–
Iron, mg	2.4	10 (men) 18 (women)	8 (men) 18 (women)	24/30 13/13

Table 7. Dynamics of clinical symptoms in patients with PC after specialized product taking

Clinical implications	Before treatment n = 30	After treatment n = 30
General well-being improvement	100%	21%*
Defecation 1 time per day	0%	85%*
Defecation rarer than 1 time per day	25%	10%*
Defecation rarer than 1 time in 3 days	60%	5%*
Defecation rarer than 1 time in 5 days	15%	0%*

Note. * – difference is significant in comparison with results before treatment at $P < 0.05$.

The studies were carried out with the help of the Nottingham Health Profile which is one of the methods of the quality of life assessment.

The World Health Organization defines the quality of life as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".

The study of quality of life allows to identify factors that contribute to life improvement and finding its meaning.

Respondents were asked to reflect the impact of their health state on the activity in seven areas of everyday activities, reflecting the quality of life.

Energy level: "I quickly get tired" (24.00); "I do everything by forcing myself" (36.80); "I feel tired all the time" (39.20).

Sensation of pain: "I feel pain when I go up and down the stairs and walk" (5.83), "I feel pain when I stand" (8.96), "I feel pain when I change body position" (9.99), "I feel pain when I sit" (10.49), "I feel pain when I walk" (11.22), "I feel pain at night" (12.91), "I feel unbearable pain" (19.74), "I always feel pain" (20.86).

Emotional state: "The days are like a heavy burden" (7.08), "I often feel my critical state" (7.22), "I forgot when I was happy" (9.31), "I am often in a bad mood" (9.76), "Everything disappoints me" (10.47), "I wake up depressed" (12.01), "Anxiety wakes me up at night" (13.95), "I think I can't control myself" (13.99), "I don't want to live" (16.21).

Sleep: "I wake up too early" (12.45), "I can't sleep" (15.94), "My sleep is bad" (21.48), "I take pills to sleep" (23.14), "Most of the night I lie awake" (26.99).

Social isolation: "I think that it is hard to get along with people" (15.97), "I think it is hard to communicate with people" (19.36), "I think I don't have me loved ones" (20.13), "I feel lonely" (22.01), "I feel that I am a burden to people" (22.53).

Physical activity: "I find it difficult to reach out to others" (9.30), "I find it difficult to bend down" (10.57), "I find it difficult to go up and down the stairs" (10.79), "I find it difficult to stand for a long period of time" (11.20), "I can only move around the house" (11.54), "I find it difficult to dress myself (12.61), "I need help to go outside" (12.69), "I can't go outside" (21.30).

Respondents answer "yes" and put points if they have these restrictions and answer "no" and don't put points if they don't.

The test results are shown in the Table 8. In general the dynamics is positive.

On the basis of these data the following conclusions were made:

1. The taking of the specialized product, "Light mood" bar, by the patients with PC 1–2 type has a positive effect on clinical implications.
2. The patients with PC 1–2 type noted the improvement of mood and well-being, and therefore quality of life.
3. Dietetic therapy helps to normalize defecation, improve the quality of life of this group of patients.
4. The product has good acceptability and does not cause any side effects.

The obtained data give reason to recommend the product for a dietetic therapy to improve the body's resistance to adverse environmental influences, stressful situations, emotional and physical stress, and restoring normal defecation. This specialized product can be recommended in complex treatment and for prevention of constipation in combination with other somatic diseases in the compensation stage.

The clinical trials on the BAA "Green Star" were carried out. Despite the lack of evidence of any acute or chronic disease all patients at the beginning of the observation noted fatigue; in some cases there were complaints of somatic and vegetal character. There were certain signs of dysfunction of the digestive system, which included complaints about irregular bowel movements, belching or episodic nausea. The taking of specialized products allowed to change the pattern in the direction of decreasing of the presented complaints frequency, and in some cases "Green Star" had a more positive effect than a multivitamin complex, in particular, fatigue as one of the symptoms of intoxication significantly reduced (Fig. 1).

After taking the "Green star" volunteers from the experimental group noted restoration of normal levels of hemoglobin. A positive effect was observed in the form of improved pigment metabolism and functioning of hepatic cells in the main group of patients taking the "Green Star", according to terms of the content of bilirubin in the blood (Fig. 2).

Table 8. Dynamics of clinical symptoms in patients with PC after taking of "Light mood" bar

Parameter	Before treatment, points n = 30	After treatment, points n = 30
Energy level	45.3 ± 1.5	34.2 ± 1.3
Sensation of pain	23.5 ± 1.7	21.5 ± 2.4
Emotional state	74.2 ± 1.8	34.6 ± 1.5*
Sleep	34.3 ± 1.8	35.1 ± 1.3
Social isolation	24.2 ± 1.5	26.4 ± 1.7
Physical activity	32.1 ± 1.6	24.3 ± 1.3
Total	233.6 ± 4.1	176.1 ± 3.4

Note. * – difference is significant in comparison with results before treatment $P < 0.05$.

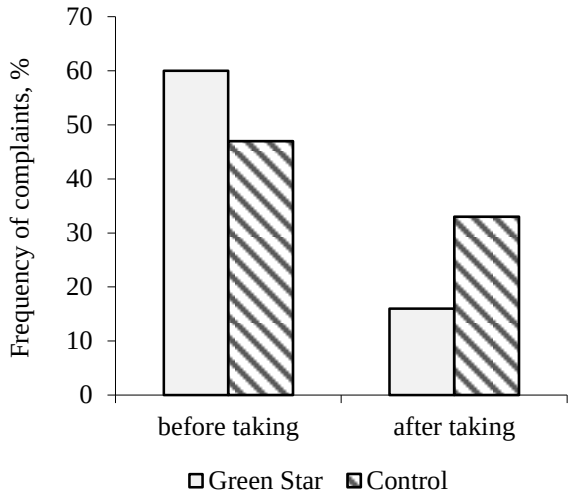

Fig. 1. Patients' fatigue frequency.

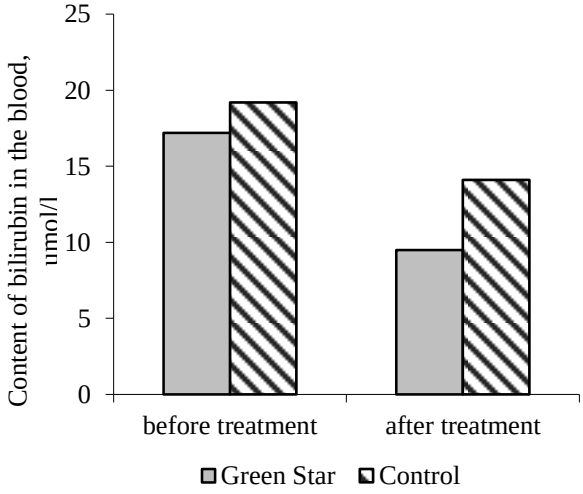

Fig. 2. The content of bilirubin in the blood.

After the coprological survey the following conclusions were made: the "Green Star" taking helps to restore digestion health and reduces the frequency of registration of undigested muscle fibers in the test material samples in 1.6 times in comparison with the benchmarks, and mucus in 2.2 times.

It was concluded that the BAA has the ability to improve digestive health, reduces overall toxicity, has a positive effect on metabolic processes. It can be taken as enterosorbent and to improve metabolic indicators.

Technical documents are developed and approved, the expert opinion of the Institute of Nutrition RAMS and Rospotrebandzor is received.

Developed products' formulation and technology were tested under production conditions in the SPA "Yug" and the "Art-Life" company (Russia), certified under the international standards ISO 9001, 22000 and GMP requirements, which allows, in addition to novelty and demand on the market , to present the products as innovative.

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STUDY OF PROCESSES OF OXIDATION OF LIPIDS AND PROTEINS OF HALF-SMOKED SAUSAGES AT THE STAGES OF TECHNOLOGICAL PROCESSING DEPENDING ON THE COMPOSITION OF CURES

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Abstract: The formation of qualitative characteristics of sausages is significantly effected by the oxidizing processes of fatty and protein fraction of meat raw materials. The orientation and intensity of processes depends both on the type of used raw materials and nutritional supplements and the parameters of each stage of technological process. The decrease in intensity of processes of peroxide oxidation of lipids is aimed at the increase in safety of ready-made products and lengthening of terms of their storage. This article presents the results of researches of effect of composition of cures on the anti-oxidizing potential of meat raw materials at the stage of salting and on the dynamics of oxidizing processes in half-smoked sausages in the course of cold storage. The properties of source raw materials, pork and beef and the properties of the combined mincemeat subjected to salting by salt and cures consisting of 70% of chloride of sodium and 30% of the composition of $KCl+CaCl_2$ in the ratio of 1 : 1 and also with the addition of yeast extract are studied. The effect of conditions of salting on the intensity of oxidizing changes of lipid fraction and haem pigments in half-smoked sausages within 20 days of storage at a temperature of (2–6)°C is established. It is established that the decrease in the amount of salt as part of cures provides an increase in the activity of antioxidant enzymes of meat raw materials and, as a result, a decrease in the intensity of processes of oxidation of lipids and haem pigments. The addition of yeast extract to the weight of raw materials in the amount of 2% provides the strengthening of inhibiting effect on oxidation processes.

Keywords: lipids, oxidation, antioxidant system, enzymes, catalase, peroxidase, meat, myoglobin, methmyoglobin, yeast extract

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INTRODUCTION

The oxidation of lipids of muscular tissue and proteins is the interconnected processes that have an effect on the organoleptic characteristics and nutrition value of meat and meat products. It is possible to claim that the decoloration of meat and the duration of storage are considerably caused by the complex of reactions between lipids and pigments. The rate of oxidizing changes of lipids of meat depends on a lot of external factors, including the parameters of technological processing, a packaging method, storage temperature and the use of antioxidants. The antioxidant system which consists of antioxidant enzymes and also of such components as glutathione, ascorbic acid, tocopherols, carotenoids, coenzyme Q 10 and others belongs to the internal protective factors which effectively protect the lipids of muscular tissue from oxidation. The most significant antioxidant enzymes of muscular tissue are catalase, peroxidase, superoxide dismutase and glutathione peroxidase which form an intracellular barrier for free radicals [1, 2, 3, 4, 5].

Catalase is a haem-containing enzyme which catalyzes hydrogen peroxide disintegration according to the following reaction $H_2O_2 \rightarrow 2H_2O+O_2$. The disintegration of hydrogen peroxide inhibits the processes of oxidation of oxymyoglobin and prevents the formation of H_2O_2-activated methmyoglobin which is considered as one of the important factors of lipidic oxidation of meat raw materials during storage [9].

The enzyme peroxydase has the same mechanism of action as catalase and is characterized by high specificity concerning such hydroperoxide compounds as methyl- and ethylhydroperoxide and methyl, ethyl and other alcohols. A protohaem which, unlike the haem groups of the majority of haemproteins, is very poorly bound with an apoenzyme is a prosthetic group in peroxidase. In the reaction catalyzed by the peroxidase $2H_2O_2+AH_2 \rightarrow 2H_2O+A$, hydrogen peroxide is reduced due to the bonds acting as the

donors of electrons, such as an ascorbate, quinones or cytochrome C [9, 15].

Peroxidase, as well as catalase, also performs the detoxication of active oxygen radical, with the formation of hydrogen peroxide of superoxide, at the same time they differ with the affinity to a substratum. In case of a low content of hydrogen peroxide, organic peroxides are mainly catalyzed by peroxidase, whereas catalases act at high concentrations [16].

Superoxide dismutase is a tetrameric protein consisting of four identical subunits, each of which contains one residue of selenocysteine which catalyzes the metabolism of hydrogen peroxide and a series of organic hydroperoxides, including cholesterin. Superoxide dismutase is an enzyme conjugated with catalase. There are conformational changes of oxymyoglobin in meat raw materials during the postlethal period as a result of change of pH and development of peroxide oxidation, as a result of which an anion radical with the formation of methmyoglobin is formed. The formed superoxide-anion is neutralized effected by superoxide dismutases according to the following reaction: $O_2+2H_2O \rightarrow H_2O_2$ [5, 7, 8, 10].

In turn, according to the mechanism of action, peroxidase and glutathione peroxidase supplement each other, providing the protection against the effect of peroxide oxidation of lipids at the stage of branching of chain reactions and formation of secondary peroxide products [17].

Glutathione peroxidase is a selenium-containing enzyme capable to inhibit and prevent oxidizing reactions in muscular tissue both during lifetime and during the postlethal period by the control of formation of free radicals of the hydroperoxides contained in raw materials [13]. The enzyme glutathione peroxidase provides the disintegration of active forms of oxygen including that of hydrogen peroxide, as a result of several consecutive reactions. At the first stage there is the oxidation of selenium-anions E-Se or E-SeH which are two catalystically active forms of selenocysteine contained in glutathione peroxidase (Reaction 1).

$$E\text{-}SeH + H_2O_2 \rightarrow E\text{-}SeOH + H_2O. \quad (1)$$

After the oxidation of active center of glutathione peroxidase, an enzyme reacts with a reduced glutathione with the formation of the enzyme-glutathione complex (Reaction 2).

$$E\text{-}SeOH + GSH \rightarrow E\text{-}Se\text{-}SG + H_2O. \quad (2)$$

Further, the formed complex reacts with another molecule of glutathione which, being oxidized, reduces the enzyme glutathione peroxidase (Reaction 3).

$$E\text{-}Se\text{-}SG + GSH \rightarrow E\text{-}SeH + GSSG. \quad (3)$$

In turn, glutathione peroxidase reduces once again the oxidized glutathione in the presence of NADP [14].

Glutathione, being a cofactor of glutathione peroxidase, is capable to have an independent antioxidant effect as the main low-molecular antioxidant of cell. Glutathione (GSH) is a tripeptide γ-glutamylcysteinylglycine which contains an unusual peptide bond between an amino group of cysteine and a carboxygroup of side chain of glutamate. Meat raw materials are characterized by a sufficiently high content of glutathione from 50 mg/kg to 200 mg/kg (175–600 μmol/kg) which is present both in the oxidized and reduced form [1, 17, 19]. The sufficient solubility in water solutions provides the fast penetration of glutathione through a cellular membrane. It is active in relation to a wide range of free radicals and products of peroxide oxidation of lipids – hydrogen peroxide and organic radicals ROO, single oxygen and also an extremely reactive hydroxyl radical OH [15, 18].

Endogenous antioxidant enzymes, especially catalase, glutathine peroxidase and peroxidase, are potential inhibitors of peroxide oxidation of lipids of meat. The activity of antioxidant enzymes is determined by a lot of factors, including the type of meat, type of muscles, duration of cold storage, and also the presence of various nutritional supplements both decreasing and increasing their antioxidant potential.

The type of meat, type of muscles and their anatomic origin have an effect on the content of lipids in general and on the fatty acid composition of meat raw materials, as well. The scientific researches testify that phospholipids play an important part in the development of peroxide oxidation of lipids both in raw and thermally processed meat [20, 21, 22]. Thus, in case of storage of raw beef and pork in a frozen state, the value of thiobarbituric number was higher than in the frozen fowl, which is the result of high content of haem iron in it, as well. At the same time, it is established that thermally processed chicken meat is more susceptible to peroxide oxidation of lipids than beef and pork are. Thus, 90% of malonic aldehyde are formed of polynonsaturated fatty acids of phospholipid fraction of chicken meat and provide the formation of taste of rancidity. On this basis, it is possible to say that the intensity of peroxide oxidation of raw meat, first of all, effect the content of haem pigments and activity of catalase whereas the content of polynonsaturated fatty acids is the main factor that determines the lipid oxidation in meat products [3, 23, 24, 25].

The nutritional supplements which are traditionally applied in technology of meat products can decrease the antioxidant potential of enzymes. One of such supplements is salt (sodium chloride) which has a positive effect on the formation of functional and technological and flavoring characteristics of meat products, and also inhibits the development of spoilage microorganisms. At the same time, sodium chloride in the concentration, applied in the technology of meat products, accelerates the oxidation of myoglobin, having a negative impact on the color of raw meat, and also provides the oxidation of lipids [26]. The results of works by Lee S.K. and others testify that in the presence of 2% of salt the activity of catalase, glutathione peroxidase and superoxide dismutase in its presence decreases by 8%, 32% and 27%, which can provoke the peroxide oxidation of lipids [35]. Sarraga, Karreras and Regueiro, studying the effect of concentration of salt on the activity of glutathione peroxidase, established that in the samples with 2% of

sodium chloride the activity of enzyme was higher than that in the samples with 3% of salt [33]. Therefore, a decrease in the level of addition of sodium chloride in meat products should be considered as one of the possible methods of decrease in prooxidant effect of sodium chloride.

It is especially urgent in view of the fact that the overconsumption of table salt as a sodium source provides the development of cardiovascular diseases [28, 29]. Today the production of foodstuffs, including meat products, with the lowered content of sodium is one of the priority directions of food technology. This tendency can be realized by such methods as the decrease in the formulation quantity of salt, the partial or complete replacement of salt by other substitutes and the use of intensifiers of flavor and spices. Chlorides of potassium, calcium, more rarely – chloride of magnesium and ammonium [30, 31, 32] gained the widest spread as substitutes of salt.

The positive effect of substitutes of salt on the quality and safety of meat products can be due to their capability to affect on the activity of antioxidant enzymes in meat. The researches by Hamid Reza Gheisari and Hossien Motamedi established that the replacement of salt by potassium chloride has no significant effect on the activity of catalase and glutathione peroxidase. The most significant factor is the value of ionic force. Thus, during the increase in ionic force from 0.175 to 0.7 the activity of catalase in beef and fowl decreases, respectively, by 3.2% and 7.5%, whereas the activity of glutathione peroxidase decreases by 14.6% and 32%, respectively. Along with it, it is established that the absolute values of activity of antioxidant enzymes were higher in the samples of beef and fowl processed by potassium chloride [27]. Similar results are received by Hernandez and others [34].

Nevertheless, the analysis of available information testifies that the data about the factors effecting the activity of antioxidant enzymes in meat raw materials are not enough.

The purpose of their own researches was the study of effect of composition of salting mixtures on the activity of antioxidant enzymes as the factor of regulation of intensity of oxidation of lipids of sausage mincemeat in the course of salting and during the storage of sausages.

OBJECTS AND METHODS OF STUDY

The objects of research were second-grade beef and semifat pork stored in the frozen state no more than 3 months. The meat raw materials were defrozen up to the temperature (-1 ÷ +1)°C, crushed and mixed in the ratio of 1 : 1 with the addition of sodium chloride 3% (the control sample is Sample K). In the test samples 30% of sodium chloride were replaced by the mixture KCl+$CaCl_2$ in the ratio of 1 : 1 (test sample A1). For the purpose of strengthening the antioxidant effect, yeast extract in the amount of 2% was added to the weight of raw materials as a source of glutathione in another test sample (Sample A2). The prepared samples were cured at a temperature of (0–4)°C for 48 hours with sampling in 24 hours. Half-smoked sausages with the formation of artificial protein coat of mincemeat were produced of cured mincemeat. The depth of oxidizing changes was estimated in sausages by the value of thiobarbituric number within 20 days of cold storage.

Determination of activity of peroxidase using the colorimetric method based on the determination of rate of reaction of oxidation of benzidine before the formation of blue coloring of its oxidation in the presence of peroxide and peroxidase [36].

Determination of activity of catalase using the spectrophotometric method based on the determination of rate of disintegration of hydrogen peroxide by the catalase of studied sample with the formation of water and oxygen [37].

Determination of total of pigments using the method of Lee B.J., Hendricks D.G. and Cornforth D.P. based on the extraction of meat pigments by water solution of acetone and the subsequent measurement of optical density of extract using the spectrophotometer SF PE-5400UF with the wavelength of 640 nanometers concerning muriatic acetone [38].

Determination of content of methmyoglobin using the method of Krzywicki and others based on the extraction of pigments by ice phosphatic buffer solution with the subsequent measurement of optical density of solution with the lengths of waves of 525, 545, 565 and 572 nanometers [39].

Oxidizing spoilage of lipids of meat raw materials by the determination of thiobarbituric number (TBN) using the distillation modified method of Tarlagis B. with the use of a sulfanilic reagent [40].

Color characteristics of products in the system Lab using the non-destructive testing method with the use of spherical color comparator KTs-3, which can work in the mode of comparator and spectrophotometer. The preparation of samples consisted in the cutting of slices of samples of the correct form from 3 to 5 mm thick with a plain surface of cutoff and without emptiness. The measurement of intensity of light reflection from the measured samples throughout the whole visible range of wavelengths and the summing of intensity of light reflection in case of the selected ordinates during the operation in the mode of spectrophotometer are performed, which is achieved by the use of integrating light filters – orange, green and blue – which are fixed in the instrument. When operating automatically, the instrument calculates the values of chromaticity coefficients (X, Y, Z) in the presence of source C reproducing the conditions of day lighting. Taking into account the coordinates of chromaticity of the source (X_0, Y_0, Z_0), the calculation of color indicators in the system of Lab is performed using the following formulas:

$$L = 116 \cdot (Y/Y_0)^{0.33} - 16,$$

$$a = 500 \cdot \left[(X/X_0)^{0.33} - (Y/Y_0)^{0.33} \right],$$

$$b = 200 \cdot \left[(Y/Y_0)^{0.33} - (Z/Z_0)^{0.33} \right].$$

The color saturation or brightness is calculated using the following formula:

$$S = \left[(a)^2 + (b)^2 \right]^{0.5}.$$

Hue is determined using the formula:

$$H = arctg(b/a).$$

The integrated assessment of identity of coloring is performed on the basis of indicator of full color distinctions that are calculated using the following formula:

$$\Delta E = \left[(\Delta a)^2 + (\Delta b)^2 + (\Delta L)^2 \right]^{0.5},$$

where *L* is the lightness, *a* is the redness degree, *b* is the blueness degree.

The content of nitric oxide pigments is determined using the method based on the extraction of pigments by water solution of acetone of homogenized sample weight and the subsequent measurement of optical density of extract using a Spekol spectrophotometer in case of wavelength of 540 nanometers concerning an 80% water solution of acetone [43].

RESULTS AND DISCUSSION

The oxidation of lipids is a chain reaction which consists of the initiation, development and break of chain. The mechanism of oxidation proceeds under the influence of both internal and external factors, such as the concentration of prooxidants, endogenous bivalent iron, myoglobin, enzymes, pH, temperature, ionic force and the reaction of consumption of oxygen and fatty acid composition of meat.

The intensity of process of oxidation in meat raw materials is regulated by various endogenous antioxidatic factors, such as reducing bonds (for example, ascorbic acid), natural antioxidants (carnosine, anserine, glutathione, α-tocopherol, etc.) and antioxidatic enzymes, including catalase and peroxidase.

The antioxidant potential of meat raw materials was estimated by the activity of catalase and peroxidase, and also by the content of glutathione and haem pigments in raw materials (Table 1). According to the obtained data, the activity of catalase and peroxidase of beef is on average higher than in pork by 30% and is 325 U/g and 12.4 U/g for beef and 255 U/g and 7.5 U/g for pork, respectively.

The high activity of enzymes in beef is, on the one hand, a consequence of a higher content of contained proteins, including sarcoplasmic proteins which are antioxidant enzymes. The hyperactivity of catalase in the studied raw materials, unlike peroxidase, is due to the high affinity of this enzyme concerning a substratum which hydrogen peroxide is. The formation of hydrogen peroxide in meat raw materials also occurs as a result of the oxidation of haem pigments, in particular, of myoglobin which being oxidized, turns into ferrylmyoglobin and initiates the peroxide oxidation of lipids with the formation of peroxides and hydroperoxides. On the other hand, the higher content of muscle fibers of oxidizing type in beef characterized by a high quantity of myoglobin also provides a higher activity of antioxidant enzymes [41].

Glutathione peroxidase, except catalase and peroxidase, belongs to the antioxidant enzymatic system of meat. An enzyme is one of the main antioxidants of cell that allows to consider it as one of the important participants of antioxidant system, active in relation to a wide range of free radicals and products of peroxide oxidation of lipids (hydrogen peroxide, organic radicals and a reactive hydroxyl radical). The main cofactor of glutathione peroxidase is glutathione the quantity of which is 10.42 mg/100g in beef and 8.06 mg/100g which is coordinated with the available literary data [42].

According to the obtained experimental data the total of pigments in beef is 240.04 mg/100g, at the same time the content of methmyoglobin is 42.4% of total of pigments that corresponds to 101.7 mg/100g. Proceeding from it, the total quantity of myoglobin is 138.34 mg/100g. The total of haem pigments in the studied pork at the initial point of time was 108.3 mg/100g with the content of methmyoglobin of 49.9%, that is 54.1 mg/100g, and the quantity of myoglobin is 54.2 mg/100g. The obtained results are coordinated with the available literary data concerning the muscular tissue of different types of meat raw materials, including beef, broilers and flounder [3, 5, 6, 15].

The assessment of effect of conditions of salting on the activity of antioxidant enzymes of meat raw materials was performed at the following stage of researches (Table 2). The research was performed using combined forcemeat consisting of beef and pork in the ratio of 1 : 1.

Table 1. Characteristics of the antioxidant potential of meat raw materials

Name of food raw material	Activity of enzymes, U/g of protein		Glutathione, mg/100g	Pigments		
				Total, mg/100g	Methmyoglobin	
	catalase	peroxidase			%	mg/100g
Beef	325.0 ± 11.3	12.40 ± 0.61	10.42 ± 0.49	240.04 ± 11.40	42.4	101.70 ± 5.24
Pork	255.0 ± 9.2	7.50 ± 0.28	8.06 ± 0.18	108.30 ± 8.42	49.9	54.10 ± 2.87

Table 2. Effect of conditions of salting on the activity of catalase, peroxidase and the content of haem pigments

Test sample	Activity of peroxidase, U/g	Activity of catalase, U/g	Total of pigments, mg/100g	Quantity of methmyoglobin, mg/100g
unsalted mincemeat	10.10 ± 0.34	295.00 ± 11.40	139.40 ± 6.87	59.60 ± 1.45
24 hours of brine treatment				
Sample K	7.40 ± 0.28	282.00 ± 10.30	139.60 ± 5.48	67.66 ± 2.31
Sample A1	7.70 ± 0.26	289.00 ± 9.25	138.70 ± 6.12	56.36 ± 2.97
Sample A2	7.80 ± 0.34	291.00 ± 9.65	139.70 ± 5.67	53.96 ± 1.97
48 hours of brine treatment				
Sample K	6.10 ± 0.42	276.00 ± 8.75	139.20 ± 4.27	69.34 ± 2.54
Sample A1	6.60 ± 0.37	281.00 ± 9.24	138.40 ± 5.74	58.96 ± 1.82
Sample A2	6.90 ± 0.21	288.00 ± 7.42	138.70 ± 6.54	57.55 ± 1.77

It is established that the salting of mincemeat by sodium chloride (Sample K) during 24 hours and 48 hours provides a decrease in the activity of peroxidase by 26.7% and 39.6%, respectively, as compared to the unsalted mincemeat. The decrease in the activity of catalase in Sample K was 4.4% and 6.4% as compared to the unsalted raw materials which is the result of effect of table salt on sarcoplasmic proteins which are the studied enzymes.

The decrease in the quantity of salt as part of cures provides some increase in the activity of enzymes. The replacement of 30% of chloride of sodium as part of cures by premix ($KCl+CaCl_2$) (Sample A1) has a positive effect on the activity of studied enzymes. Thus, an increase in the activity of peroxidase by 4.05% and by 8.2% occurs in Sample A1, as compared to Sample K, in 24 hours and 48 hours, respectively. The strengthening of activity of catalase, as compared to the control sample (Sample K) occured in Sample A1, thus, the increase in the activity was 2.48% in 24 hours and 1.81% in 48 hours as compared to Sample K.

The strengthening of reactive capacity of antioxidant enzymes of meat raw materials was provided by the use of yeast extract during salting, which is confirmed by the obtained results. Thus, the activity of peroxidase in Sample A 2 increased by 5.4% and 13.1% as compared to Sample K during the studied periods of salting. In turn, the increase in the activity of catalase was 3.19% and 4.34%, as compared to Sample K, in 24 and 48 hours of salting. The increase in the activity of peroxidase was 1.30% and 4.55%, as compared to the sample with a lower content of salt, in 24 hours and 48 hours, respectively. The obtained relation is explained by the fact that glutathione, being the substratum of true peroxidases, provides an increase in their activity. The results of researches allow to judge about a higher stability of catalase in the environment of chlorine-containing salts which is coordinated with the available literary data [27].

In view of the fact that haem pigments can act both as synergists and prooxidants in relation to the antioxidant system, the effect of salting on the processes of transformation of meat pigments were studied. It is established (Table 2) that the composition of cures and the duration of salting does not effect the total of pigments which remains almost the same for all the samples throughout the studied salting period, the revealed changes are within the limits of test error.

At the same time, it should be noted that against the background of constant presence of common pigments, changes in the ratio of various forms of myoglobin have been revealed. According to the obtained data, the curing of meat (the control sample) is followed by an increase in the content of methmyoglobin by 13.5% and 16.3% within 24 hours and 48 hours, respectively.

The decrease in the amount of chloride of sodium as part of cures provides a decrease in the amount of irreversibly oxidized form of myoglobin by 30%. The quantity of methmyoglobin in Sample A1 decreased by 16.7% and 14.97% as compared to the control sample during the studied salting periods. It should be explained by the decrease in the quantity of ions of chlorine in the test cures, which, according to the calculations, is 0.0427 mol in the control cures whereas it is 0.038 mol in the test cures. The addition of yeast extract, as the source of glutathione, to the studied systems, has an additional inhibiting effect on the process of oxidation of myoglobin. It is established that the quantity of methmyoglobin in Sample A2 decreased by 20.2% in 24 hours of salting and 17.0% in 48 hours of salting as compared to the control sample. The decrease in the quantity of methmyoglobin in Samples A1 and A2 should be regarded as occuring due to a higher activity of catalase and peroxidase. The decrease in the quantity of methmyoglobin is a positive prerequisite for stabilization of the processes of peroxide oxidation of lipids. There is an assumption that the intensity of oxidation of lipids depends on the level of haem pigments in raw materials, the high content of oxymyoglobin provides the formation of methmyoglobin and hydrogen peroxide, and, as a result, of ferrylmyoglobin, a compound which is a strong prooxidant.

Thus, the use of cures with the lowered content of salt at the stage of salting provides an increase in the activity of antioxidant enzymes and a decrease in the content of methmyoglobin as a prooxidant factor, at the same time the effect amplifies in the presence of yeast extract.

Further, the effect of composition of cures on the stability of lipid and protein fraction of half-smoked sausages in the course of cold storage was studied, as from the point of view of the process of hydrolysis and oxidation the quantity of initiating factors in meat products is much higher than that in the source raw materials. Haem iron, free fatty acids, free moisture, salt and also the modes of thermal treatment and storage conditions belong to them.

The sausages were cooled and stored in the refrigerator at a temperature of (2–6)°C within 20 days after thermal treatment.

The intensity of formation of primary products of oxidation was estimated by the dynamics of peroxide number (Fig. 1) as regards the reference value. The value of peroxide number of the control sample (Sample K) increased by 26.5% in 10 days of storage at low positive temperatures, and by 41.9% in 20 days. In the sausages with the partial replacement of salt (Sample A1) the value of peroxide number increased by 24.5% and by 36.1%, as compared to the reference value, in 10 days and 20 days, respectively. In the sausages with the addition of barmy extract (Sample A2) the increase in the peroxide number was 23.2% in 10 days of storage, as compared to the reference value, and 31.0% in 20 days.

It should be noted that the values of peroxide number for all the studied samples throughout all the process of storage remain in the values which do not exceed the established norm and hygienic standards of safety equal to in the course of storage no more than 10 mmol of O_2/kg.

The intensity of formation of secondary products of oxidation in sausages was estimated by the change of thiobarbituric number (TBN) that reflects the amount of formed malonic aldehyde (Fig. 2).

According to the obtained data, the accumulation of products of secondary disintegration of fats proceeds more intensively in the control sample, than in the test samples. Thus, the value of peroxide number increased by 13.5% in 10 days and by 36.5% in 20 days of storage, as compared to the reference value. The decrease in the amount of salt as part of cures and the addition of yeast extract provided a decrease in the rate of formation of secondary products of oxidation in the samples. It is established that in Sample A1 the increase in the value of peroxide number was 9.7% in 10 days and 15.3% in 20 days of storage, as compared to the reference value, whereas in Samples A2 the increase was 5.4% and 11.2% in 10 and 20 days, respectively, as compared to the reference value.

The relation of change of forms of pigments in half-smoked sausages in the course of storage (Table 3) is coordinated with the results of determination of activity of catalase and peroxidase depending on the composition of cures.

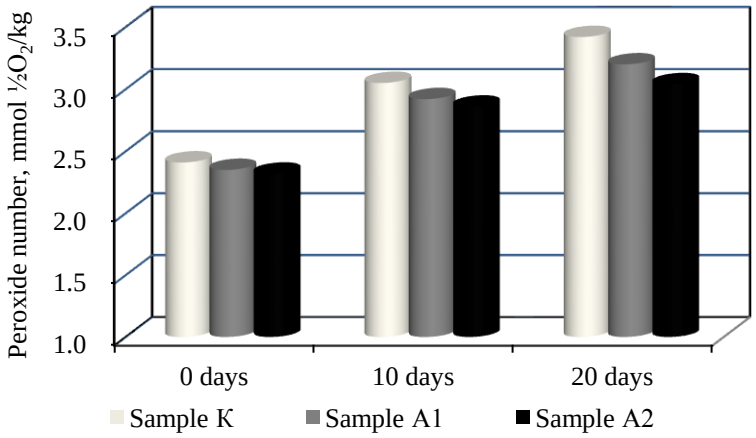

Fig. 1. Change of peroxide number in the course of storage of sausages.

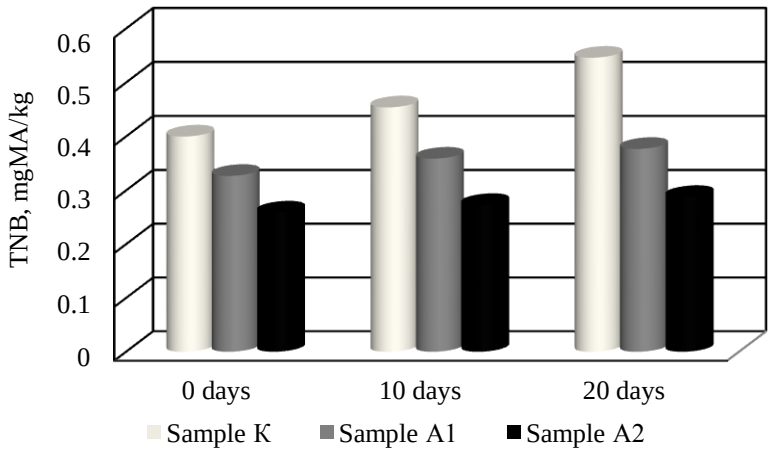

Fig. 2. Change of TBN in the course of storage of sausages.

Table 3. Dynamics of change of quantity of haem pigments in the course of storage half-smoked sausages

Test sample	Storage duration, days		
	0	10	20
Total of pigments, mg/100g			
Sample K	127.24 ± 6.35	126.96 ± 5.23	129.88 ± 6.12
Sample A1	124.72 ± 6.21	125.60 ± 4.89	126.00 ± 5.95
Sample A2	126.24 ± 5.62	124.30 ± 4.62	126.40 ± 5.58
Quantity of methmyoglobin, mg/100g			
Sample K	49.47 ± 1.68	52.38 ± 2.12	56.57 ± 1.97
Sample A1	45.47 ± 1.62	49.78 ± 1.97	50.61 ± 1.82
Sample A2	41.82 ± 1.72	43.00 ± 1.83	46.86 ± 1.79
Quantity of nitric oxide pigments, mg/100g			
Sample K	77.77 ± 2.54	74.58 ± 2.16	73.31 ± 2.34
Sample A1	79.25 ± 2.89	75.82 ± 2.45	75.39 ± 2.63
Sample A2	84.42 ± 2.71	81.30 ± 2.86	79.54 ± 2.45

According to the results of researches, the total of pigments in the studied samples of sausages was within the limits of 124–129 mg/100g irrespective of the duration of storage and the composition of cures.

The content of methmyoglobin in the control sample (Sample K) increased by 5.88% and 14.3% in 10 days and 20 days of storage, respectively. The increase in the content of methmyoglobin in half-smoked sausages containing combined cures (Sample A1) was 9.47% and 11.3%, as compared to the initial content, during the studied storage periods, which is less than in the control product with salt (Sample K) by 4.9% and 10.5%. In the sausages with combined cures and yeast extract (Sample A2) an increase in the content of methmyoglobin by 2.8% and 12.0%, as compared to the initial content, was revealed on the 10th and 20th days of storage, which is less than in Sample K by 17.9% and 17.1%.

The results of determination of nitric oxide pigments testify to the positive effect of decrease of amount of salt as part of cures in the combination with yeast extract on the stability of color of half-smoked sausages in the course of cold storage. It is established that in the control sample (Sample K) the quantity of nitric oxide pigments decreased by 4.1% and 5.73% in 10 days and 20 days of storage, respectively, whereas in the sausages with combined cures (Sample A1) the decrease in the amount of nitric oxide myoglobin was 4.3% and 4.8% during the studied storage periods, as compared to the initial content. This is 1.6% and 2.8% more than in the control product with salt (Sample K). The combination of cures with yeast extract as the source of glutathione (Sample A2) provided the stabilization of coloring of sausages. According to the obtained data, the content of nitric oxide myoglobin in Sample A2 decreased by 3.6% and 5.7% on the 10th and 20th days of storage, as compared to the initial content, and was higher than in the control sample by 9.0% and 8.5%. At the same time the coloring of Samples A1 and A2 with the studied compositions of cures was more stable and attractive after 20 days of cold storage. The objective confirmation to that were the data of study of coloring by a nondestructive method.

The color indicators are presented by integrated characteristics in the CIE system recommended by the International Organization for Standardization and related to the three-component theory of the colored sight according to which the perception of color with an eye of the person is caused by the existence of three types of cones in the retina: red, green and blue sensitive. The main color indicators in the CIE system are lightness (the amount of color), "*a*" and "*b*" – the degree of redness and blueness indicating the quality of color. These data are initial for the determination of more evident indicators – saturation (*S*) and hue (*H*).

Table 4 gives the results of determination of indicators of coloring of the studied samples.

Table 4. Indicators of color of test samples

Sample	Lightness (*L*)	Saturation (*S*)	Hue (*H*)	Redness index *a*/*b*	Indicator of full color distinctions Δ*E*
0 days of storage					
Sample K	55.07 ± 2.72	28.67 ± 1.23	0.56 ± 0.03	1.59 ± 0.07	–
Sample A1	55.84 ± 2.43	29.15 ± 1.43	0.56 ± 0.01	1.61 ± 0.05	1.37 ± 0.06
Sample A2	57.01 ± 2.01	30.49 ± 1.27	0.53 ± 0.04	1.70 ± 0.04	2.57 ± 0.02
10 days of storage					
Sample K	53.95 ± 2.86	28.42 ± 1.54	0.58 ± 0.02	1.52 ± 0.03	–
Sample A1	54.71 ± 1.97	28.72 ± 1.34	0.58 ± 0.04	1.54 ± 0.02	0.79 ± 0.04
Sample A2	56.62 ± 1.68	30.06 ± 1.74	0.54 ± 0.70	1.65 ± 0.05	3.25 ± 0.03
20 days of storage					
Sample K	53.08 ± 1.23	27.95 ± 1.26	0.60 ± 0.02	1.46 ± 0.03	–
Sample A1	53.98 ± 1.87	28.48 ± 1.32	0.59 ± 0.01	1.51 ± 0.04	1.13 ± 0.07
Sample A2	55.87 ± 1.67	29.43 ± 1.42	0.57 ± 0.03	1.57 ± 0.06	3.30 ± 0.05

The main color indicators in the CIE system are lightness, which characterizes the intensity of coloring and is a quantitative assessment of color. According to the obtained results, the intensity of coloring of test samples A1 and A2 had been higher by the beginning of storage by 1.4% and 3.52%, respectively, than that of control sample K.

In the course of storage a decrease in the value of indicator of lightness for all the studied samples of sausages was revealed. In 10 days of storage the decrease of lightness, as compared to the reference value, was 2.03% for control sample K, 2.02% for Sample A1, and 0.68% for Sample A2. In 20 days of storage there is still a tendency of change of the indicator, at the same time the test samples have a higher value of indicator of intensity of coloring.

An increase in the indicator of saturation in the test samples, as compared to the control sample, testifies to the improvement of color quality, that is to its higher purity with the almost equal color tone. The full color distinctions of test samples A1 and A2 are 1.4 and 2.5 of color sensitivity threshold, respectively, which suggests that the distinctions can be revealed visually.

The indicators of color tone and saturation suggest the condition of pigments in sausages. The color tone in the values up to $\pi/4$ characterizes the color of products as belonging to the red area, to the orange area in the range from $\pi/4$ to $\pi/2$ and to the yellow area when it approximates $\pi/2$.

According to the results of determination, the color tone of half-smoked sausages is 0.53, 0.53 and 0.56 for control sample K and test samples A1 and A2, respectively, by the beginning of storage, which corresponds to the red and orange area. At the same time, the values of indicator of color saturation allow to regard the coloring of all the samples of sausages as the saturated and pleasing one.

In the course of storage an insignificant decrease in the saturation of red color and a tendency to the shift of color tone to the orange area of range was revealed for all the samples. Thus, the color saturation of control sample K and test samples A1 and A2 decreased by 0.87%, 1.47% and 1.12%, respectively, as compared to the reference value, in 10 days of storage. The similar relation remains in 20 days of storage. It should be noted that the decrease in the amount of salt as part of cures, including that with barmy extract, provided the improvement of color characteristics of half-smoked sausages. The obtained results are coordinated with the experimental data of determination of quantity of haem pigments of meat.

For better understanding about the color of sausages the indicator "redness index" – the relation of degree of "redness" to the degree of "blueness" - has been determined. The higher the value of the indicator, the redder the color. According to the obtained data, the redness index for control sample K was 1.52 in 10 days of storage, which is lower than the reference value by 4.4%. The decrease in the index of redness for test sample A1 was 4.34%, as compared to the reference value, in 10 days of storage, and, respectively, 2.9% for Sample A2. This is 1.3% and 8.5% higher than in control sample K. In 20 days the decrease in the redness index, as compared to the reference value, was 6.2% and 7.6%, in samples A1 and A2, respectively, which is 3.4% and 7.5% higher than in control Sample K.

On the basis of analysis of obtained experimental data, it is possible to claim that the replacement of 30% of salt as part of cures by the premix $KCl+CaCl_2$ in the ratio of 1 : 1 and the addition of yeast extract to the formulation in the amount of 2% to the weight of raw materials provided an increase in the antioxidant potential of meat raw materials at the stage of salting and, as a result, the inhibition of processes of oxidation of lipids and pigments in half-smoked sausages in the course of storage and the improvement of their color characteristics.

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BIOTECHNOLOGY

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF CORN HYBRIDS GRAIN OF DIFFERENT PIGMENTATION

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Abstract: The article presents the results of studies of the chemical composition and antioxidant activity of corn hybrids grain of different pigmentation, created in All Russian Research Scientific Institute of Corn (Pyatigorsk). We included kernels of yellow corn as control samples (Uralskiy 150 hybrid). Test kernel samples were differently pigmented: kernels of white color (White), of orange color with yellow tops (Orange), of brown color with yellow tops (Brown), with grey tops and light yellow sides (Grey), of purplish-red color (Rubin). Botanical studies of differently-colored corn kernels revealed that the kernel color depends on the outer layer (pericarp) pigmentation. It was shown that corn kernels Orange, Rubin, and Grey contained three groups of biologically active compounds: flavonoids: 80, 70, 73 mg/%, carotenoids: 2.40, 1.70, 1.60 mg/%, and anthocyanins: 30, 120, 30 mg/%, respectively. Corn kernel samples Orange and Rubin demonstrated high antioxidant activity (1.0 and 0.76 mg/l in gallic acid equivalents, respectively). Antioxidant activity of common yellow corn (Uralskiy 150 hybrid) amounted to 0.09 mg/l in gallic acid equivalent, which is about ten times less than in the test samples. Thus, presence of biologically active substances – carotenoids, flavonoids, and anthocyanins – in differently-pigmented corn kernels is correlated with high antioxidant activity. Our results suggest that corn kernels Orange and Rubin might be recommended for further use in food industry producing products with high content of biologically active compounds.

Keywords: grain, corn, chemical composition, anthocyanins, flavonoids, antioxidant activity

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INTRODUCTION

Search for new types of plant-based raw materials containing biologically active substances contributes to diversification of feedstock used for healthy foods production, which corresponds to the basic provisions of the Strategy for Improving Food Products Quality in the Russian Federation until 2030. In order to achieve the Strategy goals, we should prioritize research on dietary profiles among the population, including prophylaxis of most common non infectious diseases, and development of food processing technologies that target food products quality improvement and promotion of healthy dietary habits [1].

In recent years, research in antioxidant activity of different plant-based raw materials and processed products seems to be particularly relevant. Findings show that antioxidant and antiradical properties of the products under investigations result from bioflavonoids these contain: flavonoids, isoflavonoids, anthocyanins, catechins, and other phenolic compounds. Low dietary antioxidants intake leads to free radical pathology, which is a concomitant condition of various diseases. Bioflavonoids, when included into the diet in physiological concentrations, demonstrate anti-allergic and adaptogenic properties, which enhances body's resistance to common illnesses [2, 3, 4].

Along with main nutrient elements synthesized during the corn growth, we can distinguish a separate class of biologically active substances that impart certain color to corn kernels. Xanthophylls (lutein and

zeaxanthin) are yellow pigments that, when accumulated, make the corn kernels yellow, and anthocyanins provide different coloring, from red to purple.

Anthocyanins have exceptional antioxidant properties: they can scavenge free radicals and protect cells against membrane damage. They are also known to strengthen capillary walls and to reduce edema naturally. Anthocyanins inhibit lipid peroxidation, thus protecting them against oxidative stress, which, in turn, contributes to cardiovascular prophylaxis. Health-promoting properties of anthocyanins are used in pharmaceutical industry, for producing various biologically active food supplements mostly used in ophthalmology. Anthocyanins are easily accumulated in retinal tissues, at the same time, they strengthen blood vessels and reduce capillary permeability and fragility within the retina. Anthocyanins improve fibers and cells structure in connective tissue, restore outflow of intraocular fluid and reduce intraocular pressure, which facilitates glaucoma treatment [2, 5].

A number of studies have been performed on specificity of anthocyanins accumulation in different parts of corn plant [6, 7], and on effects of anthocyanin corn extracts onto physiological functions of body systems [8].

However, complex research of locally-bred corn hybrids with differently pigmented kernels has not received sufficient attention in Russia.

Thus, a study of chemical composition and antioxidant activity of biologically active substances in differently-pigmented corn kernels shall determine types of corn of major biological value.

OBJECTS AND METHODS OF STUDY

Studies were conducted in the Corn Quality and Processing laboratory of the All-Union Scientific Research Institute of Corn, and in the scientific research laboratory for Nanobiotechnologies and Biophysics at the public access center within the North-Caucasus Federal University.

Sampling materials were differently-pigmented hybrid corn kernels developed in the All-Union Scientific Research Institute of Corn. A sample of yellow corn (hybrid Uralskiy 150) was used as control. Test kernel samples were differently pigmented: kernels of white color (White), of orange color with yellow tops (Orange), of brown color with yellow tops (Brown), with grey tops and light yellow sides (Grey), of purplish-red color (Rubin).

Feedstock materials were planted on testing fields of the All-Union Scientific Research Institute of Corn, in Predgorny district of Stavropol Krai in 2014–2015. Corn hybrids were seeded on 2-row-plots (S=7.8 m^2, with planting density of 4–5 plants per 1m^2), within the optimal seeding time, from April, 26 to May, 6.

Soils of the testing field were represented by common chernozem, thick, with heavy loam texture. Topsoil layer contained 55.96% of physical clay, with prevailing slit fraction (31.00 %), fine sand (21.69%), coarse slit (21.32%). As for humus content, these soils are low-humic.

Agricultural technology included plowing (to the depth of 23–25 cm), spring-tooth harrowing (BZT-1.0), tilling (KPS-4), marking (SUPN-8), two inter-row cultivations (KRN-5,6). Soil-applied herbicide Merline (150 g/ha) was introduced under preplanting tilling. Plant nutrition was administered at the phase of 8–10 leaves: Humistim (2.0 l/ha) + Nibid (0.4 g/ha) + Megamix (0.2 l/ha) (spraying device: MTZ-X2 – OP-200). Harvesting was performed manually, at firm ripe stage.

Kernels were soaked and sectioned manually with blades, then colored with an alcoholic phloroglucinol solution and sulphuric acid solution (50%). Temporary slides were mounted in glycerine solution and observed under a Biolam microscope (zoom x4; x10; x40). Sections were photographed with a digital camera SONY CS 5.1.

In hybrid corn kernels, protein, starch, and fats content was determined by infrared spectroscopy with InfraLUM FT-12 analyzer.

Elemental analysis of minerals was performed by MPU 4 – C method: semi-quantitative spectroscopy for minerals derived from carbon electrode crater of AC plasma arc (DG-2). Mineral spectrum was obtained with a spectrophotograph ISP-28.

In order to determine flavonoids content in hybrid corn kernels, we used ethanol (95%) as extracting agent. For quantitative analysis, we used differential spectrophotometry method based on flavonoid-aluminum chloride complexation, which allows to exclude any influence coming from related compounds.

In order to determine carotenoids content in hybrid corn kernels, we used ethanol (95%) as extracting agent. Optical density of the solution was measured by a spectrophotometer at 440 ± 5 nm wavelength, in a 10mm cuvette cell. Total carotenoids content (mg%) was evaluated in the amount of β–carotene.

In order to determine anthocyanins content in hybrid corn kernels, we used ethanol (50%) with hydrochloric acid (1%). Optical density of the permeate was measured by a spectrophotometer at 510 ± 5 nm wavelength, in a 10 mm cuvette cell. Total anthocyanins content was evaluated in the amount of cyanidin-3,5-diglucoside.

Antioxidants content was quantified in the "Cvet Jauza-01-AA" measuring instrument plotting the output to a gallic acid calibration curve.

An amperometric method for total antioxidants content estimation in plant-based raw materials was developed by Khimavtomatika SPA JSC and certified by the All-Russian Research Institute of Metrological Service. A stock solution of gallic acid (100 mg/l) was prepared. 1 ml of stock solution was poured into a volumetric flask, making up the volume to 10 ml with distilled water. The diluted solution was further mixed. The solution was prepared immediately prior to calibration. Preparing the calibrating solutions of gallic acid (0.2, 0.5, 1.0, 2.0, 4.0 mg/l). 20, 50, 100, 200, 400 µl of gallic acid solution (100 mg/l) were introduced into 10 ml volumetric flasks, making up the volume with distilled water, and further mixed.

Mobile phase (eluent) used was orthophosphoric acid solution (0.0022 mol/l). 150 µl of concentrated orthophosphoric acid were added to approximately 700 ml of distilled water in a 1 l volumetric flask. The volume was made up with distilled water and stirred.

Tested solution was introduced into the instrument, and electrical currents produced on the electrode were transduced into digital output displayed as peaks on the screen.

RESULTS AND DISCUSSION

Hybrid Uralskiy 150 is an early-season hybrid belonging to the normal corn variety (*indentata sturt.*). Kernels are small and flat, elongated in shape, with pronounced facets. There is an indentation on top of the kernel, the surface is smooth and shiny (Fig. 1a). Horny endosperm is only present on the lateral sides, while floury endosperm develops in the center and in the top of the kernel, beneath the indentation. Kernels are marigold yellow.

Seed coat consists of several layers of heavily deformed cells elongated in tangential direction. Aleurone layer is located just beneath the seed coat and consists of one layer of thick-walled cells, rounded-square in shape. The main part of the seed, endosperm, is located under the aleurone layer. Endosperm is made up of thin-walled cells with 4 to 6 straight facets; these cells are filled with starch. Endosperm cells nearest to the layer are smaller than other cells inside the seed (Fig. 2a).

White corn belongs to the normal corn variety (*indentata sturt.*). Kernels are large and flat, elongated in shape, with pronounced facets. There is an indentation on top of the kernel, the surface is smooth and lustrous (Fig. 1b). Kernels are white.

Other test samples (Orange, Broun, Grey, Rubin) belong to the flint corn variety (*Indurata sturt.*). Kernels are smallish, rounded and elongated in shape, compressed, both dorsal and ventral side concave, rounded tops, surface flat, lustrous (Fig. 1c–f). Horny endosperm is scarcely present only on the lateral sides of the kernel, while floury endosperm is present in the center and in the top. Seed coat consists of several layers of cells elongated in tangential direction, arranged in neat rows. Cross section reveals three distinct layers within the pericarp: these are exocarp, mesocarp, and endocarp. Exocarp is a single layer of cells elongated in tangential direction, with thickened walls. Mesocarp consists of 2–3 layers of parenchyma cells with thickened walls, elongated in tangential direction, and arranged in neat rows. Endocarp is made up of thick-walled cells elongated in tangential direction fused into the seed coat. Cell walls constituting the hull are heavily pigmented from dark orange to raspberry brown (Fig. 2 c–f).

Botanical studies of differently-pigmented corn seeds have revealed differences in internal organization of the seed and in the hull coloring. Color of a corn kernel depends on pigmentation of the outer layer (pericarp), while aleurone layer and endosperm are not relevant for this parameter [9]. Corn color might be modified by crossing with some differently-pigmented feedstock. However, pericarp being the maternal tissue of the seed, pollen of the paternal variety from the year of crossing does not participate in its formation. Dominance of paternal traits in kernel pigmentation depends on amount and composition of biochemical compounds introduced by the pollen.

In corn kernels, protein, starch, and fats content was determined by infrared spectroscopy with “InfraLUM FT-12” analyzer. Findings of the analysis are presented in Fig. 3.

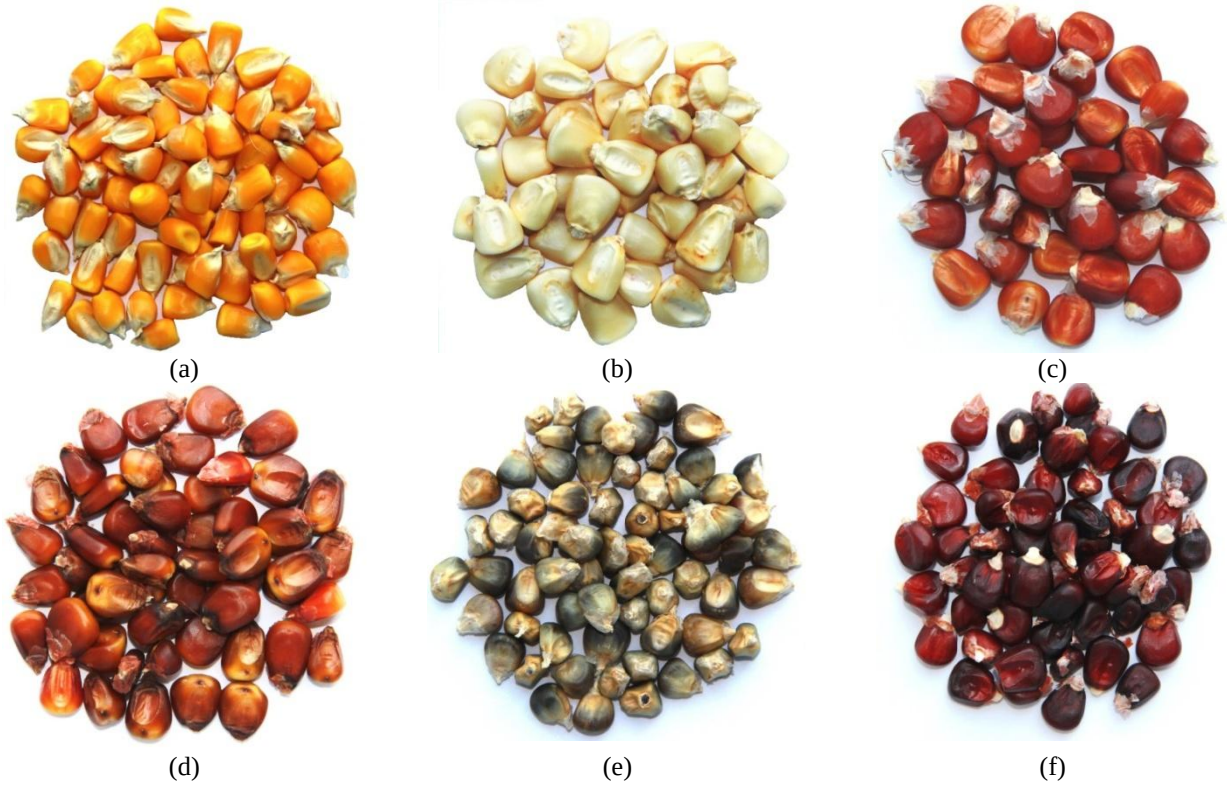

Fig. 1. Differently-pigmented corn kernels: (a) Uralskiy 150; (b) White; (c) Orange; (d) Brown; (e) Grey; (f) Rubin.

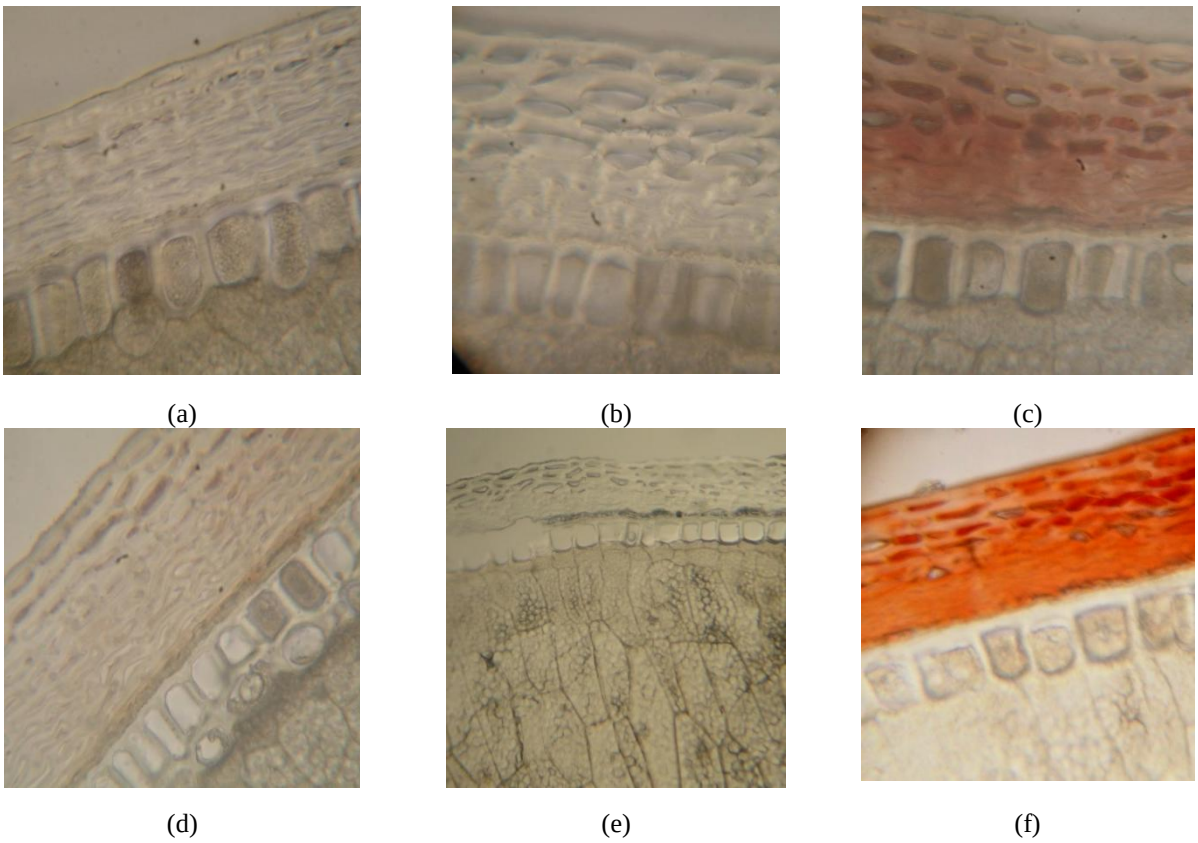

Fig. 2. Section view of differently-pigmented corn kernels: (a) Uralskiy; (b) 150 White; (c) Orange; (d) Brown; (e) Grey; (f) Rubin.

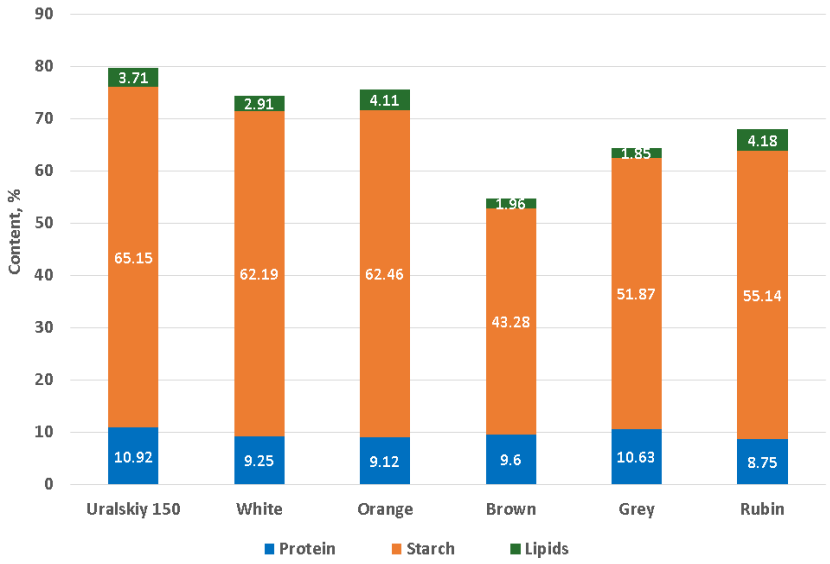

Fig. 3. Chemical composition of differently-pigmented corn kernels

The data provided in Fig. 3 indicate that protein content in corn kernels varied from 8.75% to 10.92%. Maximum protein content (10.92%) was found in Uralskiy 150 corn sample. High protein content (10.63%) was determined in Grey corn kernels.

In corn kernels, main substance is starch, its average content amounting up to 60–75%. Maximum starch content (65.15%) was found in Uralskiy 150 corn kernels. White and Orange corn samples contained approximately 62% of starch. Minimum starch content (43.28%) was found in Brown corn kernels.

Increased fat content in corn kernels is particularly relevant for corn oil extraction, and also for kernels used as fodder. Among the test samples, increased fat content was observed in Rubin and Orange corn kernels: 4.18% and 4.11%, respectively. Grey and Brown corn contained under 2% of fat.

Minerals amount to 2–5% of dry solids content within a kernel. These form ash particles during sample combustion. Table 1 presents minerals content in hybrid corn kernels.

Table 1. Minerals content in corn kernels, mg/100 g

Sample name	Copper	Zinc	Lead	Phosphorus	Manganese	Titanium	Iron	Potassium	Sodium	Calcium	Magnesium	Aluminum	Silicon
Uralskiy 150	2.45	7.8	0.52	32.4	9.5	2.60	3.90	73	54.2	59	186	11.2	47
White	1.22	9.2	0.48	52.3	12.3	3.20	1.90	92	38.9	63	162	9.4	63
Orange	0.84	8.4	0.54	42.0	8.4	4.20	4.20	84	50.4	84	168	25.2	84
Brown	0.85	8.5	0.51	33.6	8.5	4.25	2.55	85	42.5	85	170	8.5	51
Grey	3.15	15.7	0.63	52.5	31.5	5.25	5.25	128	53.5	105	210	10.5	105
Rubin	5.10	51.0	0.44	168.0	25.2	8.40	16.80	240	84.0	168	252	25.2	168

As per the data presented in Table 1, Rubin corn kernels contain maximum amount of: phosphorus – 168 mg/100 g; iron – 16.8 mg/100 g; potassium – 840 mg/100 g; sodium – 84 mg/100 g; calcium – 168 mg/100 г; magnesium – 252 mg/100 г; zinc – 51 mg/100 g; silicon – 168 mg/100 g.

Macro- and microelements found in Grey corn kernels were as follows: phosphorus – 52.5 mg/100 g; iron – 5.25 mg/100 g; potassium – 128 mg/100 g; sodium – 53.5 mg/100 g; calcium – 105 mg/100 g; magnesium – 210 mg/100 g; copper – 3.15 mg/100 g; zinc – 15.7 mg/100 g.

Other samples had lower minerals content, therefore, Grey and Rubin corn might be recommended as a source of macro- and microelements for enhancing mineral value of food products.

Flavonoids contain anthocyanins, a group of plant pigments responsible for hues of vegetative tissues. Anthocyanins are colored crystals readily soluble in water and other polars. Free anthocyanins and anthocyanidins bases are colored in purple. Red, blue, and purple hue of some vegetative tissues might come from the same anthocyanidin type involved in different cell sap reactions. In blue parts of a plant, it might be present as potassium or other alkali salt, in red ones, as oxonium salts of an organic acid, in purple ones, it is mostly a pigment base [10].

Quantities of biologically active substances in corn kernels were determined. Findings are presented in Table 3.

As per the data provided in Table 2, Orange, Rubin, and Grey corn kernels contain all three groups of biologically active compounds: flavonoids: 80, 70, 73 mg/%, carotenoids: 2.40, 1.70, 1.60 mg/%, and anthocyanins: 30, 120, 30 mg/%, respectively. Maximum concentration of flavonoids was registered in Orange corn sample: 80 mg/%, while Brown and Orange corn kernels were characterized by high content of carotenoids (2.40 mg/%), which is 2.2 times higher than the respective value in conventional yellow corn varieties. Maximum anthocyanins content was found in Rubin corn kernels: 120 mg/%.

Use of Orange and Rubin corn kernels in food industry seems promising for producing food products enriched with biologically active substances.

Recent years have witnessed a growing interest in natural antioxidants and their application in food industry. Apart from maximizing shelf life of foods, they also have antioxidant properties and boost human immune system. Considerable advances have been made in structural research of many complex natural compounds of plant origin [3, 6]. Phenolic compounds-flavonoids, anthocyanins, carotenoids, occurring widely in plants, and in corn kernels as well, have received much attention.

Express integral assessment of their quality should be based on antioxidant activity value reflecting content and impact of any organic reducing agent present within the test item.

Antioxidant activity of biologically active substances in corn kernels was quantified by amperometric assay in the "Cvet Jauza 01-AA" measuring instrument (Fig. 4).

Table 2. Biologically active substances content in corn kernels, mg/%

Sample name	Contained in a corn grain		
	flavonoids	carotenoids	anthocyanin
Uralskiy 150	76	0.90	–
White	67	–	–
Orange	80	2.40	30
Brown	–	2.40	–
Grey	73	1.60	30
Rubin	70	1.70	120

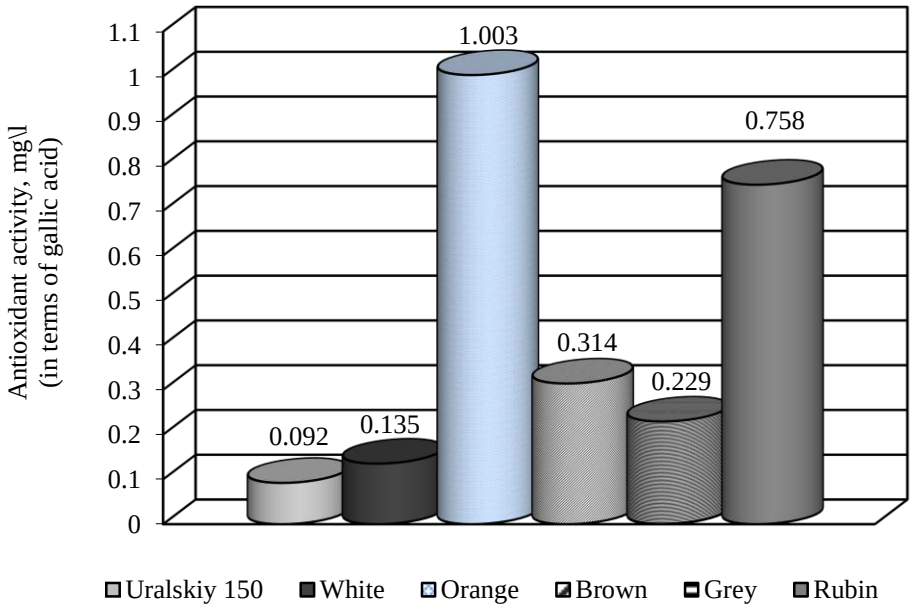

Fig. 4. Antioxidant activity of differently-pigmented corn kernels

According to the data presented in Fig. 4, high antioxidant activity (1.0 and 0.76 mg/l in gallic acid equivalents) was found in Orange and Rubin corn kernels, respectively. It might be explained by presence of all three groups of biologically active substances in these corn samples: carotenoids, flavonoids, and anthocyanins. Antioxidant activity of common yellow corn (Uralskiy 150 hybrid) amounted to 0.09 mg/l in gallic acid equivalent, which is about ten times less than in the test samples. Antioxidant activity of Grey and Brown corn kernels amounted to 0.23 and 0.31 mg/l in gallic acid equivalent, which exceeds control sample data over two and three times, respectively.

Thus, this complex study of chemical composition and antioxidant activity in differently-pigmented hybrid corn kernels suggested options for their differentiated targeted use in food industry. We showed that protein content in Grey hybrid corn kernels is similar to that of the hybrid yellow corn Uralskiy 150, conventionally used in food processing, which allows to diversify feedstock base for food processing industry. Starch content values deserve particular attention, as corn kernels represent a top priority feedstock in starchy foods products. Our research suggests that White and Orange hybrid corn kernels might be recommended for these specific technological purposes. Orange and Rubin hybrid corn kernels are particularly suitable for corn oil extraction. Grey and Rubin hybrid corn kernels can be used as a relevant source of macro- and microelements in developing food products with enhanced nutritional value. We observed increased antioxidant activity of tested hybrid corn samples in comparison to the control sample of hybrid corn Uralskiy 150. Findings showed certain groups of biologically active substances: flavonoids, anthocyanins, and carotenoids. Maximum flavonoids content was registered in Orange hybrid corn kernels (80 mg/%), maximum anthocyanins, in Rubin hybrid corn (120 mg/%). High antioxidant activity of the above hybrid corn kernels suggests their processed derivatives might be used in dietary prophylaxis as promising sources of biologically active substances. In conclusion, differently-pigmented hybrid corn cells are a valuable plant feedstock for food industry. Processed foods derived from differently-pigmented hybrid corn kernels shall amplify significantly the scope of grain feedstock application and shall contribute to the growth of domestic foods product assortment, which is particularly relevant under current economic circumstances.

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INVESTIGATING ANTIBIOTIC ACTIVITY OF THE GENUS *BACILLUS* STRAINS AND PROPERTIES OF THEIR BACTERIOCINS IN ORDER TO DEVELOP NEXT-GENERATION PHARMACEUTICALS

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Abstract: In recent years, we have witnessed a considerable growth in number of strains resistant to antibiotics. Therefore, research on new antimicrobial components that might be used for development of new-generation drugs is currently very important. We have studied antibiotic activity of *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis strains*, isolated their bacteriocins, and evaluated their properties. The study was carried out in the scientific research institute for biotechnology, Kemerovo Institute of Food Science and Technology, in the city of Kemerovo. Strains of microorganisms were isolated from vegetables grown in Krasnodar region, namely, samples of Manas onions, Big Beef tomatoes, and Capia bell peppers. Antibiotic activity of the strains was evaluated in liquid nutrient medium. All test strains demonstrated some level of antimicrobial activity which varied from 18 to 91%. We established minimum inhibitory concentrations for the isolated strains based on measured optical density; MIC for *Bacillus safensis* was $1.5*10^6$ CFU/cm^3, for *Bacillus endopheticus*, $1.5*10^6$ CFU/cm^3, for *Bacillus subtilis*, $1.5*10^8$ CFU/cm^3. We then isolated respective bacteriocins and purified them by HPLC method. During disk diffusion tests, bacteriocin preparations proved active against *Micrococcus luteus* strain. Molecular weight was determined by PAGE electrophoresis. Molecular weight of bacteriocins varied from 3.6 through 4.21 kDa. Isolated bacteriocins were proved to belong to the lantibiotics class.

Keywords: antibacterial activity, *Bacillus* strains, bacteriocins, pathogenic strains

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INTRODUCTION

This article considers some research that seems promising for future application in medicine and in developing the next generation of drugs. The aim of this work is to investigate antibiotic activity of the genus Bacillus strains and to study properties of their bacteriocins in order to create next-generation pharmaceuticals.

Bacteriocins are heterogeneous antibacterial complexes, rather different in their activity level, action spectrum and mechanisms, molecular weight, physical and chemical properties. The main biologically active part of any bacteriocin is a protein component. Action mechanism of bacteriocins is based on creation of unregulated pores in the membrane of targeted cells, which interferes with the membrane potential and kills the cells. Bacteriocins are normally synthesized by strains as a defense system, and they inhibit the development of microorganisms related to the producing strain. Bacteriocins were classified in accordance with their chemical, structural properties, and method of killing.

It is proposed to subdivide bacteriocins from lactic acid bacteria into three classes. The first class includes lantibiotics: small peptides with molecular weight below 4–5 kDa, such as nisin and subtilin. The second class is represented by thermostable proteins with molecular weight below 10 kDa, these include coagulin and turicin. The third class includes thermostable proteins with molecular weight below 30 kDa, representatives of this group being lacticin and helveticin. The fourth class of bacteriocins includes glycoproteins or lypoproteins, representatives of this group being lactocin and lacstrepticin [1].

Ample potential for bacteriocin synthesis is known to be present in numerous strains of various micro-organisms. Main bacteriocin-producing species are lactic acid bacteria and the genus *Bacillus* strains.

Lactic acid bacteria are in the focus of interest for many researchers, due to their high potential for

bacteriocins synthesis [2, 3]. The following strains are known as the main bacteriocin-producing lactic acid bacteria: *Lactococcu, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus, Carnobacterium*. Bacteriocins produced by lactic acid bacteria include the following: helveticin, lactacin, bavaricin, sacacin [4].

Some bacteriocins produced by lactic acid bacteria hold the GRAS status, which confirms their safety. Therefore, bacteriocins prooduced by lactic acid bacteria are widely used in food industry and in pharmaceutical industry [5].

Bacillus strains have a capacity to synthesize a wide spectrum of different bacteriocins. Main bacteriocins-producing strains are *Bacillus licheniformis, Bacillus pumilus, Bacillus circulans, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Bacillus amyloliquefaciens* [6, 7, 8].

Thus, *Bacillus* strains are producers of peptide and lypopeptide antibiotics [9] and they demonstrate capacity for synthesis of an ample spectrum of bacteriocins, such as: bacilin and fungumicin [10] plipastatin and sufractin, [11], coagulin [12], tochiin, [13], amylolychin [14].

The ample spectrum of antimicrobial activity demonstrated by *Bacillus* strains-produced bacteriocins allows them to inhibit not only gram-positive bacteria, but also gram-negative bacteria, yeast or fungi that are pathogenic for humans or animals.

Thus, this capacity of *Bacillus* strains to synthesize a great number of antimicrobial substances with an ample spectrum of antimicrobial activity, corroborates the importance of further research on new types of bacteriocins.

Bacteriocins are natural substances, harmless for humans, with a wide activity spectrum. All this makes their application in food industry and medicine rather promising. In food industry, bacteriocins are mostly used as biopreservatives to control growth of pathogenic microorganisms. Bacteriocin concentrates are both added into fermented products and used for treatment of foods during storage [14]. One of the bacteriocins that are widely applied in food industry is nisin, it is used as preservative for sauces and cheese production. [15].

Occurrence and distribution of antibiotics-resistance strains is a problem that has acquired global relevance and represents a significant threat for public health.

Resistance is a capacity of microorganisms to withstand greater concentration of antibiotics than other microorganisms of the same species, and to grow when exposed to high concentrations of antibiotics above their respective therapeutic values.

There are several reasons for resistance development in bacterial strains. One of the main reasons is the uncontrolled self-administration of antibiotics in daily life. Improper uses or early discontinuation of antibiotics results in development of resistant microorganisms. Resistant bacteria might be disseminated through direct contact with humans or animals [14].

Yes another reason is the use of antibiotics in agricultural industry. Antibiotics are included into nutrition supplements and used as drugs for animals and poultry [15]. Antibiotics do not accumulate in the body, however, whenever norms for their use are not respected, they remain in meat, milk, and eggs, and all these products are getting consumed. Nowadays, there are now measures that would allow to control antibiotics content in food products, and manufacturers are solely responsible for monitoring the compliance with applicable norms and standards.

Distribution of resistant bacteria leads to such consequences as rise of new untreatable infections, and resistance development in bacteria that cause severe diseases, such as tuberculosis, diarrhea, respiratory disorders, and malaria [16].

At present, a number of studies have centered on several antimicrobial agents that might become alternative to antibiotics, such as bacteriophages [17], probiotic bacteria [18], antimicrobial peptides [19], and bacteriocins [20]. Bacteriocins are one of the most promising components for further development of antibiotics.

Development of new antimicrobial components is an important direction for current research. Due to their capacity to produce bacteriocins with an ample activity spectrum, *Bacillus* strains represent a promising object of reseach in the field of new-generation drugs development.

OBJECTS AND METHODS OF STUDY

We performed surface strain recovery in vegetables grown in Krasnodar region: onion Manas, tomato Big Beef, bell pepper Capia. The study was carried out in the scientific research institute for biotechnology, Kemerovo Institute of Food Science and Technology (University), in the city of Kemerovo.

At different stages of the study, the following chemicals were used: Bacto Peptone, meat extract, enzymatic dry peptone, yeast extract, dry nutrient agar (LLC Lab-Biomed, Russia); HPLC columns XK16 with Phenyl Sepharose 6 Fast Flow carrier, column Octyl HR 16/60 (GE Healthcare, USA); ENrich S (BioRad, USA); $(NH_4)_2SO_4$ (Reakhim, Russia); tris, tricine, N, N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); acetic acid and boric acid (LLC Component-Reactiv, Russia); hydrochloric acid, sodium chloride, sodium acetate (LLC Component-Reactiv, Russia; coomassie R250 (Amresco, USA); ammonia persulfate, acrylamid and TEMED (Bio-Rad, Great Britain); glycerin (LLC Belkhim, Belarus); bromphenol blue (LLC Cation, Russia); tris (Applichem, USA); tris, N,N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); DNA-marker (Sibenzyme, Novosibirsk); kitfor Gram coloring (LLC Lab-Biomed, Russia); chemical kit PROBA-NK for DNA purification from biologic materials (LLC DNA Technology, Moscow); tris, N,N'-methylene-bis-acrylamid, acrylamid, 2-mercaptoethanol, sodium dodecylsulfate (Helicon, Russia); DNA-marker (Sibenzyme, Novosibirsk); набор Gene clean (MP Biomedicals, USA); GFXTM PCR DNA and Gel Band Purification Kit (GE Healthcare, USA); purification kit for amplified DNA (Sigma, USA); ABI

Prism Big Dye Terminator Cycle Sequincing Ready Reaction Kits (Applied biosistems, CIIIA); GTG-agarose (Lonza Rockland, USA); crystal violet (MERCK, Germany); *BigDye Terminator v1.1/3.1* Sequencing Buffer (Applied biosystems, England); Terminator v3.1 cycle. Sequencing RR-100 (Applied biosystems, England); Sephadex G50 Superfine (Biosciences, Sweden).

All experiments were replicated three times. Data provided in the table represent an average of the obtained results.

Strains recovery. Three strains of microorganisms, *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis*, were recovered from onions, tomatoes, and bell peppers. The recovery procedure was as follows: triturated vegetables were placed into a liquid nutrient broth medium (meat-peptone broth) and cultivated at 30 ± 2°C, 37 ± 2°C and 45 ± 2°C for 1–5 days. When sufficient biomass was grown, inoculation was performed onto nutrient agar (meat-peptone agar). In order to obtain pure cultures, streaks were inoculated daily, until single colonies were produced. Isolated colonies were identified by Gram coloring, spores formation, morphological properties, cell size, oxidase and catalase tests. For more precise identification, genetic analysis of the strains of performed, with 16S RNA sequencing.

Determination of antimicrobial activity. To evaluate antagonistic activity, we measured optical density by spectrophotometry. Test culture was grown onto the MPA for 24 hours, at optimal temperature for each strain. Cells from the agar surface were re-suspended in NaCL solution until 10^9. Purified strains were grown into the MPB medium for 24 hours at 37 ± 2°C. Then, the cultural liquid was centrifuged at 7000 rpm for 10 minutes, and supernatant was separated. In order to isolate the cells, the supernatant was filtered with Millex-GV filters (0.22 μm, Millipore, USA). The supernatant was neutralized by adding sodium hydroxide. 150 μl of each *Bacillus* strain culture filtrate were introduced into a plate, then 150 μl of test strains culture solution were added to each culture. The plate was incubated at 37 ± 2°C for 24 hours. 150 μl of sterile water, and 150 μl of pathogenic strain solution were used as controls. Optical density of the mixture was measured during cultivation. All experiments for antagonistic activity evaluation were replicated three times.

Determination of minimum inhibitory concentration. Minimum inhibitory concentration of strains was determined by spectrophotometry. Test strains were cultivated onto the MPB nutrient medium, then cells were separated by centrifugation at 7000 rpm for 10 minutes. Supernatant was separated, and pH was made up to 7.0 by adding NaOH 1M solution. Turbidity of the cultural liquid was controlled, as it achieved the value of 0.5 in McFarland units (containing approx. $1.5 \cdot 10^8$CFU/cm^3). Optical density level was controlled by spectrophotometry: absorption was within the same range as 0.5 in the McFarland units (at OD 625 nm, optical density was within 0.08–0.13). Optical density of the obtained microbial suspension was regulated by adding sterile nutrient medium. Then, a series of consequent dilutions 1 : 10 was performed, thus reaching a metabolite concentration from 10^7 to 10^1 CFU/ cm^3. In order to prepare the microbial suspension of test cultures, the colonies were diluted by sterile nutrient broth up to 0.5 in the McFarland units.

500 μl of the diluted test culture were mixed with 500 μl of the microbial suspension obtained from the tested strains. Minimum inhibitory concentration was assumed to be minimum metabolite concentration that slows down the metabolite growth.

Separation and purification of bacteriocins. In order to obtain bacteriocins, cells were separated from the cultural liquid, prior to purification, by 30 minutes centrifugation at 4200 rpm. Bacteriocins were sedimented with ammonia sulfate up to 90% of saturating concentration. Sediment and cultural liquid were separated by centrifugation at 4200 rpm for 40 minutes. The sediment was dissolved in 20 mmol acetate buffer pH 5.0. Undissolved sediment was separated by centrifugation at 4200 rpm for 30 minutes. Then the sediment waswashed again in 20 mmol acetate buffer pH 5.0, and the undissolved part was separated againby centrifugation.

Bacteriocin purification by HPLC. Purification was performed with AKTAfplc system (Amersham Biosciense, Sweden). At the first stage of purification, XK16 column was used, with PhenylSepharose 6 FastFlow as a carrier. The column with Phenyl-sepharose was balanced by initial buffer: 20 mmol acetate buffer pH 5.0 + 1 mol $(NH_4)_2SO_4$. $(NH_4)_2SO_4$ concentration in the applied preparation was made up to 1 mol. The preparation was applied onto the column at 3 ml/min. The second stage of purification was performed at OctylHR 16/60 column (GEHealthcare, USA). The column was balanced by initial buffer: 20 mmol acetate buffer pH 5.0+, application rate 1 ml/min. The final stage of purification was performed at ENRichS column (BioRad, USA). The column was balanced with initial buffer: 20 mmol acetate buffer, pH 5.0, application rate 1 ml/min. Electrophoresis was performed in Tricine-SDS, PAGE 16%, in the Mini-PROTEAN II cell (BioRad, USA).

Evaluation of antimicrobial properties in purified bacteriocin preparations. Antimicrobial activity of the purified bacteriocin preparations was determined by disk diffusion test. Test culture was streaked onto the MPA. Bacteriocin preparations were applied onto disks placed on the nutriuent agar. Nutrient agar was left to dry for some time. After 24 hours of cultivating the test microorganism at 30ºC, inhibition zone diameter was evaluated around the wells.

Protein analysis. Electrophoresis was performed in Tricine-SDS, PAGE 16%, as per Schagger & von Jagow procedure (Schagger H., von Jagow G., 1987). The following markers were used: triosephosphate isomerase (26.625 kDa), myoglobin (16.95 kDa), alfa-lactalbumin (14.437 kDa), aprotinin (6.512 kDa), insulin chain B oxidized (3.496 kDa), bacitracin (1.423 kDa).

RESULTS AND DISCUSSION

Determination of antimicrobial activity. We performed a study of antimicrobial properties in bacteriocins of *Bacillus* strains purified at the previous stages of our research. Antagonistic properties of these strains were evaluated through cultivation of test cultures together with the metabolites produced by the strains under consideration. Biomass growth was monitored in liquid medium for 24 hours. Results are presented in Fig. 1–3.

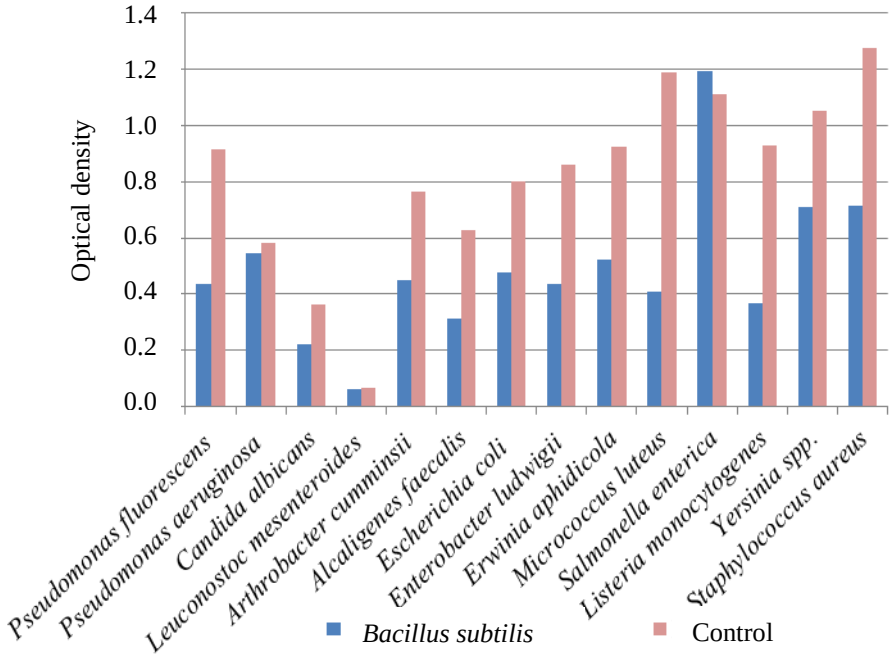

Fig. 1. Antagonistic activity of *Bacillus subtilis* strain.

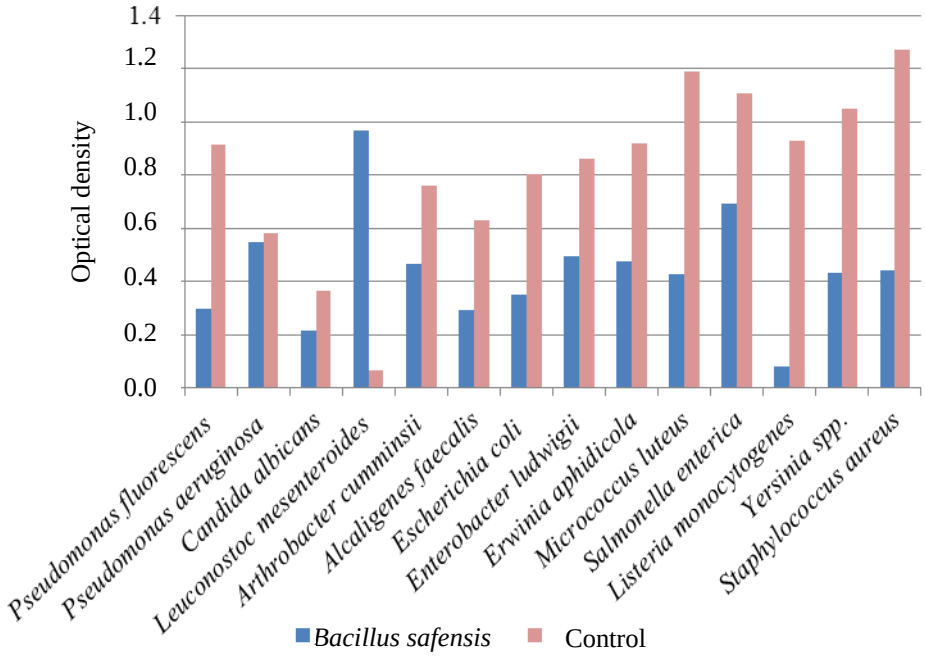

Fig. 2. Antagonistic activity of *Bacillus safensis* strain.

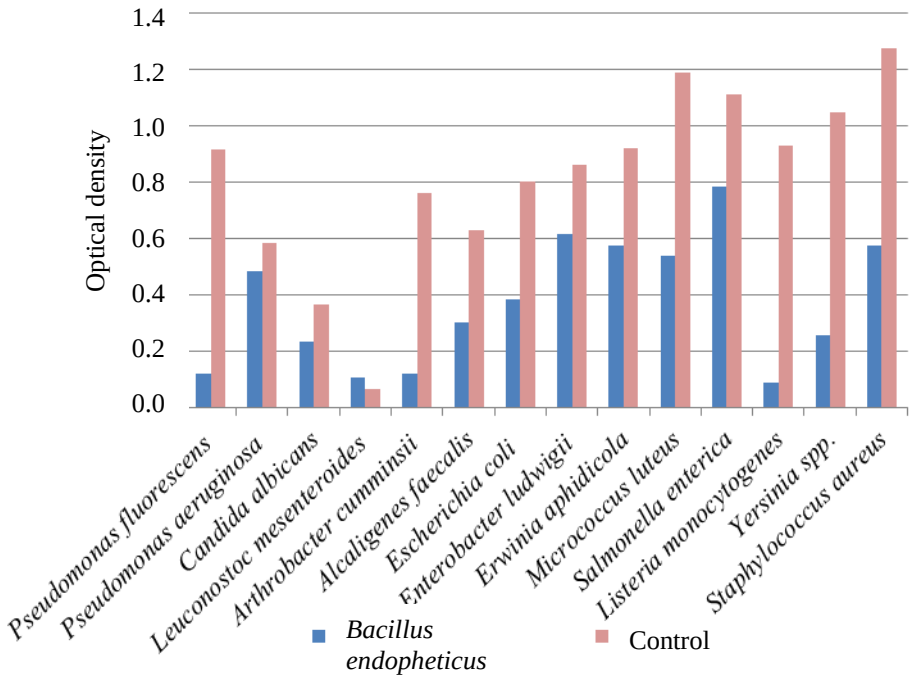

Fig. 3. Antagonistic activity of *Bacillus endopheticus* strain.

Purified cultures demonstrated high antagonistic activity again most pathogenic strains. Thus, during the study, *Pseudomonas fluorescens* growth was inhibited by *Bacillus safensis*, *Bacillus endopheticus* strains (from 53 to 88%). Activity against *Arthrobacter cumminsii* and *Staphylococcus aureus* was found in *Bacillus pumilus* and *Bacillus endopheticus* strains (from 55 to 85%). *Micrococcus luteus*, *Listeria monocytogenes* were inhibited by *Bacillus endopheticus*, *Bacillus safensis*, *Bacillus subtilis* strains (from 55 to 91%). Growth of *Yersinia spp.*, *Escherichia coli* was inhibited by *Bacillus endopheticus* and *Bacillus safensis* strains (from 54 to 76%). Growth of *Enterobacter ludwigii* and *Erwiniaa phidicola* strains was inhibited by the following bacterial cultures: *Bacillus safensis*, *Bacillus endopheticus*, (from 28 to 49%). Growth of *Salmonella enterica* was restrained by strains of *Bacillus safensis*, *Bacillus endopheticus* (from 25 to 38%), while antimicrobial activity against *Alcaligenes faecalis* was demonstrated by *Bacillus safensis*, *Bacillus endopheticus*, *Bacillus subtilis* (52–54%). *Pseudomonas aeruginosa* was inhibited by strains of *Bacillus endopheticus*, *Bacillus subtilis*, *Bacillus safensis* (48–55%).

Determination of bacteriocin minimum inhibitory concentration. Determination of minimum inhibitory concentration is an important step in studying the spectrum of antimicrobial activity of the strains and in further development of antibiotics. Findings are presented in Tables 1–3.

Table 1. Minimum inhibitory concentration of *Bacillus subtilis* strain

Cultivation time, hours	Concentration of *Escherichia coli* metabolites, CFU/cm^3				
	$1.5*10^8$	$1.5*10^7$	$1.5*10^6$	$1.5*10^5$	control
	Optical density				
2	0.0725 ± 0.0036	0.0658 ± 0.0033	0.0507 ± 0.0025	0.0419 ± 0.0021	0.0755 ± 0.0038
4	0.0893 ± 0.0044	0.1013 ± 0.0051	0.0977 ± 0.00488	0.0905 ± 0.0045	0.1329 ± 0.0066
6	0.0948 ± 0.0048	0.1666 ± 0.0083	0.1974 ± 0.0099	0.1053 ± 0.0053	0.1843 ± 0.0092
24	0.1275 ± 0.0064	0.0802 ± 0.0041	0.3012 ± 0.0151	0.3433 ± 0.0172	0.2525 ± 0.0126

Table 2. Minimum inhibitory concentration of *Bacillus endopheticus* strain

Cultivation time, hours	Concentration of *Escherichia coli* metabolites, CFU/cm^3				
	$1.5*10^8$	$1.5*10^7$	$1.5*10^6$	$1.5*10^5$	control
	Optical density				
2	0.0741 ± 0.0037	0.0629 ± 0.0031	0.0699 ± 0.0035	0.0456 ± 0.0023	0.0755 ± 0.0037
4	0.0859 ± 0.0043	0.0895 ± 0.0045	0.0882 ± 0.0044	0.0967 ± 0.0048	0.1329 ± 0.0066
6	0.0932 ± 0.0470	0.1889 ± 0.0094	0.1553 ± 0.0078	0.1034 ± 0.0052	0.1843 ± 0.0018
24	0.0544 ± 0.0027	0.0809 ± 0.0041	0.1051 ± 0.0053	0.1966 ± 0.0098	0.2525 ± 0.0126

Table 3. Minimum inhibitory concentration of *Bacillus safensis* strain

Cultivation time, hours	Concentration of *Escherichia coli* metabolites, CFU/cm^3				
	1.5*10^8	1.5*10^7	1.5*10^6	1.5*10^5	control
Optical density					
2	0.1251 ± 0.0062	0.0889 ± 0.00444	0.0767 ± 0.0038	0.0655 ± 0.0033	0.0755 ± 0.0038
4	0.1328 ± 0.0066	0.1323 ± 0.0066	0.1009 ± 0.0051	0.0938 ± 0.0047	0.1329 ± 0.0066
6	0.1596 ± 0.0079	0.1897 ± 0.0095	0.1102 ± 0.0055	0.2228 ± 0.0111	0.1843 ± 0.0092
24	0.0291 ± 0.0014	0.0913 ± 0.0046	0.1283 ± 0.0064	0.2515 ± 0.0126	0.2525 ± 0.0126

Our findings suggest that higher optical density, and, consequently, a higher biomass growth was observed when test culture was cultivated without adding the bacterial suspension of the strain under investigation. Growth of test culture was being inhibited during the 24 hours cultivation. The lowest biomass growth was observed during their joint cultivation, which means that the test culture growth was inhibited. Thus, for *Bacillus subtilis* strain, minimum concentration that inhibited the growth of *Escherichia coli* was 1.5*10^8 CFU/cm^3 (Table 1).

Findings show that optical density was decreasing - in comparison to the controls when metabolite concentration reached 1.5*10^5 CFU/cm^3. Thus, for *Bacillus endopheticus* minimum inhibitory concentration was 1.5*10^5 CFU/cm^3 (Table 2).

Decreasing optical density of the medium, and, as a result, decreasing text culture biomass concentration, in comparison to the controls, was observed at *Bacillus safensis* concentration of 1.5*10^6 CFU/cm^3 (Table 3).

Bacteriocins purification. Bacteriocins were obtained from cultural liquid by multi-step sedimentation with ammonia sulfate and washing in acetate buffer. Bacteriocins were purified by HPLC, and putification parameters were suitably adjusted. Purification in several steps proved to be optimal. The first stage was performed with XK16 column, with Phenyl Sepharose 6 Fast Flow used as a carrier, the second stage, with the column Octyl HR 16/60 (GE Healthcare, USA), and the third, with the column ENrich S (BioRad, USA). Application rate varied in dependence with the type of the column. Preparation was washed by initial and acetate buffers. Adjustment of purification parameters revealed the hydrophobic nature of bacteriocins. Bacteriocin preparations obtained by purification were analyzed for protein concentration and output. Findings are presented in Table 4.

Purification of bacteriocins produced by *Bacillus subtilis* resulted in a bacteriocin preparation with protein concentration of 13.900 ± 0.278 mg/cm^3, while the product output was 1.26 ± 2.00%.

Upon purification of bacteriocins produced by Bacillus safensis, protein concentration in the resulting preparation was 0.067 ± 0.001 mg/cm^3, with the product output of 16.60 ± 2.00%.

Table 4. Results of bacteriocins purification

Purification stage	Protein concentration, mg/cm^3	Output, %
Bacillus endopheticus	1.750 ± 0.035	46.30 ± 2.00
Bacillus subtilis	13.900 ± 0.278	1.26 ± 2.00
Bacillus safensis	0.067 ± 0.001	16.60 ± 2.00

Purified preparations of *Bacillus endorpiticus* bacteriocins had a protein concentration of 1.750 ± 0.035 mg/cm^3, the end product output being 46.30 ± 2.00%.

Protein analysis. In order to determine molecular weight and to identify the isolated and purified bacteriocins, we performed electrophoresis in Tricine-SDS PAGE 16.50%. Findings are presented in Fig. 4.

Electrophoresis results detemined that molecular weight of *Bacillus subtilis* was 3.6 kDa, while for *Bacillus endorpiticus* strain molecular weight was 3.8 kDa. *Bacillus safensis* strain had molecular weight of 4.21 kDa (Table 5).

Determination of antimicrobial activity in bacteriocins. The next important step after bacteriocins purification was determination of their antimicrobial activity. We studied the capacity of the bacteriocin preparations to suppress the growth of *Micrococcus luteus* strain. The study was carried out on MPA, at cultivation temperature of 30°C. Specific activity of the bacteriocins under consideration was evaluated by changed in their inhibition areas. A relative unit of activity was assumed to be the amount of bactericin that creates a lysis area of 1 cm in diameter.

Bacteriocin activity is an important characteristic for describing bacteriocins in bacterial cultures. Antibiotic activity study in bacteriocins preparations revealed that *Bacillus safensis* strain demonstrated the highest specific activity, which amounted to 115.6 RU. A lower activity in relation to the test culture was observed in *Bacillus subtilis* strain, with specific activity of 13.64 RU. Activity value in *Bacillus endopheticus* strain was 13.64 RU.

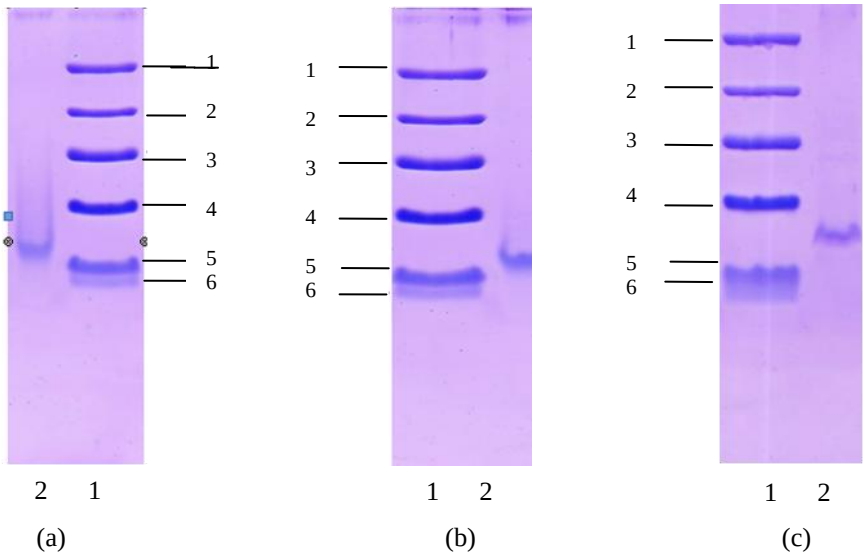

Fig. 4. Tris-tricine electrophorsis for bacteriocins after purification: (a) *Bacillus subtilis*, (b) *Bacillus safensis*, (c) *Bacillus endorpiticus*. Markers, kDa: (1) triosephosphate isomerase – 26.625; (2) myoglobin – 16.95; (3) alfa-lactalbumin – 14.437; (4) aprotinin – 6.512; (5) insulin chain B oxidized – 3.496; (6) bacitracin – 1.423.

Table 5. Results of activity evaluation in purified bacteriocin preparations

Bacteriocins of strains	Volume, ml	Activity, RU/ cm^3	Overall activity, RU	Specific activity, RU/mg
Bacillus safensis	3.40 ± 0.07	34.00 ± 0.68	115.60 ± 2.31	503.70 ± 10.07
Bacillus endopheticus	2.90 ± 0.06	28.00 ± 0.56	81.20 ± 1.62	4.48 ± 0.09
Bacillus subtilis	4.40 ± 0.09	3.10 ± 0.06	13.64 ± 0.28	7.30 ± 0.15

CONCLUSIONS

In this work, we found strains of the following microorganisms present on the surface of fresh onions, tomatoes, and bell peppers: *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus*.

The studies we performed revealed antibiotic properties in bacteriocins produced by purified strains of *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus*. All strains under consideration demonstrated antibiotic activity against common pathogenic strains that provoke numerous human diseases. Antibiotic activity present in these strains allows us to consider them as bacteriocin producers.

We carried out an evaluation of minimum inhibitory activity for bacteriocins produced by *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus* strains. As a result of this study, we determined minimum concentrations of purified bacteriocins that are sufficient to inhibit the growth of bacterial cultures.

Adjustment of HPLC parameters targeting the purification of bacteriocins produced by *Bacillus safensis*, *Bacillus subtilis*, *Bacillus endopheticus* strains allowed to select proper columns, to determine main purification stages and their respective specifications. As a result, we obtained bacteriocin preparations with a high protein concentration; besides, this specific purification procedure contributed to the increase of the end product output.

PAGE electrophoresis of the purified bacteriocin preparations was carried out in order to determine their molecular weight and, consequently, to perform their further identification. Specific activity present in purified preparations confirms that they belong to bacteriocins. Molecular weight of all bacteriocin preparations varied from 3.60 to 4.21 kDa. Thus, we found out that all the bacteriocins we obtained belong to the class of lanbiotics, the peptides with molecular weight below 5 kDa.

Lanbiotics comprise such well-known antibiotics as nisin, subtilin, epidermin, mercacidin, cinnamycin, mutacin II, and lacticin 481. It is considered that they kill cells by making pores in cell membrane, by interfering with the membrane potential, and by inhibiting the biosynthesis of enzymes within the cell wall.

Classifying the isolated protein products as lanbiotics, a group which includes many of the previously described antibiotics, together with their capacity to suppress the growth of pathogenic strains, makes their further research rather promising.

Development of new-generation drigs is an important direction for current medicine. Results that we have gathered at the present stage of research prove that the metabolites produces by test strains have a

wide spectrum of antimicrobial activity, and might be used in future for developing pharmaceutical products of the new generation.

Further studies of the isolated bacteriocins produced by the genus *Bacillus* strains shall provide a better understanding of their properties, which, in turn, will give way to opportunities for new-generation drugs development.

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PROCESSES, EQUIPMENT, AND APPARATUS FOR FOOD PRODUCTION

ULTRAFILTRATION OF MODIFIED MILK WHEY

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Abstract: The current trend of human community evolution comes to addressing two main tasks: to provide people with full-value food and environmental conservation. The tasks may be addressed only by the appropriate management of rational use of natural raw resources that cannot be disposed in full to practically limited models of food equipment of new generation manufactured with the use of latest scientific achievement. The activity to enhance the urban interference in the society development results in processes where the significance of mineral substances, vitamins and proteins is undervalued in production of purified food for human use that may be obtained by reprocessing of secondary dairy products during baromembrane separation. The study aimed to determine the best parameters of membrane-associated re-processing of the milk whey modified with plant extracts and validation of further use of retentate and permeate obtained. The milk whey was used obtained during production of the grained curd of the normalized cow milk. Employment of membrane methods in the milk whey re-processing may be useful to maintain the non-waste production and avoid ecological pollution. The use of these methods in modern food industry is the upcoming trend that allows opportunities to manufacture a wider range of dairy products, drinks, forage and other resource and energy saving solutions. This article describes the dependency of the membrane permeability and selectivity of type UAM-150 of the modified milk whey of the working pressure value and circulation rate of separated system in the feeder of the baromembrane machine. The impact of pH of the separated liquid polydisperse system on the value of this membrane rejection rate. The viability to use the permeate and retentate of the modified milk whey as the base for drinks manufacture.

Keywords: milk whey, membrane technology, ultrafiltration, herbal extracts

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INTRODUCTION

The milk whey is the waste product of curd, cheese and casein production process. It contains up to 50% of dry solids of the whole milk [8, 9] and this allows considering it as the valuable secondary raw material. The discharge of the milk whey to the atmosphere considerable impacts the environment [8, 9]. This kind of so-called waste of dairy industry may be re-processed through ultrafiltration. Since baromembrane separation of the milk whey is of low cost-efficiency for most standard and, in particular, low capacity dairy processing plants [8, 9], one of solutions to address this problem is to change its physical and chemical characteristics by adding herbal substance extracts. During ultrafiltration of the milk whey modified in this way the permeability of polymer membranes increases while retaining the specified selectivity parameter.

The study aimed to determine the best parameters of membrane-associated re-processing of the milk whey modified with plant extracts and validation of further use of retentate and permeate obtained.

OBJECTS AND METHODS OF STUDY

The milk whey was used obtained during production of the grained curd of the normalized cow milk. Main physical and chemical parameters of the whey pre-purified by conventional method and modified with the extract of herbal raw mixture (stevia herb + licorice rhizome), performance parameters of the polymer membrane are shown in Tables 1 and 2. The literature [8, 9] and results of in-house researches were analyzed and it was identified that in current conditions it is reasonable to use the following types of polymer membranes for ultrafiltration separation of the caseous whey: UPM-P, UPM-67, UAM-500 and UAM-150 of “Vladipor” manufacture.

Table 1. Physical and chemical whey parameters as the ultrafiltration item.

Parameter	Serum	
	Baseline	Modified
Content of dry substances, %, not less	6.5	6.3
Including:		
Lactose	5.0	5.0
Protein	1.1	0.7
Fat	0.1	0.1
Minerals	0.3	0.5
Acidity, ºT	45	42
pH value	5.0	5.1
Density, kg/m^3	1023	1024
Absorbancy of 10% solution	0.26	0.20

Table 2. Performance parameters of polymeric membranes of type UPM and UAM (Russia, "Vladipor").

Main parameters	Type of membrane			
	UPM-P	UPM-67	UAM-150	UAM-500
Pressure, MPa	0.1–0.4	0.1–0.4	0.1–0.5	0.1–0.5
Retention rate, kDa	30–35	45–50	20–25	50–55
Temperature, °C	5–40	5–40	10–50	10–50
Washing medium pH	2–10	2–10	2–12	2–12
Service life, h	up to 3500	up to 3500	up to 3000	up to 3000

By reference parameter (retention limit), these membranes may be used for ultra-filtration of dairy primary products. All other things constant, polyamide membranes (UPM) differ from cellulose acetate membranes (UAM) in higher cost and extended life cycle. However, while using membranes of type UAM-500 and UAM-150, the process is possible with the higher operation pressure and harder cleaning is practicable. The process of ultra-filtration was performed using the special laboratory unit. The area of membrane surface is up to 0.5 m^2. The value of operation pressure, circulation rate of the liquid polydisperse system to be separated in the pipeline of the baromembrane machine, its temperature varied within $\Delta P = 0.1 \div 0.4$ MPa, $V = 0.05 \div 0.45$ m/s and $t = 8 \div 18$ºC, accordingly. Permeability Q and selectivity φ parameters of the membrane of type UAM-150 were found by testing. The value Ψ was estimated by the formula 1 below:

$$\varphi = \frac{V_2 \cdot C_2}{V_1 \cdot C_1} \cdot 100\%, \quad (1)$$

where V_1 is the reference volume of the system to be separated and C_1 is the weight ratio of particles dispersed in it; V_2 and C_2 is the volume of retentate and weight ratio of particles dispersed in it.

The confidence factor of results obtained is 95%.

RESULTS AND DISCUSSION

As per modern knowledge on self-organization of protein molecule structure [1], protein globules of the milk whey may be estimated as the spherical particles of the disperse phase having adsorptive properties. During the process of the milk whey ultra-filtration, the concentration polarization phenomenon depends on the conventional supply of such globules to the membrane surface, their further agglomeration and the polymer film formation that, in fact, is the dynamic membrane that increases the flow resistance to permeate.

In compliance with the filtration concept (at constant pressure), the permeate flow rate is determined by the expression 2:

$$w\ t\ = \frac{1}{S}\frac{dV}{dt} = \frac{\Delta P}{(R_m + R_g)\eta}, \quad (2)$$

where ΔP is the operation pressure in the baromembrane machine, R_m and R_g is the flow resistance of polymeric and dynamic membranes, η is the permeate viscosity, S is the area of membrane, V is the permeate volume, t is the time. Resistance R_g may be determined as:

$$R_g = r\delta_g, \quad (3)$$

where r is the filtration resistance and δ_g is the thickness of the dynamic membrane layer.

Filtration resistance is expressed in the dependence:

$$r = a(1 - \varepsilon)\rho, \quad (4)$$

where a is the specific resistance, ε is the porosity, ρ is the density of the deposit layer on the membrane surface.

Thickness of the dynamic membrane is determined as follows:

$$\delta_g = \frac{Vk\ 1-d\ C_b}{S(1-\varepsilon)\rho}, \quad (5)$$

where k is the retention factor, C_b is the concentration of disperse phase particles in the separated system, k is the particle share of the disperse phase that is carried from the membrane surface into the main flow due to the reverse diffusion.

In respect of (3)–(5) it can be written as:

$$R_g = \frac{1-d\ aVkC_b}{S}, \quad (6)$$

Substituting for the equation (6) to the expression (2) we obtain the following:

$$w\ t = \frac{1}{S}\frac{dV}{dt} = \frac{\Delta P}{R_m\eta + aVkC_b\ 1-d\ \eta/S}, \quad (7)$$

As the equation is integrated (7), its solutions towards V and substitution for V to the equation:

$$G = \frac{1}{w^2} = \left(\frac{R_m\eta}{\Delta P}\right)^2 + \frac{2aRC_b\ 1-k\ \eta}{\Delta P}t, \quad (8)$$

At k = 1 and d = 0, the equation (8) goes over into the formula determined by the authors (Chudacek M.W. and Fane A.G.) of the acquainted work [2].

Fig. 1 shows the dependence $G = f(\tau)$ in graphic form. The plot is the straight line; a slight linear change is seen at the initial section. The line extension cuts out a section on the X-axis consistent with $\tau \approx 0.1$ hours, that is, in our opinion, relevant to the primary formation of the deposit layer on the membrane surface. In fact, similar dependence values were obtained [3] during ultra-filtration of other protein solutions. Upon baromembrane separation of 0.5÷0.6 kg of the modified whey on the membrane, about 0.1^{-3} kg of dry deposits. This allows to determine that the factor (1 – d) = 0.2. At C_b = 1.8 kg/m^3, k = 94÷96%, $\eta = 2\cdot10^{-3}$ ns/m^2 and $\Delta P = 10^5$ N/m^2, the calculation by the slope of straight line in Fig. 3 shows the value of specific resistance $a \approx 2.2\cdot10^5$ m/kg. Specific resistance of high-molecular deposit layer on membrane surfaces may correlate with the size of protein globules as per the Carman-Kozeny equation [4]

$$a = \frac{180\ (1-\varepsilon)}{\rho d^2 \varepsilon^3}, \quad (9)$$

where *d* is the mean specific diameter of protein globule.

If the value of density ρ = 1÷1.13 kg/m³ and d = 40÷43 nm are taken, the formula (9) shows that the porosity of high-molecular deposit layer will be equal ε = 0.5÷0.52, which is lower but comparable to the relevant value of cellulose acetate membranes [6]. This suggests that initially polarizing processes (including intrapore area) on the membrane surfaces with further jellification are the main reason of the membrane permeability reduction. This is consistent with conclusions in the work [7] on the comprehensive nature of impact caused by the disperse phase structure on the permeability of ultra-filtration membranes when separating liquid high-molecular systems.

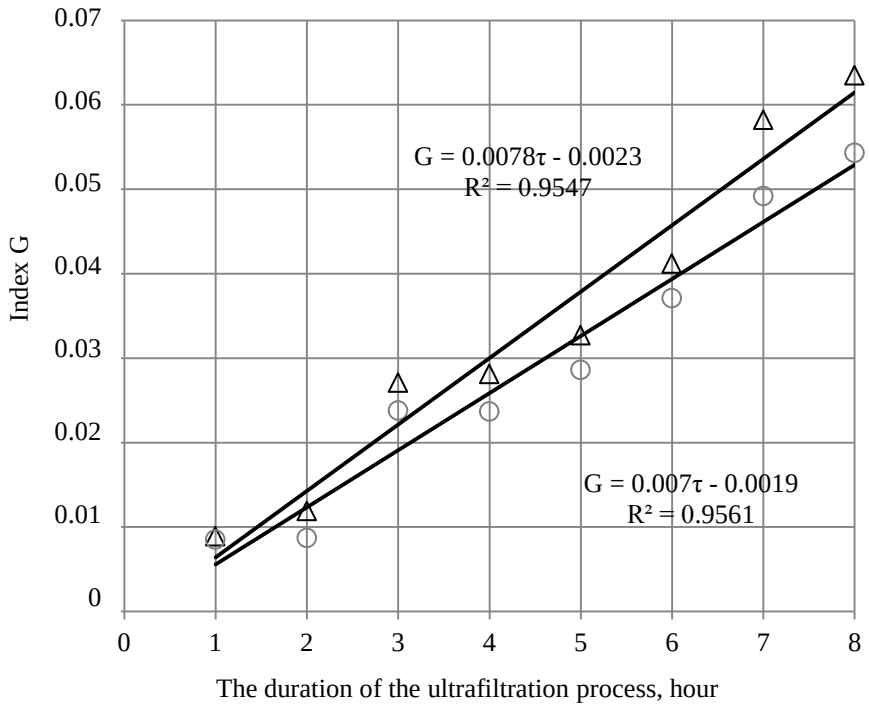

Fig. 1. Dependence of the value G on the time of ultrafiltration process of common (○) and modified (Δ) milk whey on the membrane UAM-150.

It should be noted that sizes of colloidal particles of the disperse phase that change into the whey during the milk re-processing, vary within quite a wide range (nm): α-lactoalbumin – 15÷20, β-lactoglobulin – 25÷30; casein micelle – 40÷300. Macromolecules of whey proteins are folded in dense globules with the negative charge and quite steady hydration shells. They are highly stable in the dispersed medium but in certain conditions they may coagulate when they reach the relevant isoelectric point, and with the reduction in pH, they form associates of several monomers. Due to H-particle adsorption, such globules are positively charged [1]. Since the intensity of globule interaction with the membrane surface and the pore walls may be due to the globule charge, the study was conducted to assess the impact of the separated system pH on the value of the membrane UAM-150 retention factor during ultra-filtration of the common (○) and modified (□) milk whey. With the increase of pH value, the membrane retention rate gradually increases and reaches its highest value at pH = 4.5÷5.0. The, it decreases remaining constant at pH = 6.5÷7.5 (Fig. 2). This way to change the dependence of the retention factor of the disperse phase protein particles in the common milk whey on pH of the system to be separated is easily explained based on the frictional translation model. In case if the protein globules travel in the pore space of the dynamic membrane, they repulse from each other and from the pore walls at similar charge indexes. This results in the decrease of the friction ratio between the membrane and globules. As a result, at lower pH values of the separated system, the value of the retention factor of the membrane increases. Within the range of pH > 7.5, the friction factor between the membrane and globules presumably decreases to a considerable extent which causes reduction in its value of retention rate. This explanation is supported with the fact that as a result of milk whey modification with the *stevia extract*, the physical and chemical properties of the separated system considerably change. This mainly relates to interaction between the protein particles and several components as parts of this plant [10]. In case of separation of the modified milk whey by ultra-filtration, the value of the membrane retention factor is practically the same at changes in the pH of the separated system; its slight increase is reported within pH = 4.5÷5.5, that does not contradict the frictional translation model.

To develop practical recommendations to increase the performance of the modified milk whey ultra-filtration, comparative experiments were conducted to evaluate the dependence of permeability Q and selectivity φ of UAM-150 membrane on the operation pressure index of the separated system in the pipe of the baromembrane machine (Fig. 3 and 4).

As it is seen from data shown, the way the dependence of type Q=f(Δp) changes for the UAM-150 membrane during separation of the common and modified milk whey by ultra-filtration is quite similar: an increase in permeability is propportional to the increase in the operation pressure in the pipe of the baromembrane machine. However, the values $tg\varphi_i=dQ/d\Delta p$ for relevant plots differ. Since the physical significance $tg\varphi_i$ is the rate of increment of function Q=f(Δp), this suggests that both the membrane permeability Q and its growth intensity increase during ultra-filtration of the modified whey. This phenomenon is derived from the fact that the stevia's extract added to the milk whey obtained from the production of the grained curd by the conventional method causes changes in the whey physical and chemical properties due to separation by ultra-filtration method. Review of plots for the function Q=f(Δp) show that, all other things constant, the increment in the membrane permeability is seen with the increase in the operation pressure within Δp = 0.1÷0.12 MPa to Δp = 0.3÷0.32 MPa. The, the Q value remains practically stable. The increase in the value of Δp parameter over 0.42÷0.44 MPa insignificantly impacts the membrane permeability during ultra-filtration of both liquid systems, and when the common whey is used, as the object of separation, the Q value tends to decrease.

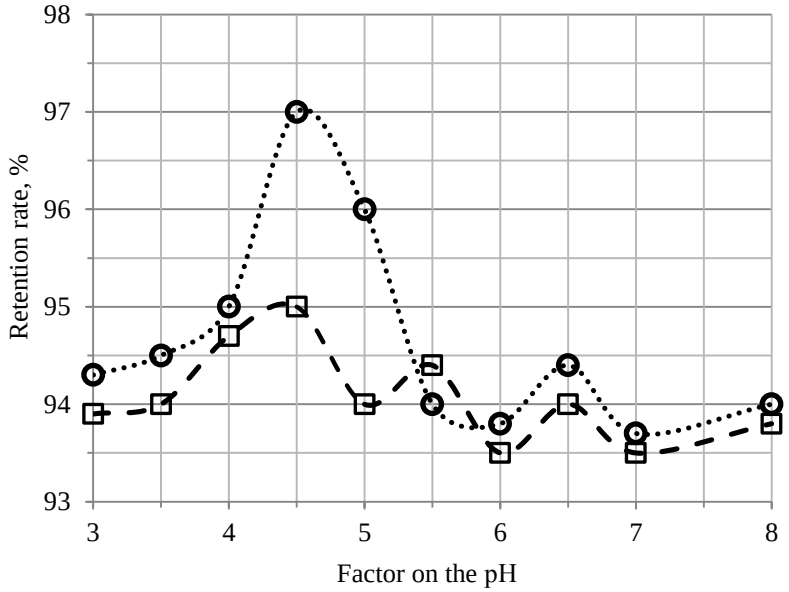

Fig. 2. Dependence of the membrane retention factor on pH value of the separated system during ultrafiltration of the common (○) and modified (□) milk whey on the UAM-150 membrane.

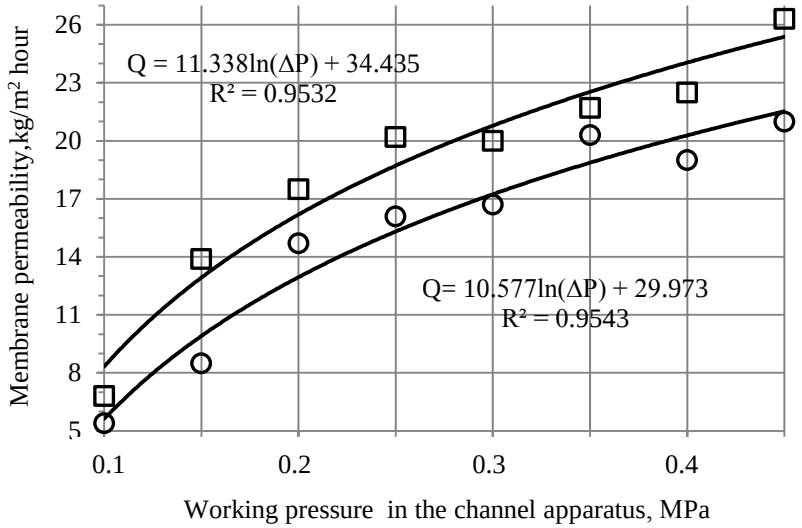

Fig. 3. Dependence of permeability Q of UAM-150 membranes on the operation pressure index Δp (t = 10÷12°C, *v* = 0.1÷0.3 m/s, $C_{d.m.}$= 8÷8.2%) during ultra-filtration of common (○) and modified (□) milk whey.

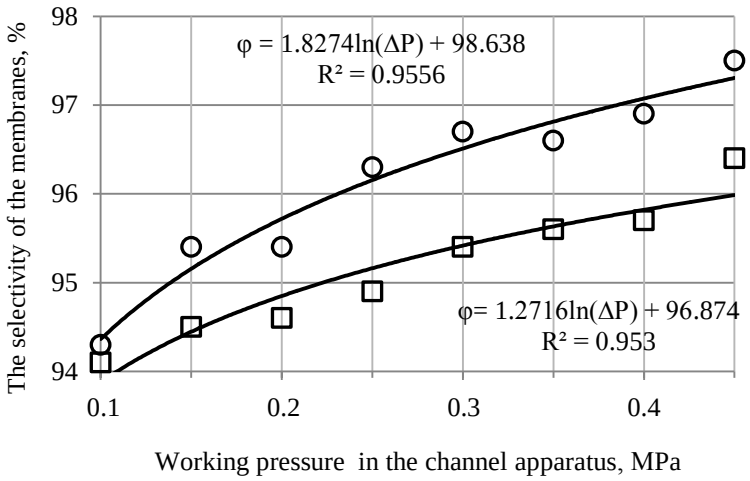

Fig. 4. Dependence of selectivity φ of membranes of type UAM-150 on the value of operation pressure Δp (t = 10÷12°C, *v* = 0.1÷0.3 m/s, $octal_{notation}$= 8÷8.2%) during ultra-filtration of common (○) and modified (□) milk whey.

A significant increase in the membrane selectivity at Δp > 0.42÷0.44 MPa in case of sieve model of ultra-filtration most probably relates to the start of mechanic obstruction of pores with disperse phase particles complicated with partial deformation of polymer membrane structure. In total, this causes reduction in the effective size of the flow section of pore space. This suggests that the area of the efficient operation pressure value in the pipeline of the baromembrane machine should be limited within Δp = 0.3÷0.4 MPa. The validity of such conclusion is proved by the results of experimental finding review to assess the dependence of the membrane selectivity φ on the value of operation pressure. In general, the pattern of changes in the parameter φ for both wheys is also identical, but, during ultra-filtration of the modified whey, the rate of increase in φ is somewhat lower as compared with that of the common whey as the object of baromembrane separation. This suggests that the intensity of adsorptive intermolecular interactions "disperse phase particles – membrane" identified by physical and chemical properties of the modified milk whey, is lower against that in the common whey.

It should be noted, that in case of tangential flow of the separated liquid system, the permeability value Q and the membrane selectivity φ are considerably affected by the value of circulation rate *v*, apart from the operation pressure, in the circuit of the baromembrane machine. Characteristic curves expressed as Q=f(*v*) and φ=f(*v*) of membranes of type UAM-150 obtained from ultra-filtration of the modified and common milk whey are shown in Fig. 5 and 6.

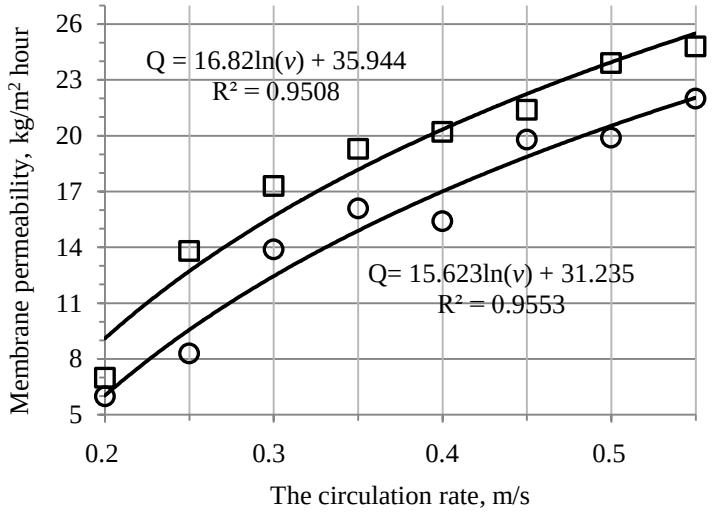

Fig. 5. Dependence of permeability Q of UAM-150 type membranes during ultrafiltration of the common (○) and modified (□) milk wheys on the rate of the separated system circulation in the membrane pipe of the machine (t = 10÷12°C, Δp = 0.1÷0.4 MPa, , $C_{d.m}$ = 8÷8.2%).

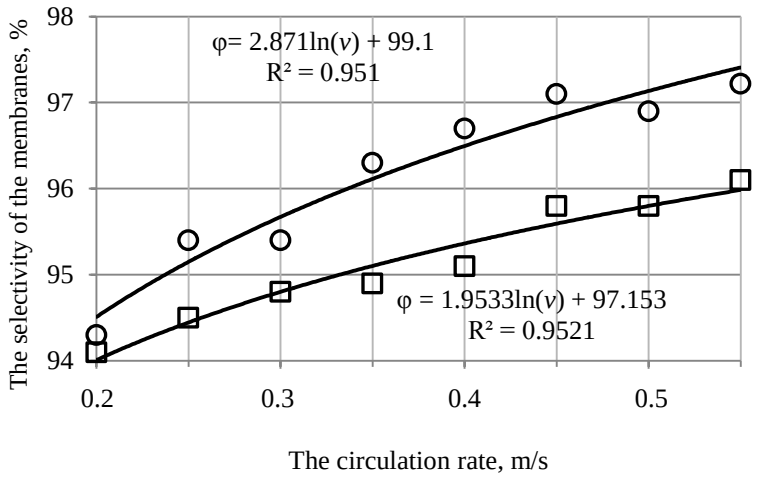

Fig. 6. Dependence of selectivity φ of UAM-150 membranes during ultra-filtration of the common (○) and modified (□) milk whey on the rate of separated system circulation in the membrane pipe of the machine аппарата (t = 10÷12°C, Δp = 0.1÷0.4 MPa, , $C_{d.m.}$= 8÷8.2%).

The results of analysis of dependence curves Q=f(*v*) and φ=f(*v*) allow concluding that, at all other things constant, the obvious increase in the membrane permeability is seen against an increase in the circulation rate for both common (○) and modified (□) milk whey within *v*=0.2÷0.4 m/s. If the sieve ultra-filtration is used, the changes in the membrane selectivity φ at *v*>0.4 m/s may be explained by the fact that, this circulation rate causes destruction of poorly attached deposit layers in the pipe of the membrane unit that formed on the membrane surface [8, 9]. However, this parameter increased higher than *v* =0.6 m/s in cassette units is not adequate to remove mainly "primary" protein fields, strongly attached to the membrane. This conclusion may be proved by the fact that the flow rate rise over *v* = 0.5 m/s has practically no impact on membrane permeability during ultra-filtration of the milk whey. And, when the modified whey is used as the object of separation, the Q value tends to rise insignificantly which may be due to probable changes in physical and chemical properties of the separated system and, as a result, to the reduction in intramolecular binding between the membrane and the disperse phase particles of the separated system. Therefore, it may be suggested that the scope of the best circulation rate MES in the baromembrane unit pipeline should be limited to within *v* = 0.4÷1.0 m/s.

To identify the potential to use the permeate of modified milk whey as the base for production of drinks, its physical and chemical properties have been investigated and, first of all, organoleptic evaluation has been performed in comparison of the permeate of the common milk whey obtained using one and the same type of UAM-150 membranes. The investigation results are shown in Table 3.

As per the overall evaluation, the permeate of the modified milk whey 0.25 scores excels the common whey permeate. Physical and chemical properties of permeates are given in Table 4.

Table 3. Comparative organoleptic evaluation of modified and common milk whey permeates

Parameter	Caseous whey permeate	Modified milk whey permeate
Appearance	Homogenous liquid, clear, of greenish to yellow color	Homogenous liquid, clear, of light-yellow color
Odor	Fermented-milk, clean flavor	
Taste	Sour, with strong wheyish after-taste	Slightly acid to sweet flavor, with weak wheyish flavor, with no after-taste
Overall evaluation	0.5	0.75

Table 4. Physical and chemical properties of permeates of modified and common milk wheys (p = 0.95)

Parameter	Modified whey permeate	Common whey permeate
w/w of dry substances, %	5.0	5.1
Total protein, %	0.10	0.15
Lactose, %	4.4	4.4
Minerals, %	0.5	0.5
Acidity, pH	4.3	4.2
Acidity, ºT	88	85
Density, kg/m^3	1020	1018

During ultra-filtration of the secondary milk product, the structure of permeate is considerably affected by the main separation processes and concentration factor. Tables 5 and 6 show the results of experimental testing of this parameter impact on the content of nitrogen compounds, weight ratio of dry substances, lactose and mineral substances in the permeate of the modified milk whey.

It is specified that the content of dry substances in the permeate increases during ultra-filtration of the modified milk whey. This is presumably due to an increase in the membrane selectivity parameter caused by the pore obstruction with the whey protein molecules and the relevant increase in the concentration of the disperse phase particles of the retentate. This results in the partial rise of weight ratio of lactose, nitrogen compounds and mineral complex components in the permeate. In addition, higher content of potassium, calcium, sodium, phosphor and magnesium ions in the permeate shows its value as the source of macro and micro elements of the similar significance for the dietary structure as other components of the secondary diary stock. It may be supposed that the hydrous and mineral complex of such permeate may be used as the base to produce a different class of natural mineralized drinks.

The complete cycle of the modified whey comprehensive re-processing should be arranged so that to ensure efficient application of both permeate and retentate obtained in less volumes but being the valuable source of native serum proteins.

To assess the competitively of the modified milk whey retentate with selected types of protein products used to produce different food products, its organoleptic parameters (Table 7) are shown against the concentrate of serum proteins obtained by the conventional method of thermal denaturation (KSBT).

Comparison of organoleptic parameters of the retentate of modified milk whey and KSBT showed that stevia extract added commit to neutralize the wheyish flavor and taste in the semi-finished food obtained. Since concentrates of serum proteins are used to produce various food staff [8, 9] to a wider extent with their consumer features mostly specified by physical and chemical parameters of such raw material, one of the goals of this stage of study was to assess the relevant parameters of the retentate obtained during separation of the modified milk whey by ultra-filtration. Table 8 shows main parameters which allow, upon analysis thereof, defining main paths of further use of such retentate to manufacture food products or for other use.

Thus, it may be suggested that the modified milk whey retentate is superior to KSBT in its organoleptic characteristics and does not yield to KSB-UF in terms of physical and chemical parameters.

The capacity to use protein semi-finished products as one of components in food manufacturing process should be assessed in terms of heavy metal content. The raw stock to produce stevia extract used for MES production was obtained from the scientific-experimental farm of the Stavropol State Agrarian University. The test results of the retentate obtained from the modified milk whey separation by ultra-filtration method are shown in Table 9.

Table 5. Dependence of the physical and chemical structure of the modified milk whey permeate on the concentration level (p=0.95)

Concentration factor	Weight ratio, %				
	Dry substances	Protein	Non-protein nitrogen	Lactose	Mineral salts
1.5	5.20	0.1	0.07	4.22	0.49
2	5.20	0.1	0.08	4.25	0.49
2.5	5.21	0.1	0.08	4.29	0.50
3	5.21	0.1	0.09	4.31	0.50
3.5	5.22	0.1	0.09	4.33	0.50
4	5.22	0.1	0.10	4.40	0.51

Table 6. Mineral structure of permeates of modified and common milk wheys (p=0.95)

Description of speciment	Mineral structure mg/100 ml (mg, %)				
	Na^{+}	K^{+}	Ca^{+2}	Mg^{+2}	P^{+5}
Common whey permeate	38.8	156.0	92.4	9.2	48.0
Modified permeate	57.4	187.0	146.7	10.8	85.0

Table 7. Organoleptic evaluation of MES and KSBT retentate

Parameter	Modified milk whey retentate	KSBT
Appearance	Homogenous mass of creamy tint	Non-homogenous mass with fine factures
Consistence	Delicate	Arenaceous, coarse
Color	Light beige	Yellowish to cream-colored
Odor	Wheyish, with slight herbal flavor	Obvious wheyish
Taste	Sour to sweet, milky, with fruit flavor	Sour, with wheyish after-taste

Table 8. Main characteristics of MES retentate (p = 0.95)

Parameter	KSB-UF	Modified milk whey retentate
Weight ratio of dry substances, %	10.20	10.25
Weight ratio of protein, %	3.83	3.78
Lactose, %	5.10	5.08
Minerals, %	0.71	0.76
pH	4.7	4.8
Acidity, ºT	50	48

Table 9. Content of heavy metals in the MES retentate (p = 0.95)

Name of heavy metal	Concentration in the retentate of modified milk whey, mg/kg	Allowable level of concentration as per SanPiN 2.3.2.1078-01 for similar products, mg/kg
Lead	not detected	0.3
Arsenic	not detected	0.5
Cadmium	0.01	0.2
Mercury	not detected	0.03

Accessibility efficiency of protein substances in the organism may be qualified at a first approximation by the main parameter – balanced state for amino acid content. This evaluation is taken as a basis to classify proteins as per their biological value which is the basic criterion of the protein potential to fit human needs in amino acids. For such evaluation, the amino-acid score method is traditionally applied which ensures to give a comparative description of any protein against the reference protein as per the content of amino acids in it. The test results of the amino acid content in the retentate of the modified milk whey is shown in Table 10.

It should be noted that certain amino acids (for example, lusine) in proteins may form compounds hardly accessible in the organism during long-term raw material storage or treatment. It means they become practically fully unavailable to the action of digestive enzymes which results in considerable decrease in the value of proteins themselves. And, since the study used the process of ultra-filtration of the modified milk whey at higher pressure but at the temperature up to

10 ± 2°C, the process length should be considered as the limiting external factor to process the modified milk whey. It is specified that amino acids (leucine, valine, histidine, phenylalanine and others) contained in proteins of milk whey basically transfer to the retentate of the modified milk whey to the fullest extent.

As specified in the official SanPiN document 2.3.2.1078-01 (Hygienic requirements to safety and value of food products), all food products should, apart from the direct intent to fit the human physiological needs in nutrients, comply with standards in terms of the accessible level of potentially hazardous microorganisms for the human health. This is what explains the necessity of the study of microbiological parameters of the modified milk whey retentate both directly upon its production and in process of storage at (4 ± 1)ºC in the sealed package (Table 11).

Based on the results of experimental studies reviewed, it is established that the retentate obtained by separation of the modified milk whey by ultra-filtration method, may be referred to valuable protein semi-finished products, in terms of physical and chemical parameters, microbiological points and content of amino acids, that differ from closest analogs in higher organoleptic parameters. This allows using the retentate in production technique of food products.

Table 10. Amino acid content of the modified milk whey retentate as compared with serum proteins (p = 0.95)

Amino acid	Content of amino acids, g/100 g proteins	
	Proteins (milk whey)	Modified milk whey retentate
Asparagine acid	10.6	8.6
Threonine	5.2	4.7
Serine	5.2	4.1
Glutamic acid	17.1	16.1
Glycine	1.7	1.2
Alanine	5.3	4.4
Valine	5.7	5.2
Methionine	2.3	1.7
Isoleucine	6.5	6.1
Leucine	12.3	9.8
Thirosine	3.8	3.5
Phenylalanine	4.4	4.1
Histidine	1.7	1.6
Lysine	9.1	7.4

Table 11. Microbiological parameters of the modified milk whey retentate (p = 0.95)

Parameter	Modified milk whey retentate		
	Fresh	4-day	8-day
Total viable count, CFU/g	$0.7 \cdot 10^3$	$0.9 \cdot 10^3$	$3.0 \cdot 10^4$
	(at least $5 \cdot 10^4$ is allowed)		
Coliforms, in 1 g	not detected		
Yeasts, CFU/g	absent		
Fungi, CFU/g	absent		

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IMPROVEMENT OF LOCALLY MANUFACTURED EQUIPMENT FOR NON-STANDARD OAT PROCESSING

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Abstract: Analysis of the existing oat groat and Hercules flakes production processes suggests that oat processing technology largely corresponds to the conventional technologies prescribed in the applicable regulatory documentation. This process only allows for processing oat grains which conform to standard specifications. Use of off-spec grain results in production of low grade or non-conformant products, which considerably reduces the profit margin of such processing. This paper presents results of studies on non-standard oat grains processing. This grain differs from standard type in moisture content, in mass fraction of double grains, in mass fraction of small grains, and in grain admixture content (grain mixtures). A distinctive feature of the technologies we propose is the lack of this grain preparation stage preceeding the processing part. Use of the proposed technologies allows to increase both utilization and profitability of oat processing and groat production facilities. Our findings suggest that the proposed technologies provide considerable advantages. We have calculated the economic effectiveness of processing grains with four types of non-conformity. We demonstrated that use of this technology allows to reduce production costs of non-standard grain processing in producing Hercules oat flakes by up to 17.8% and result in a fully conformant product.

Keywords: oat grain, humidity, small grain, double grains, substandard grain, Hercules flakes, small grains, grain properties

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INTRODUCTION

Grain products belong to the staple food due to their distinctive features: capacity to synthesize a great number of dry substances, to retain their properties for a long period of time, their transportability and affordability. With their significant nutritive value – proteins, carbs, minerals, and vitamins they contain – grain derivatives account for approximately 1/3 of the human diet, providing for more than half of the daily energy intake [1].

Use of oats in food industry (oat groats, flakes, flour, oatmeal, etc.) is related to high digestibility of its nutritional elements and vitamins; diverse benefits of oats make this product equally suitable for healthy people, individuals suffering from diabetes and hypertension, for kids and people with specific dietary needs. Oat derivatives consumption is on the third place after rice and buckwheat, and keeps growing constantly.

However, flour & groats industry of Altai Krai is faced with a series of systemic challeges, such as [2]:
– Shortage of quality grain, with specifications complying with groats production codes;
– Considerable wear of process equipment;
– Dwindling markets: recently, Altai Krai has been very active in developing local production, however, Altai groats are not really in demand in the European part of the country;
– Low profit margin does not allow to invest in efficient advertising and marketing campaigns;
– Altai enterprises have no export opportunities;
– Storage, transportation, and logistics for groats products is underdeveloped: rehabilitation and startup of new facilities allows for utilization of max. 45.6% of the total production capacity of groats industry [3].

Low utilization rates of oat grain processing facilities result in use of substandard grains (grains that do not comply with code specifications) for groats production. In the last 12 years, work experience of Biyskiy elevator JSC, located in the Biysk region of Altai Krai, showed that the quantity of such substandard grain might reach up to 50%. Grain processing companies face the shortage of quality grain suitable for groat production. New grain processing methods and technologies are being elaborted in order to address the challenge of processing poor quality grains. Due to low cost-efficiency of such processing, this type of grain had always been used as forage, and never in food industry applications.

New technological solutions are being designed in order to respond to such objectives as production costs and price reduction, product quality improvement,

higher utilization efficiency for grain and grain derivatives, development of cost-effective equipment and resource-saving grain processing technologies, elaboration of a new series of healthy food products with targeted modifications introduced into their chemical composition [4].

Production process taking place at an oat processing facility consists of a series of interrelated operations, each one of them is performed by designated equipment and corresponds to the specific anatomy of a grain, with respective distribution of nutrient and non-nutrient substances within. Analysis of the existing groat production processes suggests that groat processing technology largely corresponds to the conventional classifications prescribed in the applicable regulatory documentation.

This technology only allows to process the grain that conforms with the code specifications. Results of non-standard grain processing allow to conclude that grains with off-spec quality undergo a significant change of their technological properties. That is why processing such grain with the help of standard technology results not efficient or altogether impossible.

Use of non-standard grain leads to deficient production output and low profit margins. Therefore, it seems reasonable to investigate quality parameters and technological properties of non-standard grain, which will contribute to a better organization of technological processes for obtaining groat from this grain.

As of today, no data have been reported on rehabilitation of assemblies and equipment targeting non-standard grain to groat processing.

The objective of this work is to improve home-made equipment for processing non-standard oat grains, and to evaluate the efficiency of the proposed technological solutions.

OBJECTS AND METHODS OF STUDY

For our experiments, we selected batches of non-standard oat grains grown in the Biysk region of Altai Krai between 2002 and 2014 and delivered for oat flour processing to be further distributed via retail and wholsale.

All studies were carried out in shop conditions, at an oat processing facility with 50.0 ton/hour capacity. We used grains of spec quality, complying with groat processing code requirements, as controls.

Cost efficiency of a grain processing facility depends on various factors, including end product output. End product output, in turn, depends on quality parameters of the grain, its moisture content, kernel content, weedy and grain admixtures, uniformity, shape and size of the grain. Grains with the above parameters below the specification requirements are classified as non-standard. Struggling to meet specification requirements in preparing such grains for processing is rather difficult and not really cost effective. Processing such grains for groat results in off-spec products, or products of low quality, with poor nutritional value and taste qualities, which makes these products hardly competitive on the market. However, in market conditions, competitive success of a product is mainly determined by its quality.

In our study, we focused on oat grain batches delivered for processing with the following non-conformities:

– With moisture content of 15.6–18.0%;
– With double grains mass fraction up to 10.0%;
– With small grains mass fraction up to 20.0%;
– Oat admixtures.

RESULTS AND DISCUSSION

Groat quality parameters and profit margin of a processing facility are positively related to the technology used for grain processing, and for the quality parameters of the grain in use. Analysis of current technologies suggests that the existing oat grain processing technologies are based on prior conditioning of the grain until it meets the process requirements. A distinctive feature of the technologies we propose is the lack of this grain preparation stage preceeding the processing part. Implementation of the proposed technologies would allow to use non-standard oat grains in producing oat flour, to ensure certain profit margin for the industry, to reduce production costs and the price of the product, to improve the quality of the product, and to make a rational use of the grains with non-conformities under consideration. As a quantitative analysis, we provide an analysis of product costs for end product calculated in accordance with the technology recommended by applicable codes and standards. All studies were replicated 4-6 times and statistically processed. Across this text, average values are provided for each parameter. To determine these parameters, standard research methods were used.

Processing grain with moisture content above 15.6%. In order to investigate opportunities for improving oat grain processing technology and to adjust it for grains with higher moisture content, we tested a new method based on reduced grain preparation time prior to processing grains into groat and flakes [5].

Of all the grain delivered for processing, batches with moisture content over 15.6% were selected (deviation range 1.0 % max.). These batches were sent directly to the oat processing facility, without prior drying process. Instead of drying the grain until moisture content reaches 13.0–13.5%, in this method we used steaming [6].

According to the proposed technology, oat grains with higher moisture content are supplied into a steaming unit A9-BPB, intermittently operated. Prior to steaming, the grains are pre-heated for 20 minutes up to 20–30ºC, which accelerates the hydrothermal treatment process of the grains. Steaming is performed at 0.2–0.3 MPa pressure for 1.5–3.0 minutes. Steaming duration is calculated from the moment steam is supplied to the steamer until steam supply is stopped. From the steamer, grains are fed into a dryer for 30–40 minutes, with drying agent at 60–90ºC, and dried until the moisture content of 16–18%, then for the next 60–80 minutes they are dried in presence of drying agent at 90–110ºC. Upon drying, grains are

cooled with air, down to maximum temperature of 45ºC. When steaming and drying up to 13.0–13.5% moisture content is over, oat grains are fed into a hulling machine with further groats grading and production of Hercules oat flakes.

In order to ensure maximum separation of entire kernels, we need to account for technological properties of the grain, including structural mechanic and morphological ones [7]. Surface morphology was studied for kernels with different moisture content. Moisture content of the grain samples under study is provided in Table 1.

Kernel surface morphology in test samples was studied on a scanning electron microscope JSM-840 (Jeol, Japan), images are presented in Fig. 1.

Microphotographies in Fig. 1b, 1c, 1d allow to discern hairs of various size located across the entire surface; these might make up to 1.5–2.0% of solid mass fraction.

As we can see in Fig. 1, at ×500 magnification, the kernel surface demonstrates a regular pattern seen as longitudinal ribbing, and this pattern is more pronounced when the kernel is dry. Modification of oat kernel surface structure suggests that increased moisture produces changes in its plastic properties, which should lead to a lower hulling factor of the oat.

The grain we used for testing the proposed technology had the following quality parameters (Table 2).

Provided data suggest that grain with higher moisture content might be used for groat production, if the proposed technology is applied.

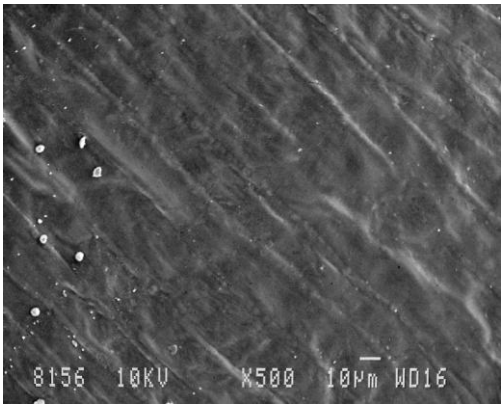
During long storage, Hercules oat flakes are known to undergo changes of both organoleptic and biochemical parameters, which results in overal deterioration of their quality. In order to evaluate changes in acidity and acidity index associated with storage duration, we performed a comparative analysis of these parameters in products manufactured by conventionally recommended and proposed hydrothermal treatment (HTT) technologies [8], results are provided in Table 3

Analysis of the data presented in Table 3 suggests that increasing temperature and pressure of saturated steam used for HTT of oats grain has impact on quality parameters of the end products. Indicated HTT regimes [9] allow for processing grains with the proposed technology, with drying substituted for steaming, which stabilizes acidity modification both in the grain and in the end product.

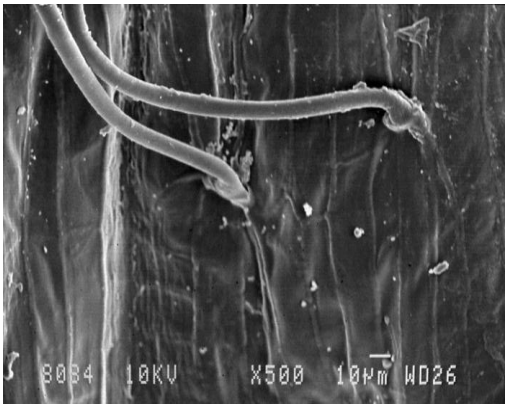
Studies of relationship between acidity / acidity index of end products (Hercules oat flakes) and initial moisture content of grains processed as per the proposed technology, within the guaranteed shelf-life period, and organoleptic properties in accordance with standard requirements, are summarized in Table 4.

Table 1. Kernel moisture content in test oat samples

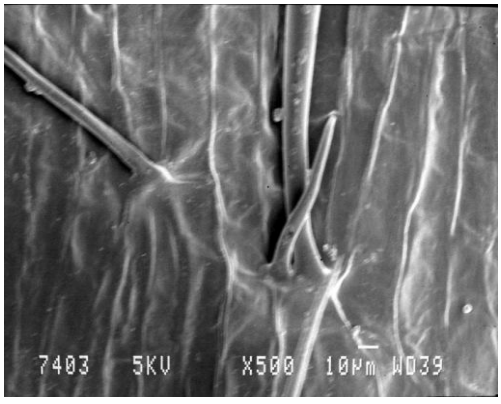
Description	Moisture content by weight, %
Sample 1a	26.7
Sample 1b	17.8
Sample 1c	12.8
Sample 1d	11.4



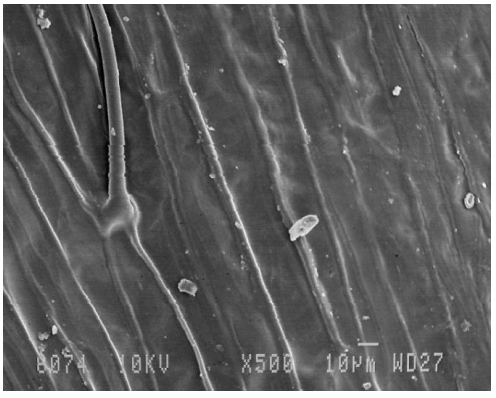
Sample 1a



Sample 1b



Sample 1c



Sample 1d

Fig. 1. Kernel surface morphology in oats with different moisture content ×500.

Table 2. Grain quality parameters in grain processed into groats with conventional and proposed technologies

Parameters	Grain quality		
	as per standard requirements	as per process specifications according to the applicable codes and standards	as per proposed technology
Moisture content, %	max. 13.5	12.5	16.8
Kernel, %	min. 63.0	64.1	63.9
Weedy admixture, %	max. 3.0	2.0	2.5
Grain admixture, %	max. 7.0	4.5	5.4
Acidity, deg.	max. 6.0	5.3	5.4

Table 3. HTT impact on shelf-life changes in acidity and acidity index of Hercules oat flakes

Saturated steam parameters		Acidity, deg.		Acidity index, mg KOH/g	
Pressure, MPa	Temperature, ºC	HTT as per standard requirements	HTT as per proposed method	HTT as per standard requirements	HTT as per proposed method
0.030 ± 0.001	81.0 ± 1.0	5.0–5.5	not determined	16.6	not determined
0.055 ± 0.001	91.0 ± 1.0	5.0–5.4		16.2	
0.070 ± 0.001	99.0 ± 1.0	4.8–5.1		16.1	
0.200 ± 0.001	120.0 ± 1.0	not determined	4.5–4.9	not determined	15.8
0.300 ± 0.001	133.0 ± 1.0		4.4–4.8		15.6

Table 4. Acidity and acidity index changes in Hercules oat flakes during shelf-life period

Parameters	Shelf-life, days								
	30			90			120		
	moisture content of feedstock grain, %								
	below 13.5	14.5	16.3	below 13.5	14.5	16.3	below 13.5	14.5	16.3
Hercules flakes moisture content, %	max. 11.3–11.8								
Acidity index, mg KOH/g	15.8	16.1	16.5	16.3	16.8	17.1	30.6	30.8	31.2
Hercules flakes acidity, deg.	3.2	3.2	3.4	4.0	4.0	4.2	4.9	5.3	5.4
Organoleptic parameters: taste, color, odor	conform to the standard, characteristic								

Analysis of the above Table 4 suggests that within the indicated period, Hercules oat flakes produced by the proposed technology are in conformity with the standard requirements.

Designing a processing technology for oat grains with double grains mass fraction below 10 %. In processing oat grains delivered to the plant we selected batches with high content of double grains [10]. When grains are processed into groats, groat feedstock is quality oat suitable for groat production. Most important traits of grains suitable for groat production are a well formed kernel with minimum hull content. According to the standard requirements, oat grains shall be uniform in size, and contain a minimum amount of double grains which are difficult to process.

Double oat grains are formed in a two floret spikelet with an undeveloped or underdeveloped kernel of the first floret, under unfavorable flowering conditions in dry years. The lower grain of the spikelet is not developed or only partially developed, and its hulls are covering the second grain. Oat kernel has an elongated spindle-like or oblong shape, and the hull is tight.

Quality parameters of double grains in comparison to average quality of oats grown in the Biyskiy region are presented in Table 5.

Table 5. Quality parameters of double grains and average quality of oats grown in the Biyskiy region

Variety	Grain unit, g/l	Grain shape	Thousand grain weight, g	Kernel content, %	Hull content, %
Double grains	380	small, well formed	21.8	43.3	38.5
Oat quality across the Biyskiy region	460	medium size	33.3	62.5	26.5

When these grains are present in the feedstock, technological properties of the oat are considerably degraded (hull content by weight might reach 40%), kernel content is decreased, and besides, certain processing challenges also arise. Double grains images are presented in Fig. 2.

Moreover, double grains have very poor sowing and foraging properties, so batches with dual grains shall be rejected.

Oat hull content (mass fraction of double grains) varies greatly and depends on the oat variety, weather conditions, cultivation practices, soil conditions, and might also be hereditary.

Grains with two hulls have poor nutritional value (low kernel content, down to 45.5%), however, while they are removed from the grain mixture, a considerable part of large, well-formed grains are removed with them, as their geometric sizes are quite similar.

According to the standard requirements, in grain to groats processing, dual grains are classified as grain admixture.

In this paper, we present studies of grain to groats processing with oats containing dual grains. For the tests, we selected batches of Korifey variety collected in the piedmont zone of Altai Krai, with various mass fractions of double kernels.

Quality parameters of grains compliant with standard requirements, and grains supplied for processing to groats, are presented in Table 6.

Fig. 2. Left to right: double grains, kernel hull, kernel with one hull and coarse hulled kernel.

Table 6. Quality parameters of oat grains compliant with standard requirements and grains supplied for processing

Parameters	Quality parameters of grain supplied for processing		
	as per standard requirements	as per process specifications according to the applicable codes and standards	as per proposed technology
Moisture content, %	min. 13.5	13.0	13.5
Grain unit, g/l	min. 460	465	430
Kernel, %	min. 63.0	63.5	61.1
Weedy admixture, %	max. 3.0	2.0	3.6
Grain admixure, % including small grains, %	max. 7.0	2.9	5.5
	max. 5.0	2.0	2.2
Double grains, %	classified as grain admixture, not regulated separately	1.4	6.4
Hull content, %	not regulated	26.6	32.6

Studies have shown that mass fraction of double grains within the feedstock grain might reach up to 10 %.

Processing such oat batches in accordance with standard requirements is not cost effective, as both end product output and capacity are reduced.

Use of special equipment to remove external hulls from double grains increases volumes of crushed grain and content of normal grain in grain screenings. When grains are graded by size, double grains fall into the coarse category, which reduces not only capacity, but also efficiency of the whole oat processing facility. In accordance with standard requirements, large grains and double grains were hulled in the same hulling machine. At the same time, in double grains only one external hull is removed.

Study of double grain geometrical parameters showed that its length is comparable to sizes of larger grains, while hulled grains are similar in size to coarse oat hulled grains and to small grains.

When hulling is over, size similarity makes machine grading of this mixture even more complicated, which results in producing off-spec or low grade products [11].

Mass fraction of unhulled grains in the end product depending on the double grains content is presented in Table 7.

The provided data suggest that in technological applications as per standard requirements, with increased mass fraction of double grains in oat feedstock, amount of unhulled grains in the end product is increased, which results in producing off-spec products. Use of grains with high double grain content for groat production brings us to conclude that, in order to obtain high quality products, we should reduce the capacity of oat processing facility down to 25%, however, increased number of cycles of the process equipment leads to higher fractions of crushed kernels and middlings, to deterioration of consumer properties, and as a result, a reduced output of the end product.

In order to resolve this issue, we deiced to separate double grains into a segregated category at the stage of fractionation. We determined geometrical dimensions and performed a comparative analysis of different types of oat grains that contain double grains. The results of studying coarse (large) grains, double grains, and small grains of oat are presented in Fig. 3.

During the study, we have experimentally confirmed the utility of separating this fraction based on the length of grains.

Corresponding geometrical dimensions suggest that these oat grain fractions might be processed together, within the same processing schemes.

In order to improve the existing technology, we carried out comparative studies of processing oat with high double grain content as per standard requirements and as per the technology we proposed.

In order to eliminate the above challenges of hulling process and further grading of hulled products, we proposed to use drum separators to segregate double grains as a separate fraction and to feed it into the hulling system together with the small grains fraction.

Table 7. Mass fraction of unhulled grains in the end product depending on the double grains content

Parameter	Double grains mass fraction, %					Standard requirement
	2	4	6	8	10	
Mass fraction of non-hulled grain in the end product, %	0.7	1.2	2.0	2.4	2.9	max. 0.4

Fig. 3. Left to right: large oat grains, double grains, and small grains.

During hulling, separating the exterior hull in the grain with double coating allowed to make the second hulling and groat grading more efficient, due to corresponding geometrical dimensions of the obtained grains and small grains.

Thus, a modified grain fractionation regime and an introduction of a new fraction into grain processing allowed to use feedstock with double grains content for producing oat flour in conformity with standard requirements.

Designing a processing technology for oat grains with small grains mass fraction below 20%. Oats used in groat production must necessarily correspond to the standard requirements, in respect to such main traits as size, uniformity, limited presence of small grains.

In processing oat grains delivered to the plant we selected batches with high content of small grains. In unfavorable years, small grains fraction might reach up to 20%. Use for such grain in groat production is not cost efficient, as studies have shown that presence of small grain fractions affects both process and economic efficiency of the processing facility. As per standard requirements, such grain is used as forage, as otherwise considerable part of small grains would be removed as screenings during preparation for processing.

However, these grains tend to have a high kernel content and a low hull content. Quality parameters of small grains in comparison to average quality of oats grown in the Biyskiy region are presented in Table 8.

Provided data suggest that small oat grains might well be used for groat production.

In order to make grains to groat processing more efficient, for oat with high content of small grains, we modified the fraction segregation method [12].

As we studied fraction segregation, we used grains with the following quality parameters (Table 9).

Provided data suggest that quality parameters of the grain used for the study are considerably different from the standard ones. In order to determine technological properties of the grain within the experiment, we selected 9 mm oat grains.

As per the standard requirements, small grain classification and fraction segregation are based on grain thickness (riddling size 1.8 × 20). However, small fraction segregation based on grain thickness has one significant disadvantage for oat grain processing.

This oat processing procedure is efficient when working with standard grains.

For processing off-spec oat batches, we proposed a processing scheme based on oat grain segregation by grain length, using a drum separator with a 9 mm cell.

A change of fractionation criteria allowed to obtain coarse oats with weedy and grain admixtures of 0.06–0.1% max.

Grain remaining from the primary segregation, with weedy and grain admixtures, was sent to the grain purification stage [12].

Grain purification performance for the second fraction, as per standard technology and as per the proposed technology, is decribed in Table 10.

Table 8. Quality parameters of small grains and average quality of oats grown in the Biyskiy region

Variety	Grain unit, g/l	Grain shape	Thousand grain weight, g	Kernel content, %	Hull content, %
Small grains	540	small, well formed	24.8	67.6	22.5
Oat quality across the Biyskiy region	460	medium size	33.3	62.5	26.5

Table 9. Quality parameters of oat grains compliant with standard requirements and grains supplied for processing

Parameters	Quality parameters of grain supplied for processing		
	as per standard requirements	as per process specifications according to the applicable codes and standards	as per proposed technology
Moisture content, %	min. 13.5	13.3	14.1
Grain unit, g/dm^3	min. 460	465	480
Kernel, %	min. 63.0	61.1	60.6
Weedy admixture, %	max. 3.0	2.0	4.6
Grain admixture, % including small grains,	max. 7.0	2.9	11.8
	max. 5.0	3.5	4.1
Oat grains: kernel length max. 9 mm, %	not regulated	9.3	12.2

Table 10. Quality parameters of oat grain before hulling as per standard and proposed technologies

Oat grain admixtures	Weight ratio, %	
	as per process specifications according to the applicable codes and standards	as per proposed technology
Weedy admixture, including: - organic impurities - oatgrass - weed seeds	0.33 – 0.16 0.17	0.35 – 0.17 0.18
Grain admixture, including: - hulled grains - crushed grains - peas - barley - wheat	0.75 – – 0.48 0.27 –	0.60 – – 0.30 0.30 –
Valid grain content in screenings, max.	65.0	3.6
Weedy admixture content in the end product	0.35	0.30

Provided data suggest that if grain is prepared for hulling as per the recommended standard technology, the content of valid grain in grain screenings achieves up to 65%. Loss of this grain in relation to physical weight of the processed batch reduces the profit margin of the oat processing facility.

Use of an admixtures-small grains segregation principle based on length and thickness of grains allowed to achieve a high degree of purification, without loosing small grains into screenings. Small oat grains purification based on grain length was performed with a Pectus K233A sieve, with drum polymer cells d 5.2 mm, manufactured by TekhMashPolimer LLC (Perm). Further use of the rotating sieve surface of ZMB-3 bolter with elongated openings 2.6 × 20 mm and 2.8 × 20 mm (depending on the oat variety: needle-like or pear-shaped) allowed to separate the remaining admixture by thickness. Small fraction was sent for processing, while weedy and grain admixtures, diverted into the storage hopper. Content of weedy and grain admixtures in small fraction before and after grain processing according to the proposed technology are presented in Table 11.

Provided data suggest that fraction segregation method we proposed allowed to efficiently purify small oat grains and to use grains with high content of small grains for groat production.

Thus, processing oat grains with the proposed technology allows to process oat batches with mass fraction of small grains (below 9 mm) up to 20%, obtaining groat that corresponds to the standard requirements.

Designing a processing technology for oat grain mixtures. A grain mixture is a mixture of two or more types of grain, when at least one of them makes up to at least 15%. In our study, we used grain batches with oat content 70–85%, while wheat, barley, and pea amounts were below 30%.

Grain mixtures are perfect forage for poultry, both in industrial and household setting. They have a high crop capacity, convenient price, and have an ample scope of various applications. Jointly planted crops are beneficial for both forage and grain nutritional values. In oat grain, protein content is increased by 1.0–1.2%, the same is applicable to fats, etc. [13].

Oat grains in such batches tend to be large, with a well formed kernel. Quality parameters of oat separated from the grain mixture are described in Table 12.

Provided data suggest that in the context of grain shortage, it seems reasonable to use grain mixtures for groat production.

Table 11. Weedy and grain admixtures in small fraction before and after grain processing according to the proposed technology

Admixtures in small oat grains	Weight ratio, %	
	before purification	after purification, max.
Weedy admixture, including: - organic impurities - oatgrass - weed seeds	1.92 0.8 0.82 0.30	0.14 – 0.10 0.04
Grain admixture, including: - hulled grains - crushed grains - peas - barley - wheat	5.4 2.1 0.55 0.6 1.1 1.05	1.4 1.2 – – – 0.2

Table 12. Quality parameters of oat segregated from grain mixtures, Korifey vatiety, and average quality of oats grown in the Biyskiy region

Variety	Grain unit, g/l	Grain shape	Thousand grain weight, g	Kernel content, %	Hull content, %	Kernel volume, mm^3
Oat from grain mixtures	530	large	38.4	65.6	23.2	32.8
Korifey	550	large	38.0	65.0	24.0	33.0
Oat quality across the Biyskiy region	460	Medium size	33.3	62.5	26.5	29.1

Grain mass supplied to the processing facility contains various admixtures, apart from the oat itself [14]. Grain batches originally represent a mixture of grains of various cultural and weedy plants.

Such admixtures as barley, wheat, and pea represent obstacles for oat purification, which results in a complicated multi-step purification procedure and decreased cost effectiveness.

As per standards requirements, in order to segregate such amount of admixtures, the grading procedure is repeated, and special grain cleaning machines are used.

Oat grain cleaning, as per standard technology, allows to process grain with mass fraction of weedy and grain admixture below the limit values for 1st category grain.

Loss of normal grain is grain screenings.

Processing grain for groat production suggests further grading of batches based on the weedy content.

That is why, during grain acceptance at the facility, actual losses of feedstock are calculated, indicated admixtures are separated, and their respective volumes are introduced into the quality certificate of the batch. Based on the feedstock loss to screenings, a batch of grain mixture is formed, for further processing into groat. During the study of grain cleaning line operation, we used grain with the following quality parameters (Table 13).

We proposed a technological procedure for cleaning this grain [15]. It differs from the technology prescribed by standard documentation, as it suggests different grain and weed admixture segregation parameters, and consists of four consecutive stages.

Our technological procedure allowed to obtain products in conformity with the standard specifications from grain with up to 24% of admixtures.

Product costs for non-standard oat grains with the above non-conformities was calculated on the basis of the actual prices at that time, 3000 rubles/ton, and 2500 rubles/ton for oat grain mixtures. Standard output of Hercules oat flakes being 60.0% and 55.0% for grain mixtures. We used standard grain as control.

Product costs calculation for processing non-standard oat grains is presented in Table 14.

Thus, a complex approach to rehabilitation of Russian-made equipment allows to use non-standard oat grain for production of oat groat in conformity with the standard requirements, with product costs decrease down to 17.8 %.

Table 13. Quality parameters of oat grain mixtures

Parameters	Quality parameters of grain supplied for processing, %		
	as per standard requirements	as per process specifications according to the applicable codes and standards	as per proposed technology
Moisture content	max. 13.5	13.2	13.4
Kernel	min. 63	63.3	55.8
Admixture fraction: weedy grain	 max. 3.0 max. 7.0	 2.5 4.4	 3.2 19.6

Table 14. Product costs of processing non-standard oat grains

Grain non-conformity	Product costs, 1 ton, rubles	Decrease of product costs, %	Profit margin, %	Grain price per 1 ton, inclusive of VAT (rubles)	Groat price per 1 ton, inclusive of VAT (rubles)
Standard grain	6265		10	3000	7580
Moist, st.	6640	–	3.8	3000	7580
Moist, prop.	6305	5.3	9.3	3000	7580
With mass fraction of double grains up to 10% st.	7233	–	-4.7	3000	7580
With mass fraction of double grains up to 10% prop.	6544	10.5	5.3	3000	7580
With mass fraction of small grains up to 20% st.	7340	–	-6.1	3000	7580
With mass fraction of small grains up to 20% prop.	6718	9.2	2.3	3000	7580
Oat grain mixtures, st.	7428	–	-7.2	2500	7580
Oat grain mixtures, prop.	6305	17.8	9.3	2500	7580

Note. *st.– standard technology, *prop.– proposed technology.

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USE OF BAR PROCESSING TO INCREASE THE SHELF LIFE OF VITAMINIZED SAUSAGES AND THEIR USE FOR THE CORRECTION OF STUDENTS' HEALTH

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Abstract: One of the priority directions of state policy in the field of healthy food is the development and integration of enriched foodstuffs with an increased expiration date into production. The purpose of researches is the study of effect of bar processing on the periods of storage of boiled sausages enriched with vitamin premix. The test samples of boiled sausages were processed under the pressure of 800 MPa within 3 minutes at a temperature of 0 … +4°C by means of a hydrostat after the end of the technological process. The control samples of boiled sausages were not processed under pressure. The safety of boiled sausages was estimated by organoleptic and microbiological indicators and pH, the content of vitamins in the product was researched in 8 and 16 days of storage. It has been established that in 16 days of storage the control samples of sausages did not conform to the requirements of regulating documentation; the shift of pH to the alkaline side, the increase in the quantity of mesophilic aerobic and facultative and anaerobic microorganisms has been noted. After 16 days of storage the content of PP and C vitamins in the control samples of sausages authentically decreased by 17.6% and 93.4% while the decrease in the test samples was 4.1% and 12.0%. The antioxidant activity of test samples of sausages is authentically 71.3% higher than that of the control samples (0.12 ± 0.04 mol equiv/dm^3). Against a background of use of vitaminized sausages an authentic increase in the antioxidant activity of catalase and ceruloplasmin is noted in the blood of students of the test group. Thus, it is established that the processing of boiled sausages enriched with vitamins, under high pressure has a bactericidal effect on microbic cells, prevents proteolysis, saves vitamins and, respectively, increases the expiration date of a foodstuff. The calculations for design of a high pressure hydrostat for foodstuff processing have been performed.

Keywords: processing under high pressure, meat raw materials, meat products, technical regulations, hydrostat, antioxidants, indicators of quality and safety

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INTRODUCTION

75% of the population of the Russian Federation feel lack of irreplaceable micronutrients in their diet which provides the deterioration of health, a decrease in working capacity, an increase in fatigue and the development of various diseases. One of the etiological factors of hypovitaminoses is a decrease in the quantity of vitamins in foodstuffs due to the use of thermal technologies of processing of food raw materials providing a decrease in a share of biologically active agents in foodstuffs.

In this regard, the development of enriched foodstuffs and the provision of stability of micronutrients in the course of storage is one of the priority directions of the modern food industry. In this case, foodstuff processing under high pressure deserves special attention. The main scope of the method of high pressures in the world today is athermic preservation ("cold pasteurization", pascalization) of foodstuffs aimed at the inactivation of microorganisms and enzymes of the processed environment. In the 90-ies of the last century in Japan the first wave of popularity of

jams of strawberry, kiwi and apples received by the use of high hydrostatic pressure began, in 1997 barotechnology was first used by the company Fresherized Foods – a world leader in the production of Guakamole (a traditional Mexican snack of avocado pulp), in 2007 approximately 120 barometric plants were put into operation for production of "new" products commercially [1] worldwide. More than 80% of the equipment functioning today were collected and produced after 2000 which testifies that this trend tends to the accelerated development and expansion of its scope [2]. Today the following countries dominate in this trend of production: North America (USA, Canada, Mexico); Europe (Spain, Italy, Portugal, France, Great Britain, Germany); Asia (Japan, China, North Korea); Australia.

The total of the products processed under pressure is steadily increasing in the world. According to the data [1] approximately 200,000 tons of this type of products (approximately 450 million pounds/year) were manufactured and delivered in sale during 2008.

The expansion of bar processing of food products is related to the fact that using the method of cold preservation the prevention of microbiological damage is possible. It has been proved during the repeated researches that the barometric effect of the pressure of 600 MPa at 20°C during 180 sec is capable to liquidate causative agents of listeriosis (*Listeria monocytogenes*) in meat and meat products, and also to inactivate other life-threatening microorganisms - colibacillus (*E. coli*), salmonellas (*Salmonella*), cholera vibrio (*Vibrio*), the most of species of mold mushrooms and pathogenic bacteria [3]. Today the considered technology is applicable only for inhibition of processes of growth and reproduction of vegetative forms of bacteria, however the combination of pressure and temperature is capable to provide the inactivation of spores of microorganisms as well. Thus, for example, the spores of *Clostridium botulinum* and some representatives of the sorts *Bacillus* and *Clostridia* can be destroyed as a result of synergic effect of the temperature and barometric factor. Such an effect provides to reduce a thermal effect by means of the pressure additionally imparted to the system [4–6]. The resistance of sporous forms is much higher than vegetative ones because of the presence of a serious protective mechanism in the first ones. Thus, it is known it is necessary to put a product under the pressure of 300–400 MPa at 25°C for several minutes to deactivate yeast, however, to destroy yeast ascospores a higher pressure and a longer effect is required. Spores of *Clostridium botulinum* are considered the steadiest among bacterial pathogenic spores, and *Bacillus amyloliquefaciens* disputes – among nonpathogenic ones [5].

It has also been proved that the pressure over 200 MPa in the temperature condition not above 45°C is capable to inactivate effectively the vegetative forms of practically all pathogenic microorganisms and those which spoil the food with no effect on flavor characteristics [7]. However, it is important to note that the efficiency of process depends, to a greater degree, on the type and complexity of the organization of microorganisms, the chemical composition and pH of the processed environment, and also on water activity. Gram-negative bacteria are more sensitive to the effect of high pressure, than gram-positive ones are. The barometric effect causes the destruction of cellular membranes and intracellular proteins playing the major role in the activity of microorganisms, this all provides the degradation of cellular structures and final destruction of a cage in general. The shift toward the acidic pH and the increase in pressure have a synergy effect during the elimination of microorganisms. Due to the increase in acidity of the environment, the inhibition of activity of water molecules occurs which provides a considerable delay of processes of inactivation induced by extra-high pressure [4]. Today the technology of high hydrostatic pressure includes two main methods – a batch and a semicontinuous one. Of practical use is, mainly, the batch technology that presumes that the packed lot of goods is placed into the chamber, then is hermetized and filled with a transfer medium (water or other low-molecular liquids). The targeted pressure imparted to the environment is transferred to the elastic walls of packing and thereof the compression of product [8, 9] occurs. The semicontinuous methods are not at all perfect today, both in the power and economic aspects. They have been created for the purpose of implementation of direct compression of liquid foodstuffs.

Fig. 1 presents the ratio between various classes of foodstuffs (%) the technology of high pressures can be applied to today.

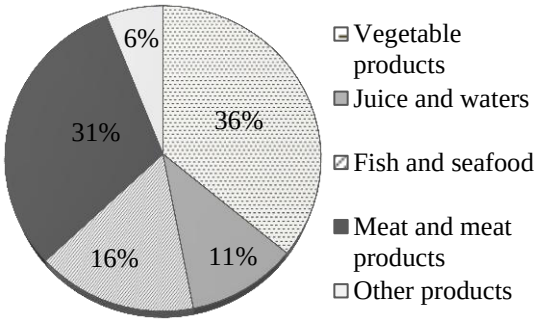

Fig. 1. Industrial use of high hydrostatic pressure for processing of different groups of foodstuffs [10].

It is established that during the processing under the pressure of 130 MPa there is inhibition of growth of microorganisms in beef for a week, the color of meat improves, the gained effect remains for 3 days at a storage temperature +4°C [9].

As a result of the researches, Han J.M. and Ledward D.A. found out that the rigidity of muscular tissue of beef increases with the increase in pressure from 200 to 800 MPa (at a constant temperature from 20 to 40°C), but considerably decreases during the use of pressure at a level of 200 MPa (at a temperature of 60 and 70°C) [10]. A Bai Y. and coauthors noted that after processing under high pressure (300–700 MPa) within 20 min. considerable changes of organoleptic properties of meat can be observed. They also recorded some modifications in the microscopic structure of myofibrils of the muscular tissue of cattle and mutton [11]. Qin H. and others proved that the activity of

calpains in the course of bar processing decreases, but the activity of acid and alkaline phosphatases does not significantly differ from the values of control samples. The pressure of about 100–200 MPa is capable to inactivate calpastatin (an inhibitor of activity of calpain) more rapidly than calpain itself is [12].

L.G. Vinnikova obtained some quite interesting results. She chose the processing under the pressure in the interval from 500 to 700 MPa during 30–60 sec as an optimum technological mode, which provided to inhibit the activity of acid phosphatases when imparting the maximum pressure (700 MPa) to the samples and thus to reach full culinary readiness with the organoleptic indicators corresponding to a boiled meat product. The mass losses of the target product in the course of processing were also reduced by 35% in comparison with the thermal effect. Thus, almost a hundred percent outcome of the finished product was stated [13].

As for the cost of modern equipment for processing, it varies from 500 000 to 2.5 million dollars depending on the power and extent of automation. The internal volume of a vessel varies from 30 to more than 600 liters [14].

The use of high hydrostatic pressure in food industry becomes more and more demanded every year. The interest of use of this particular technology is that it is capable to inactivate the action of microorganisms and fermental complexes without the inhibition of the energy and biological value.

Today one of the priority directions of state policy in the field of healthy nutrition and replacement of foreign food technologies is the development and integration of the foodstuffs enriched with irreplaceable micronutrients and also of new domestic processing equipment for food industry into production. The provision of safety of the biologically active agents put into the formulation throughout the entire period of storage deserves special attention.

The aim of work is the research of effect of bar processing on the periods of storage of boiled sausages enriched with vitamins, the assessment of efficiency of their use for correction of the state of health of students and the development of equipment alternative to import equipment capable to produce the pressure not less than 1200 MPa providing to use it in food industry.

OBJECTS AND METHODS OF STUDY

The objects of researches were boiled sausages in a nylon cover "Amilyuks" with a period of storage of 4 days at a temperature from 2 to 6°C, enriched with the vitamin premix 730/4 produced by ValetekProimpeks, CJSC in the amount of 150 g per 100 kg of the basic raw materials.

The control samples of boiled sausages enriched with vitamin premix were not processed under high pressure. The test samples of boiled sausages enriched with vitamins were processed after the end of the technological process under the pressure of 800 MPa within 3 minutes at a temperature of 0 … +4°C by means of an experimental plant – a hydrostat (Fig. 2) with the following technical characteristics: the process pressure is 800–1000 MPa; the maximum pressure is 1200 MPa, the time of process stabilization is 2 to 3 min, the process liquid is a mix of industrial oil and glycerin.

Fig. 2. High pressure plant (hydrostat).

The safety of boiled sausages was estimated according to the organoleptic and microbiological indicators and pH, the content of vitamins in the product was researched in 8 and 16 days of storage.

The microbiological indicators are in accordance with GOST R 54354-2011 “Meat and meat products. General requirements and methods of microbiological testing”, GOST 31747-2012 (ISO 4831:2006, ISO 4832:2006) “Food products. Methods for detection and quantity determination of coliforms”.Using the pH-potentiometric method, vitamins – using the fluorimetric method.The antioxidant activity – using the potentiometric method. The shift of potential Pt electrode made using screen-printing technique in the mediator system $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, observed during the administration of antioxidants (a sample) into the solution was the source of information of antioxidant activity. This shift occurs due to the change of ratio of the oxidized and reduced forms of components of mediator system as a result of the following reaction:

$$Fe(III) + AO = Fe(II) + AO_{ox}.$$

The researches were performed using a lab-scale plant in Institute of Metal Physics, Ural Department of the RAS (Yekaterinburg) and at the Department of Food Engineering of Ural State Economic University (Yekaterinburg).

The statistical processing of results was carried out with the use of the standard computer programs Microsoft Excel XP, Statistica 8.0.

RESULTS AND DISCUSSION

Table 1 presents the organoleptic indicators of boiled sausages in 8 and 16 days of refrigerating storage at a temperature of +4°C.

It follows from the data of Table 1 that after 8 days of storage the control and test samples of sausages conformed to the requirements of the Technical regulation of the Customs union “About safety of meat and meat products” (TR TS 034/2013). In 16 days of storage the control samples of sausages had a slippery and damp surface, lost their elasticity, differed in an ammoniac smell while the test samples conformed to the requirements of the regulating documentation.

Table 1. Organoleptic indicators of control and test samples of boiled sausages in 8 and 16 days of refrigerating storage at a temperature of +4 °C

Parameter	Group	
	Group 1 (control)	Group 2 (test)
After 8 days of storage		
Appearance	Bars with a clean and dry surface	Bars with a clean and dry surface
Consistence	Delicate and juicy	Delicate and juicy
Color and look in the cut	Pink, uniform mincemeat, evenly mixed	Pink, uniform mincemeat, evenly mixed
Smell and taste	Peculiar to this type of product, without foreign flavor and smell, with the aroma of spices, moderately salty	Peculiar to this type of product, without foreign flavor and smell, with the aroma of spices, moderately salty
After 16 days of storage		
Appearance	Bars with a clean and slippery surface	Bars with a clean and dry surface
Consistence	Delicate, juicy and less elastic	Delicate and juicy
Color and look in the cut	Pink, uniform mincemeat, evenly mixed	Pink, uniform mincemeat, evenly mixed
Smell and taste	Ammoniacal	Peculiar to this type of product, without foreign flavor and smell, with the aroma of spices, moderately salty

Researches of pH of sausages in the course of their storage (Fig. 3) have been performed.

Fig. 3 shows that the shift of pH to the alkaline side in the course of storage of control samples of boiled sausages is noted; the most significant changes are in the control samples which testifies an active activity of the residual microflora in a foodstuff providing proteolysis and, respectively, the accumulation of nitrogenous bases. The obtained data are coordinated with the results of organoleptic (Table 1) and microbiological researches. An authentic increase in pH has not been noted in the test samples.

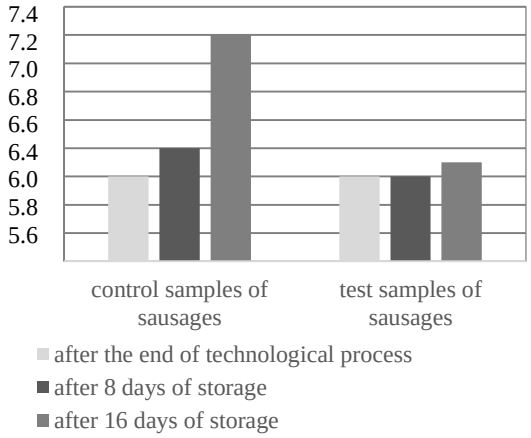

Fig. 3. Dynamics of pH of boiled sausages during their storage.

A significant increase in the content of mesophilic aerobic and facultative and anaerobic microorganisms has been noted in the control samples of boiled sausages. The quantity of bacteria was $1.6x10^3$ in 8 days of storage and $2.7x10^3$ in 16 days which exceeds the requirements of TRTS 034/2013. The processing of boiled sausages under high pressure provided to receive a sterile foodstuff. Mesophilic aerobic and facultative and anaerobic microorganisms in the test samples of sausages have not been found during the entire period of storage.

Table 2 presents the content of B_1, B_2, PP and C vitamins in the course of their storage.

It follows from the data of Table 2 that in the course of storage of boiled sausages enriched with vitamin premix a decrease in the quantity of vitamins both in the control and test samples is noted, in particular, after 8 days of storage the quantity of B1, B2, PP and C vitamins decreased by 5.3%; 1.3%; 2.7% and 13.6% in the control samples. After 16 days of storage the content of PP and C vitamin in the control samples of sausages authentically decreased by 17.6% and 93.4% while in the test samples it decreased by 4.1% and 12%. It follows from the obtained data that the processing of boiled sausages, enriched with vitamin premix, under the high pressure of 800 MPa does not only destroy the vitamins of mincemeat and premix, but also provides to keep them safe for the entire period of storage.

Table 2. Content of B_1, B_2, PP and C vitamins in the course of production and storage of boiled sausages enriched with vitamin premix, mg/100g

Boiled sausages	Content of vitamins											
	After the end of technological process				After 8 days of storage				After 16 days of storage			
	B1	B2	PP	C	B1	B2	PP	C	B1	B2	PP	C
control	0.57 ± 0.05	0.75 ± 0.08	7.4 ± 0.9	30.1 ± 0.7	0.54 ± 0.05	0.74 ± 0.07	7.2 ± 0.9	28.0 ± 1.1	0.53 ± 0.05	0.73 ± 0.05	6.1 ± 0.8	2.0 ± 0.9*
test	0.58 ± 0.04	0.75 ± 0.07	7.4 ± 0.5	30.0 ± 0.7	0.56 ± 0.05	0.75 ± 0.08	7.2 ± 0.8	28.2 ± 1.0	0.55 ± 0.05	0.73 ± 0.07	7.1 ± 0.9	26.4 ± 1.0*

The microbiological decay of boiled sausages begins even prior to lipide oxidation, however the information of the resistance of boiled sausages to oxidation is absent in this case. In the course of oxidation of lipide components there is a rancidish taste, the color and consistence worsen and the nutrition value decreases. The process of autooxidation of lipids, during which unsaturated fatty acids react with oxygen with the formation of acylhydroperoxides or peroxides of fatty acids, proceeds according to a free radical mechanism. The substances that block or slow down the process of oxidation of lipids are called antioxidants. In this regard, we investigated the antioxidatic activity of boiled sausages (AO). As a result of researches, it has been established that the test samples of sausages had a higher AOA (0.42 ± 0.03 mol equiv / dm^3), which is authentically 71.3% higher (** $P \leq 0.01$) than the AOA of the control samples (0.12 ± 0.04 mol equiv/dm^3). The obtained data are explained by the presence of vitamins C and E, which have an antioxidatic action, in the vitamin premix 730/4 and by the high stability of vitamins in the test samples of boiled sausages.

The research of assessment of efficiency of use of enriched sausages in student nutrition was performed in South Ural State Agricultural University at the department of physical training and sport in the academic year 2015–2016. Two groups of students (young men) of the first course at the age of 18–19 were formed. The criterion for including in the research was the voluntary written consent to the participation in the experiment, the provision of necessary personal health information about themselves, the accommodation in the dorm, the meals in the university canteen and attending classes of physical culture. The criterion for dismissal was the incidence of acute infectious diseases at the time of the research or within 30 days prior to the experiment, the use of vitamins and mineral substances.

Table 3 presents the scheme of researches.

The assessment of nutritional level of students was performed according to the methodical recommendations (MR) of 2.3.1.2432-08 “Norms of physiological needs for energy and feedstuffs for various groups of the population of the Russian Federation”. The statistical processing of experimental data was performed by means of the computer program Statistica-6. The assessment of state of health of students was performed with the use of method of questioning and research of the antioxidant activity of blood and the indicators of a cellular link of immunity according to the standard techniques.

The questioning of students regarding the assessment of their food is performed according to a five-mark grading system. It is established that only 8% of the students estimate their food as excellent, 35% – as good, 45% – as adequate and 12% – as inadequate. It should be noted that 27% of respondents complained about the state of their health. They complained about frequent headaches, rapid fatigability, weakness, drowsiness, a decrease in working capacity, frequent acute respiratory diseases (ARD), stomach pains, the lability of arterial pressure and others. The obtained data are coordinated with the assessment of actual diet where there is lack of the vital micronutrients.

The criterion of the state of health of students are some indicators of antioxidant activity: antioxidant activity (AOA), the content of enzyme catalase (C) and protein ceruloplasmin (CP) in the blood of students.

An authentic increase in antioxidant activity by 26.7% in the blood of students of the test group is noted against a background of the use of vitaminized sausages. Similar changes are noted in the content of catalase and ceruloplasmin. Thus, the amount of catalase and ceruloplasmin increased by 19.2% and 16.1%. The authentic changes of indicators of antioxidant protection of the organism of students of the control group are not noted. An increase in working capacity and decrease in fatigue at a background of application of vitaminized sausages is noted in the questionnaires of 61% of students of the test group.

In view of the fact that the foreign equipment for processing under high pressure in the conditions of all-round compression is expensive, it is reasonable to manufacture a domestically produced hydrostat that would not yield to foreign analogs in technological parameters. Schematically a high pressure hydrostat is presented in Fig. 4. To manufacture the hydrostat strength calculations of the hydraulic cylinder of the compression chamber have been performed.

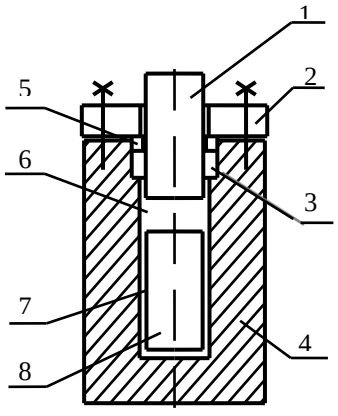

Fig. 4. Schematical design of a high pressure hydrostat for processing of objects with a liquid in the conditions of all-round compression: 1 – punch; 2 – flange; 3 – sealing; 4 – working chamber; 5 – washer; 6 – process liquid; 7 – cover; 8 – object of the research (product).

Table 3. Scheme of researches

Group	Number of students in the group, persons	Age of students, years	Correction of a diet by vitaminized boiled sausages	Dose and frequency of use of hematogen
Control	20	18 – 19	–	–
Test	20	18 – 19	Inclusion of vitaminized boiled sausages in the diet	100 g daily within 20 days

Strength calculations of the compression hydraulic cylinder of the high pressure hydrostat.Calculation of pressures in the compression hydraulic cylinder of the hydrostat.

Design pressure according to the condition of strength P_{des1}, MPa

$$P_{des1}=P_{1max} - P_{atm}, \quad (1)$$

Where P_{1max} is the maximum excessive pressure in the hydraulic cylinder of the hydrostat, MPa; P_{atm} is the atmospheric pressure, MPa.

For calculation we take P_{1max} = 1200 MPa; P_{atm} = 0.1 MPa.

Then the design pressure according to the condition of strength as per (1)

$$P_{des1}=1200-0.1=1199.9\approx 1200 \text{ MPa}.$$

As the pressures less that the atmospheric pressure are not supposed to be in the hydraulic cylinder of the hydrostat, the calculation for the chamber is performed only under the terms of strength.

Calculation of thickness of the wall of the hydraulic cylinder according to the strength theory. The cylinder is made of autofretted high-tensile steel of the brand "O-АБ".

Design thickness of the wall of the hydraulic cylinder of the compression chamber S_1, mm:

$$S_1=S_{des1} + C_1, \quad (2)$$

where S_{des1} is a design thickness of the wall of the hydraulic cylinder, mm; C_1 is an addition to the design thickness considering the process of material corrosion, mm;

Addition to the design thickness considering the process of material corrosion C_1, mm

$$C_1 = A \cdot t, \quad (3)$$

where A is the design rate of corrosion of the material of construction, mm/year; t is the planned service life of the hydrostat, year.

For calculation we take t = 10 years and A = 0.005 mm/year according to the standard for vessels and devices under pressure.

$$C_1 = 0.005 \cdot 10 = 0.05 \text{ mm/year}.$$

Design thickness of the wall of the hydraulic cylinder of the compression chamber S_{des1}, mm

$$S_{des1} = \max\{S_{n1}; S_{stab1}\}, \quad (4)$$

As the design thickness of the wall of the hydraulic cylinder the greatest of the received values under the terms of strength S_{n1}, mm, and stability S_{stab1}, mm, is chosen.

As the calculation under the terms of stability is not performed, then $S_{des1} = S_{n1}$.

Design radius of the hydraulic cylinder of the compression chamber under the terms of strength R_c, cm

$$R_c = R_0\sqrt{\frac{\sigma_{tens}+0.4P_{stab}}{\sigma_{tens}-1.3P_{stab}}}, \quad (5)$$

where R_0 is the internal radius of the body of the hydraulic cylinder sufficient for the placement of samples of foodstuffs and semifinished products in it. We take R_0 = 100 mm; σ_{tens} is the admissible tension of material of the body, for autofretted high-tensile steel "O-АБ" $\sigma_{tens} \geq 50$ MPa; P_{stab} is the design pressure of process liquid ($P_{stab} = 1.2\ P_{des}$).

$$P_{stab} = 1.2 \cdot 1200 = 1440 \text{ MPa},$$

$$R_c = 10\sqrt{\frac{5000+0.4\cdot 1440}{5000-1.3\cdot 1440}} = 13.35 = 14 \text{ cm}.$$

Design thickness of the wall of the hydraulic cylinder S_{n1}, cm

$$S_{n1} = R_c - R_0, \quad (6)$$

$$S_{n1} = 14–10 = 4 \text{ cm} = 40 \text{ mm}.$$

Design thickness of the wall of the hydraulic cylinder of the compression chamber, S_1, mm

$$S_1=40 + 0.05 = 40.5 \text{ mm}.$$

Round the received value to the nearest standard value S_1 = 42 mm. The performed calculations provide to start designing a high pressure hydrostat for processing of products in the conditions of all-round compression.

As a result of the performed complex researches of indicators of freshness and safety of vitamins in boiled sausages it has been established that the samples processed with a high pressure of 800 MPas within 3 min. after 16 days of storage conformed to the requirements of the Technical regulation of the Customs union "About safety of food products" (TR TS 021/2011). The high pressure processing of the boiled sausages enriched with vitamin premix makes the foodstuff sterile as a result of a high pressure bactericidal effect on the microbic cells, prevents proteolysis, saves the vitamins, having an antioxidant effect which provides the weakening of processes of oxidation of lipidic components. The received results show that the use of high pressure in technology of storage of boiled sausages provides an increase in the periods of their storage. Against a background of daily use of boiled vitaminized sausages within 20 days the improvement of the state of health of students is noted, in particular, the antioxidant activity of blood and catalase enzyme authentically increases and the amount of ceruloplasmin protein increases. The obtained data are coordinated with the results of questioning. 61% of students note an increase in working capacity and improvement of the general condition of their organism.

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STANDARDIZATION, CERTIFICATION, QUALITY AND SAFETY

INVESTIGATION OF KINETIC PARAMETERS OF THE DIETARY SUPPLEMENT "AMIL-ING"

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Abstract: Enzyme inhibitors are widely used in experimental studies in various spheres to evaluate the mechanism of catalytic effect of enzymes, determine the nature of protein reactive groups, to identify the role of various enzymatic processes in metabolism. But inhibitors are not stable and thus, they need to be stabilized through immobilization on matrixes. The study of immobilization using the infrared spectroscopy ensures to prove the interaction between the inhibitor and polysaccharidic matrix. The results of infrared spectroscopy showed that the linking between the matrix and the inhibitor occurs by formation of intramolecular covalent linkings, electrostatic correlation between the charged groups of agar and inhibitor. The derived comparison curve VVOP shows the reduction in the intensity of the immobilized inhibitor (dietar y supplement) in the area of 3400 cm^{-1}, that is consistent with the valent variations of the free group -OH that indicates on strengthening of the immobilized specimen hydrogen binding. Comparative study of pH-optimum of pancreatic α-amylase, native and immobilized inhibitor made it possible to conclude that pH-optimum of pancreatic α-amylase ispH 6.0, that of the native inhibitor α-amylase equals to 5.5, and the pH-optimum in the immobilized inhibitor considerably varies from 5.0 to 6.8 at the physiologic temperature (37 ± 1)ºC. Linearization methods of Michaelis-Menten equation by Lineweaver-Burk and Hanes were used to determine kinetic parameters of the dietary supplement inhibition. The kinetics of enzyme inhibition was assessed using the immobilized form of the inhibitor that resulted in the enzyme activity decrease at zero variations K_m at the decreasing V_{max} values which makes it possible to classify the inhibition to the linear uncompetitive type (catalyzed inhibition).

Keywords: pancreatic amylase inhibitor, hydrolysis kinetics, kinetic parameters of pancreatic amylase inhibition

DOI: 10.21179/2308-4057-2016-2-128-135 *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 128–135.

INTRODUCTION

As the endocrine system disease in developed countries, including the Ukraine, where the patient population is over 3% of locals, the high incidence of diabetes mellitus with severe morbidities is ranked as the civil country disease that requires comprehensive therapeutical and prevention measures to manage it. Use of dietary supplements is considered one of major measures to prevent the diabetes milletus in developed countries that contain pancreatic amylase inhibitors. In turns, these are able to lower the level of blood glucose due to the ability to suppress the segregation of starch and starch-like alimentary polysaccharides. The use of herbal α-amylase inhibitors does not cause "habituation effect" since the plant source contains contributory biologically active substances wholesome for health.

Today, scientists believe that inhibitors play a vital role in the functions of main biochemical mechanisms that specify and regulate physiological status of the cell, its reactions and interactions with neighboring cells and environmental factors. The complexity of physiological effects caused by inhibitors offer wide opportunities to produce inhibitor-based multi-functional dietary supplements.

Enzyme inhibitors are widely used in experimental tests in biochemistry, physiology, cytology, and genetics to study mechanisms of enzyme catalytic action, to specify the nature of protein functional groups, to define the role of various metabolic processes of enzymes. Recently, enzyme inhibitors are used in medicine as pharmaceutical agents and dietary supplements. The concern in amylase inhibitors is nonrandom. First of all, it is associated with the ability of efficient suppression of hydrolitic processes of alimentary polysaccharide disintegration to reduce the level of blood glucose with diabetes mellitus, obesity, hyperlipidemia and other abnormalities associated with carbohydrate metabolism disorder. Amylolytic enzyme inhibitors are able to specifically slow the reaction behavior in the human body based on the catalysis of

glycosidic linkage disruption in such substrates as starch and amyloid polysaccharides and oligo-saccharides.

Substitution therapy based on antidiabetic insulin still remains the most effective method to manage the diabetes mellitus by medication administration.

The advantage of amylolytic enzyme inhibitors is that they are not involved in the obvious stimulating effect on insulin secretion and administration of amylase inhibitors in parallel with the alimentary therapy does not result in hypoglycaemia development. Amylase inhibitors act in the intestinal lumen. It means they have peripheral mechanism of action that does not result in depletion of β-cells of the pancreas and degeneration of insula and in the diabetes enhancement.

Inhibitors of α-amylase of plant origin are responsible to maintain the activity of their amylase at the certain level, control the negative impact in the complicated glycometabolizm system and also, they decrease the level of blood glucose and insulin injection in healthy people and diabetic patients.

Amylolytic enzyme inhibitors are prevalent in the plant kingdom [1]. In certain cases, the plant-based inhibitors excel the animal and microbial analogs, they are of less toxicity, less allergenic capacity, they contain contributory biologically active component of polysaccharidic, lipidic, pigmental and other origin to be wholesome for health. Grain crops are known for the considerable content of amylolytic enzyme inhibitors [2, 3]. However, the inhibitors are not reported to have high pH values and thermal stability. This significatly reduces their effect on the human body. Various methods are used to stabilize and concentrate inhibitors as follow:

– Physisorption methods on matrixes of natural origin;

– Microencapsulation methods: complexation due to electrostatic interaction (between proteins and polysaccharides) or common coacervation; membraneless osmosis or complex coacervation;

– Method of sedimentation in the isoelectric point may be used for biologically active substances (BAS) of protein origin, though this method results in considerable drop or total loss of the biological activity of BAS.

The purpose of this study is to determine kinetic properties of the biologically active additive based on the pancreatic amylase inhibitor isolated from the oat dust.

OBJECTS AND METHODS OF STUDY

Biotechnology to obtain "Amil-ing" BAA consists of the following:

The oat dust was delipidated in the Soxhlet's extraction apparatus using ten volumes of petroleum-ether. The pancreatic α-amylase inhibitor was extracted from the oat dust 0.15 M NaCl in 0.10 M hydrocarbonate buffer (pH 9.2) at 13.4ºC (30 min, RH 7.2). The extract was heated up to 70ºC for 10 minutes for amylase inactivation that may be extracted from the oat dust. The extract was heated up to 70ºC for 10 minutes. The sediment was decanted by centrifuging at the rate of 3000 rpm for 20 minutes [6].

Water-insoluble polyelectrolyte complexs were obtained by adding to the oat dust extract containing the inhibitor so that the protein content in the mixture was stable within 0 up to 1.1%.

Ammonium sulfate precipitation with the degree of saturation within 40 and 75% was conducted at 4ºC. The deposit generated was dissolved in the distilled water, and the protein suspension was put into the porous membrane and dialyzed against 500 cm^3 of the distilled water for 3 days. The speciment obtained was then centrifugated at the rate of 3000 rpm (for 30 min) and the inhiiting activity was established [6].

4B sepharose was used as a sorbent. The sorbent was activated using the benzoquinone synthesized as per the guidelines of the pre-purified hydroquinone [7–10].

The affine sorbent "pancreatic α-amylase-sepharose 4B" was obtained by covalent binding of the activated carrier with the α-amylase of animal origin as follows: 3 cm^3 of 0.1 M hydrocarbonate buffer of pH 8 was added to 3 cm^3 of gel. The binding reaction occurred at 4ºC within 24 hours. The gel obtained was washed with the distilled water using the glass filter, and then, in the column (1x15 cm) in the sequence as follows: 1 M KCl in 0.1 M Na-acetate buffer, pH 4 for 24 hours; 1 M KCl in 0.1 M Na-bicarbonate buffer, pH 8.5 for 24 hours and distilled water until zero adsorption at 280 nm [6].

The protein solution was passed through the column (1x15 cm) with the bio-specific sorbent "pancreatic α-amylase-sepharose 4B" at a rate of 15 cm^3/min. As soon as the sorbent is saturated which is monitored by the inhibiting activity in the filtrate related to the pancreatic α-amylase, the gel was washed with 0.05 M tris/HCl buffer, pH 8. In the column, the sorbent was washed with 1 M solution of NaCl, 8 M urea in 0.05 M tris/HCl buffer, pH 8, in turn. The inhibitor was desorbed using 10^{-3} M solution of HCl. The active fraction was neutralized with 1 M solution of NaOH and lyophilized [6].

The inhibiting activity (IA) was specified by the rate of enzymatic activity suppression of α-amylase of animal origin and expressed in inhibitory units (IU). The activity of pancreatic amylase was expressed by the content of starch broken with 1 g of enzyme for 1 min.

The amylase activity was specified as follows: 10 cm^3 of 1% starch solution in 0.5 n acetate buffer pH 4.7, thermostated at 37°C was added into the tube containing 5 ml of enzyme solution or 2…10 mg of immobilized specimen in 5 cm^3 of water upon 5-min incubation at 37°C. The reference solution was 10 cm^3 of 1% starch solution in 5 cm^3 of water. In 10 minutes of incubation with the starch, 0.5 cm^3 of the incubated mixture was collected and put in the idione solution of 50 cm^3 in volume (0.0025% iodine solution). The iodine solution is stained in blue when mixed with the reference solution, and in violet color of varying intensity – when mixed with the test solution depending on the volume of starch not reacted.

The optical dense of the reference and test solutions was measured at 590 nm. Amylolytic activity was calculated by the formula:

$$AA = \frac{D_к - D_o \cdot 100}{D_к \cdot 10 \cdot н},$$

where $D_к$ is the optical dense of the reference solution; *Do* is the optical dense of the test solution; 100 is the volume of starch taken as the substrate, in kg; for testing; 10 is the incubation time; *и* is the weigh of specimen, in g.

RESULTS AND DISCUSSION

Among natural polymer carriers, agar – polysaccharide, consisting of agarose and agaropectin isolated from cellular membranes of certain algae, is often used for immobilization. It is reported to have adequate physical integrity that may be intensified by mixing with specific regaents. Agar matrix-based hydroxy groups allow immobilization of most biologically active substances (BAS) by both chemically and by sorption. Dextrane and agarose derivats form the relatively heavy gel. Dextrane derivates are mainly used as porous polymers – sephadex with various binding rate. Today, the affine chromatography is the major procedure to isolate hydrolyzing enzyme inhibitors. Nevertheless, the use of affine chromatography to isolate proteic biologically active substances to obtain biologically active additives (BAA) is not economicaly and technologically feasible. Currently, the methods are specially considered to obtain BAA with valuable BAS along with concomitant herbal source components that stabilize the basic BAS and have the biological activity. This is why, the urgent is the search of methods for BAA containing basic BAS as concentration along with other source components.

Even the minor volume of polyelectrolyte (10%) available resulted in the partial protein sedimentation; the dependence was extreme to the peak extent corresponding to 40% of the sedimented inhibitor during the polyelectrolyte concentration in the mixture $8x10^{-3}$%. The aggregated sediment that occurred during the greatest inhibitor sedimentation, contained the protein and sedimented at the ratio 0.52: 1.00 mg/mg or 40:1 mol/mol that indicates that under these conditions, only the minor portion of the polyelectrolyte forms the water-insoluble complex with the protein. The isolation of the sedimented complex even from such low concentration systems confirms towards its electroneutrality. Since the flocculation process is suppressed in presence of 6 M urea, we may suggest that flocculus develop due to hydrogen bonds between protein molecules and polysaccharide.

Consolidation of the protein globule of the inhibitor by intra-molectular covalent linking and binding with the carrier, inclusion of the carrier in concentrated gels, limit conformational motility of the BAS polypeptide chain. All this results in the higher BAS resistance to denaturation. Carrier-based immobilization with charged groups or buffer properties that ensure the best local pH value in the BAS micro-environment, prevents the protein globule unfolding that depends on its ionization modification. The consistency of immobilized BAS also increases due to prevention of unfavorable dissociation and association processes.

The process of inhibitor complexing with anionic polysaccharide agar in the acidic area of pH scale mainly occurs due to electrostatic interaction between charged groups of agar and protein (inhibitor) and hydrogen linking, and due to hydrogen linking and poor hydrophobic interactions in case if it is higher than the pI (isoelectric point) of complexing.

The inhibitor infra-red spectrum is known to have the characteristic strip of absorption 1168 cm^{-1} induced by skeletal vibrations. The absorption peak in the area of 1616 cm^{-1} is identified by binding vibrations in β-conformations, and in 2314 cm^{-1} area – by symmetric vibrations of methyl groups.The absorption in 3305 cm^{1} area occurs due to amine group vibrations associated with hydrogen linkings, and the separate peak in 3428 cm^{-1} area evidences on vibrations of free amine group. Absorption bands within 2840 ... 2900 cm^{-1} speak for valent vibrations of CH and CH_2 groups.

In the agar spectrum, within 700 ... 900 cm^{-1} area, the absorption bands are seen typical for sugar spectrum that contain galactose chains, also the absorption bands of groups -S_2-O- groups are available (strong absorption band with two peaks 1260 and 1230 cm^{-1}), induced by valent asymmetric vibrations of O = S = O groups.

An intensive wide band with the peak absorption is seen at 3400 cm^{-1} in the spectrum of immobilized inhibitor specimen that is shifted to the low-frequency area as compared with that of free OH-groups. This speaks for involvement of hydroxyls in the hydrogen linking system. Absence of absorption band at 3650 cm^{-1} indicates that almost all hydroxyls groups are involved in the hydrogen bond. We used the method of differential infrared spectroscopy (Fig. 1, 2) to compare and assess test specimens of free inhibitor and "Amil ing" BAA.

The differential infra-red spectrum of comparison (Fig. 1) is characterized by the intensive absorption within 670 ... 1225 cm^{-1} area for the free inhibitor which is due to amine groups available in the protein molecule. The reduction in the value of relative optical density (RODV) for immobilized specimens in this area may be explained by the shielding effect of the matrix (agar).

The derived comparison curve for the specimen RODV is specified by the reduction in absorption intensity of the immobilized inhibitor in 3400 cm^{-1} area which is consistent with the valent vibrations of the free OH-group. This is the evidence that the hydrogen links of the immobilized specimen are intensified.

The differential infra-red spectrum of comparison of the immobilized inhibitor specimen and matrix is also known for higher values of RODV in the hydrogen link absorption area (3000 cm^{-1}) which may be due to hydrogen link formation between the inhibitor and the matrix. Considerable absorption in the area 1230 ... 1260 cm^{-1} for the agar indicates on availability of the sulfonate group in it. The comparison of RODV absorption band for carbonyl groups (1648 ... 1690 cm^{-1}) indicates the absorption shielding in this group resulting from the agar-based inhibitor immobilization.

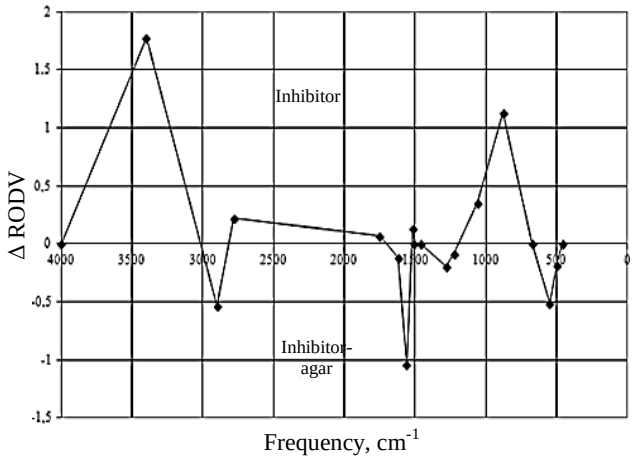

Fig. 1. Differential infra-red spectrum for "Amil ing" BAA (inhibitor-agar) against the free inhibitor.

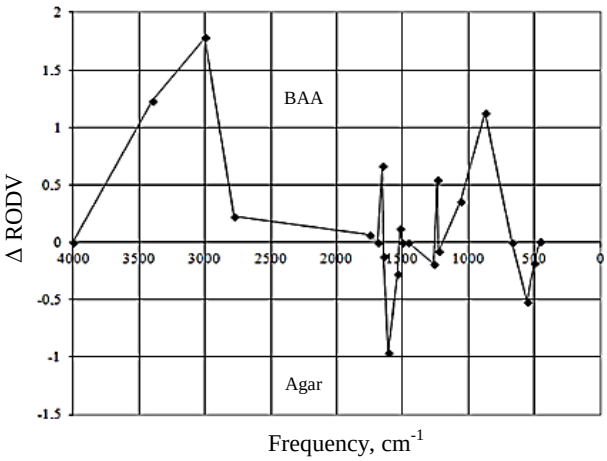

Fig. 2. Differential infra-red spectrum for BAA against the agar.

The differential infra-red spectrum of comparison of the immobilized inhibitor specimen and matrix is also known for higher values of RODV in the hydrogen link absorption area (3000 cm^{-1}) which may be due to hydrogen link formation between the inhibitor and the matrix. Considerable absorption in the area 1230 ... 1260 cm^{-1} for the agar indicates on availability of the sulfonate group in it. The comparison of RODV absorption band for carbonyl groups (1648 ... 1690 cm^{-1}) indicates the absorption shielding in this group resulting from the agar-based inhibitor immobilization.

A rise of relative absorption intensity (up to 44%) is reported in the differential spectrum of the protein within the "protein-agar" system in the area 1610 cm^{-1}, this is consistent with presence in the COOH-group system, as well as (up to 59%) in the are of absorption band 1540 cm^{-1} (Amide II) of the differential agar spectrum within the system "protein-agar" and corresponds to availability in the NH-group system.

Density and reliability of hydrogen link network were evaluated using the characteristic absorption band of 3400 cm^{-1} that is consistent with the valent vibrations of OH groups. For this purpose, the half-width of the band was identified as per wavenumbers of the tested spectrum region and the relative intensity of the band as per RODV (Table 1).

It is seen in data given in Table 1 that the reduction in the half-width of the absorption band is noted consistent with the valent vibrations of OH-groups when comparing specimens of agar and BAA. This is indicative of increase in the number of OH-group involved in strong hydrogen bonds.

Table 1. Characteristics of hydrogen links in "Amil ing" BAA and its main components

Specimen	RODV of 3400 cm^{-1} band	Half-width of band, cm^{-1}
Inhibitor	1.28	400
Agar	1.37	680
"Amil ing" BAA	1.49	650

Thus, the test results evidence that when the agar-based inhibitor is immobilized, hydrogen linkings form between the inhibitor and the matrix.

The results of colorimetric studies confirm that, in all cases of hydration of initial components, their mechanic mixtures and immobilized samples, exothermic effects occur. It is seen from the data obtained that thermal effect values of physical mixtures obtained through testing exceed the theoretically calculated by the sum of thermal effects of their component hydration.

To forecase the inhibitor action and inhibitor-based BAA andthe development of diabetic food product process, one should be aware of factors and methods that affect the inhibitor activity. Whereby, the pH value and the temperature of the environment where the inhibitor acts are of high importance. Enzyme inhibitors are used to slow down or neutralize the activity of relevant enzymes and thus, the pH value and the best termperature value for the inhibitor are main parameters.

The comparative study of pH-optimum of pancreatic α-amylase, pancreatic amylase inhibitor from the oat dust and oat dust-based BAA resulted in the conclusion that pH-optimum of pancreatic α-amylase is pH 6.0, α-amylase inhibitor from the oat dust is 5.5, and the pH-optimum of BAA is more expanded and is within pH 5.0 to 6.8 at the physiological temperature (37 ± 1)ºC. The inhibitory activity of BAA is reduced for 3.5% within this pH range.

The quantitative evaluation of the inhibitor reactivity and namely, the determination of the response kinetic parameters is the vital element of the enzyme assay. Kinetic characteristics of enzyme reaction to inhibition are based on principles of mechanic interaction between inhibitors and enzymes [4, 5, 12].

By the results of kinetic studies the best effective process conditions are determined, the affinity degree of the substrate and inhibitor to the enzyme is assessed, the origin of enzymatic process is specified, and so forth. Involvement in mechanism of enzymatic reactions of intermediate compounds

$E + S \xleftrightarrow{K_m} ES \xrightarrow{K_{кат}} E + P$ results in the following dependence of the fixed reaction rate on the substrate concentration (Michaelis equation):

$$\nu = \frac{-dS}{dt} = -d\left[S = \frac{V_{\max} [S]}{K_m + [S]} \right],$$

where V_{max} and K_m have the effective value, in most cases, since they include constant rates of elementary chemical acts of multiphase enzymatic process.

Ten sets of tests were performed to study the amylase inhibition mechanism. The first set of tests was conducted to identify the amylolytic activity of pancreatic amylase. During further sets, the amylase activity was identified by varying the volume of amylase inhibitor of plant origin 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.7, and 1.9 g/dm^3 (dry substances). The concentration of the pancreatic amylase was 0.01%. The reaction mix to determine kinetic parameteres of pancreatic amylase inibition process included pancreatic amylase solutions, solutions of its inhibitor and 0.1% of starch solution.

The data collected were reviewed by linearization methods of Michaelis-Menten equation by Lineweaver-Burk and Hanes [10, 13]. The inhibition constant value (K_i) was calculated by Dickson and Webb methods [10, 13].

Calculations made by Lineweaver-Burk and Hanes indicate on the linear type of experimental dependence proved by high linear correlation values.

Within the range of inhibitor concentrations 50 mg…3.2 g/dm^3, decrease is reported as V_{max} the concentration of inhibitor increases with no considerable changes in values K_m as compared with the intact enzyme (with the pancreatic amylase inhibitor absent).

The value K_m remains almost constant to the entire range of immobilized inhibitor concentrations; it is approximately 1.5 ... 1.6 times less than those for both intact enzyme and non-competetive inhibition by ingibitor purification.

When comparing results of kinetic studies of intact and immobilized inhibitors, the increase K_m for the immobilized inhibitor was reported against that for the intact inhibitor. Discrepancies in these values calculated by using the data of the Lineweaver-Burk plot are considered statistically unauthentic due to the great value variations; the statistical significance is seen in data obtained based on Hanes plot (value t equals to 3.487 for 1990.1–424.4 = 1565.7 that gives $0.002 < p < 0.01$ at n = 8, the value t = 3.391 for 678.4–445 = 233.3 that also gives $0.002 < p < 0.01$ at n = 11); differences are significant in case of data comparison obtained by Webb's method (t = 7.844 for 1360.4–403.8 = 956.6 that gives $p < 0.001$ at n = 48).

Since the use of immobilized inhibiting agents refers to the area of hetcrogenic processes (inhibition), the CV growth may be caused by:

– Diffusion diseases; due to that the molecular mass of the inhibitor is 25.1 kDa, and that of agar is in several orders greater, the protein binding with such a carrier should inevitably result in diffusion complications;

– Steric (spatial) restrictions; due to abrupt increase in the molecular mass of the product of inhibitor and agar interaction, its binding with the amylase may become complicated.

In addition, the following potentials should be also considered:

– Conformational changes in the inhibitor molecule resulting from its covalent immobilization of the agar; they may well impact the inhibitor binding with the enzyme;

– Electrostatic effect of the agaropectin sulphogroup;

– Impact of these groups on the pH value in the inhibitor and enzyme micro-environment varies the rate and strength of interaction in between.

The reduction in Michaelis constant is deemed as the increase in interaction between the enzyme and substrate (stabilization of enzyme-substrate complex). Thus, the review of experimental data for the pancreatic amylase inhibition by the immobilized inhibitor using mathematical analysis of kinetic studies showed no variations at reduced Vmax values that allow us consider inhibitions to the linear inhibition of non-competitive type (catalyzed inhibition).

The decrease in the enzymatic activity featured by no variations K_m at reducing V_{max} values allows us consider the inhibition process to non-competitive type (catalyzed inhibition). At such, it's quite valid to use Dixon and Webb plots to define the inhibition constant values. Baseline values to graph the Dixon plot are given in Table 2.

Statistic and kinetic paraeters of reaction of the starch hydrolysis with the pancreatic amylase over the inhibitor calculated based on the Dixon diagram as shown in the Table 3.

The Webb plot helps to perform the statistic evaluation of parameters obtained, since not only $tg\alpha = K_i$ is defined by calculations, but also its mean square deviation (S_a). Same refers to evaluation of the initial response rate since the section on the Y-axis ("*b*") is equal to one as the expression $v_0/(v_0–v_i)$ when the enzyme is saturated with the inhibitor, that is, at $v_i \rightarrow 0$ against conditions $[I] \rightarrow \infty$ and, accordingly, $1/[I] \rightarrow 0$ turns into v_0/v_0, and "S_b" is its mean square deviation value. In addition, we may state based on the Webb plot on the extent to which the inhibition constant values correlate in between as in $c = –K_i$.

Table 2. Kinetic parameters of the pancreatic amylase starch hydrolysis over the inhibitor

[I], g/dm^3	$1/V`_{max}$	
	As per Lineweaver-Burk plot data (value *b*)	As per Hanes plot data (value *a*)
0	$2.5679 \cdot 10^{-3}$	$2.5781 \cdot 10^{-3}$
$5 \cdot 10^{-2}$	$2.8763 \cdot 10^{-3}$	$2.8649 \cdot 10^{-3}$
$1 \cdot 10^{-2}$	$3.1988 \cdot 10^{-3}$	$3.3180 \cdot 10^{-3}$
$2 \cdot 10^{-2}$	$3.9772 \cdot 10^{-3}$	$4.0261 \cdot 10^{-3}$
$4 \cdot 10^{-2}$	$4.6375 \cdot 10^{-3}$	$4.9322 \cdot 10^{-3}$
$8 \cdot 10^{-2}$	$6.9369 \cdot 10^{-3}$	$6.7864 \cdot 10^{-3}$
1.6	$1.0419 \cdot 10^{-2}$	$1.0039 \cdot 10^{-2}$
3.2	$1.8181 \cdot 10^{-2}$	$1.9768 \cdot 10^{-2}$

Table 3. Statistical and kinetic parameters of pancreatic amylase starch hydrolisis over the inhibitor calculated based on the Dixon plot

As per Lineweaver-Burk plot data	As per Hanes plot data
$a = 4.8313 \cdot 10^{-3}$	$a = 5.2127 \cdot 10^{-3}$
$b = 2.7646 \cdot 10^{-3}$	$b = 2.6517 \cdot 10^{-3}$
$c = -5.7221 \cdot 10^{-1}$	$c = -5.0870 \cdot 10^{-1}$
$S_a = \pm 6.5854 \cdot 10^{-5}$	$S_a = \pm 1.5709 \cdot 10^{-4}$
$S_b = \pm 8.6028 \cdot 10^{-5}$	$S_b = \pm 2.0521 \cdot 10^{-4}$
$r = 9.9944 \cdot 10^{-1}$	$r = 9.9729 \cdot 10^{-1}$
$n = 8$	$n = 8$
$K_i = 5.7221 \cdot 10^{-1}$ g/dm^3	$K_i = 5.0870 \cdot 10^{-1}$ g/dm^3
V_{max}=361.7216 A.U./mg	V_{max} = 377.1098 A.U/mg

As per Keleti and with regard to data in Lineweaver-Burk plot (Fig. 3), the calculation formula appears as follows:

$$K_i = \frac{tg\alpha \cdot I}{tg\alpha' - tg\alpha} = \frac{\Delta Y \cdot I}{\Delta Y' - \Delta Y},$$

where the character " ' " refers to the relevant parameter for inhibition response.

In view of the physical significance of parameters given, the folowing transformations are made with regard to the Hanes plot:

$$K_i = \frac{\Delta Y \cdot I}{\Delta Y' - \Delta Y} = \frac{tg\alpha \cdot I}{tg\alpha - tg\alpha'}$$

When evaluating K_m the inhibited enzyme by Lineweaver-Burk (Fig. 3) and Hanes (Fig. 4) at 1.6 g/dm^3 inhibitor concentration, 7 points were used to consider when calculating the value of mean square deviation and standard mean square error. Results of statistic constant determinations are shown in Table 4.

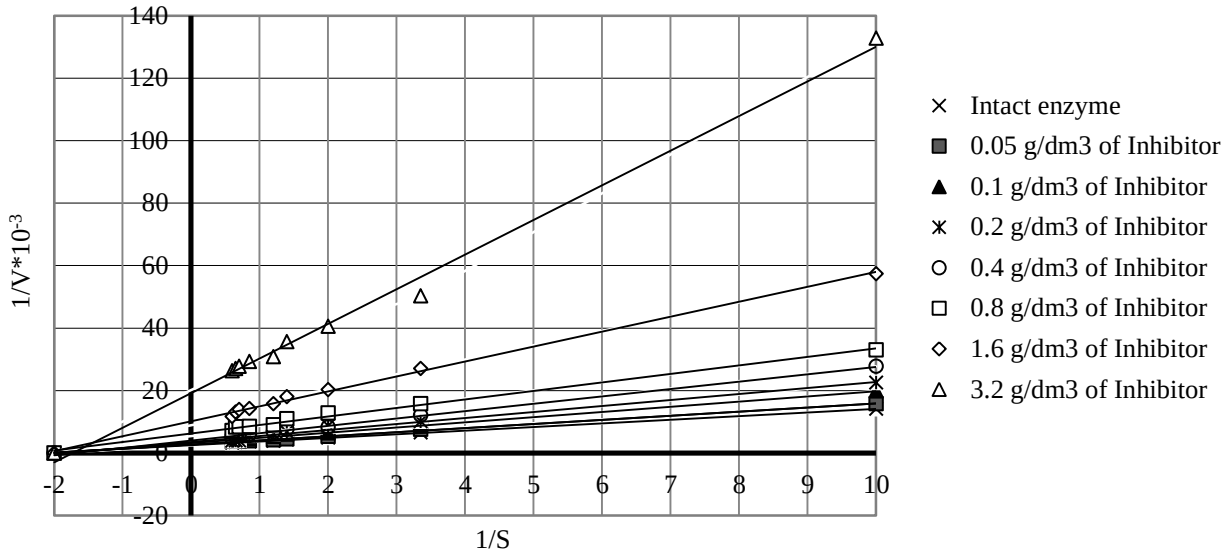

Fig. 4. Inhibition plot by Lineweaver-Burk.

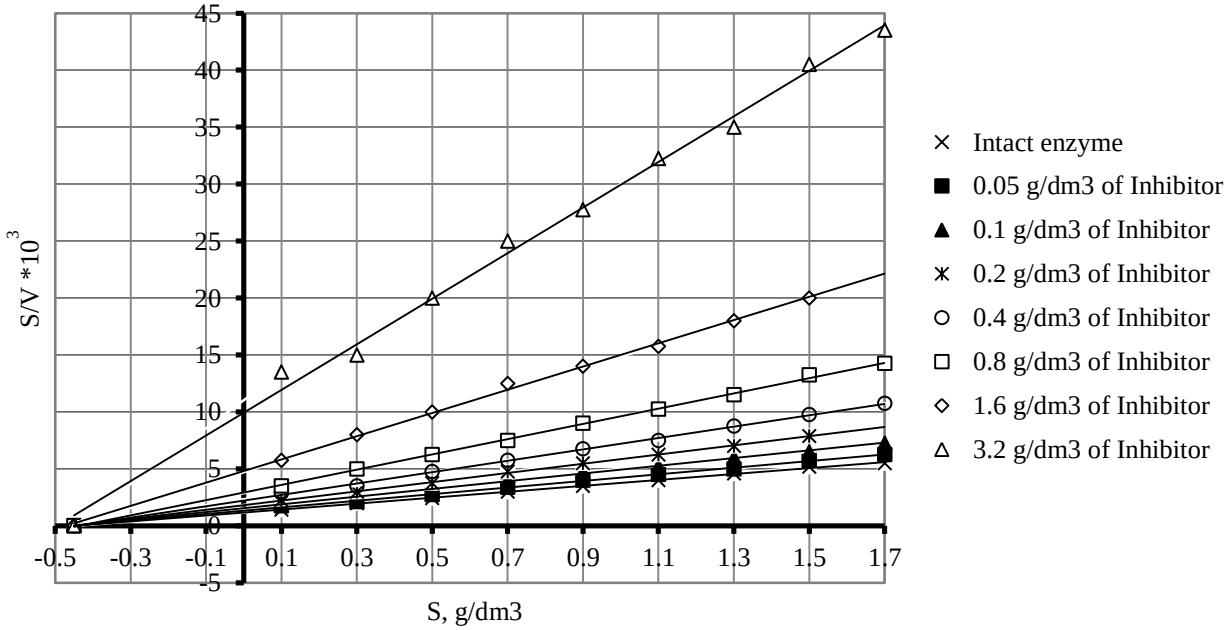

Fig. 3. Inhibition plot by Hanes.

Table 4. Statistical constants to calculate the kinetic parameters by Lineweaver-Burk and Hanes

By Lineweaver-Burk	By Hanes
$\overline{X} = 4.8677 \cdot 10^{-1}$	$\overline{X} = 4.63669 \cdot 10^{-1}$
$G_X = \pm 7.1463 \cdot 10^{-2}$	$G_X = \pm 3.6471 \cdot 10^{-2}$
$S_X = \pm 9.0758 \cdot 10^{-3}$	$S_X = \pm 4.6318 \cdot 10^{-3}$
$t = 53.6333$	$t = 100.1053$
$p < 0.001$	$p < 0.001$
$n = 7$	$n = 7$

Thus, the value K_m by Lineweaver-Burk for the enzyme over the inhibitor K_m = 486.8 ± 9.1 mg/dm^3, and by Hanes, it is K_m = 463.7 ± 4.6 mg/dm^3.

Webb plot data review is shown in Table 5.

Thus, as per the Webb plot, K_i = 403.8 ± 3.4 mg/dm^3. This method results in the reduced values of peak rate for non-inhibited enzyme equal to (92 ± 3)% of theoretical value. The process data of calculation results by Lineweaver-Burk and Hanes plots are shown in Table 6.

As it is seen in data given calculations by K_m/V_{max} give less absolute values K_i than those by $1/V_{max}$. Still, in view of errors when finding these values, the results are, in fact, similar. The analysis results ontained are summarized in Table 7.

CONCLUSIONS AND RECOMMENDATIONS

Therefore, we can state on the linear non-competetive enzyme inhibition upon processing of experimental data obtained upon inhibition of the pancreatic amylase by the proteic inhibitor based on methods of mathematical analysis above of kinetic studies.

Table 5. Statistical calculation constants K_i and V_{max} by the Webb plot

For K_i	For V_{max}
$\overline{X} = 4.0377 \cdot 10^{-1}$	$\overline{X} = 9.2006 \cdot 10^{-1}$
$G_X = \pm 2.6708 \cdot 10^{-2}$	$G_X = \pm 2.3464 \cdot 10^{-1}$
$S_X = \pm 3.3919 \cdot 10^{-3}$	$S_X = \pm 2.9799 \cdot 10^{-2}$
$t = 119.0391$	$t = 30.8754$
$p < 0.001$	$p < 0.001$
$n = 9$	$n = 9$

Table 6. Statistical constant values processed for starch hydrolysis over the maylase inhibitor by Lineweaver-Burk and Hanes methods

For Lineweaver-Burk plot	
As per tg α (K_m/V_{max})	As per Δ У ($1/V_{max}$)
$\overline{X} = 4.1921 \cdot 10^{-1}$	$\overline{X} = 4.5772 \cdot 10^{-1}$
$G_X = \pm 1.2919 \cdot 10^{-1}$	$G_X = \pm 6.2762 \cdot 10^{-2}$
$S_X = \pm 4.8833 \cdot 10^{-2}$	$S_X = \pm 2.3722 \cdot 10^{-2}$
$t = 8.5845$	$t = 19.2953$
$p < 0.001$	$p < 0.001$
$n = 7$	$n = 7$
For Hanes plot	
As per Δ У (K_m/V_{max})	As per tg α ($1/V_{max}$)
$\overline{X} = 4.2436 \cdot 10^{-1}$	$\overline{X} = 4.4498 \cdot 10^{-1}$
$G_X = \pm 1.0203 \cdot 10^{-1}$	$G_X = \pm 7.3228 \cdot 10^{-2}$
$S_X = \pm 3.8563 \cdot 10^{-2}$	$S_X = \pm 2.7678 \cdot 10^{-2}$
$t = 11.0044$	$t = 16.0773$
$p < 0.001$	$p < 0.001$
$n = 7$	$n = 7$

Table 7. Kinetic parameters of the hydrolysis response by the pancreatic amylase starch over the inhibitor based on the oat dust

Parameter to be defined	Lineweaver-Burk plot X ± m n = 7	Hanes plot X ± m n = 7	Computational approach X ± m; n = 7				Dixon plot		Plot by Webb X ± m n = 9
			As per Lineweaver-Burk plot data		As per Hanes plot data		$1/V_{max}$ by Lineweaver-Burk plot	$1/V_{max}$ by Hanes plot	
			tg α K_m/V_{max}	ΔY $1/V_{max}$	ΔY K_m/V_{max}	tg α $1/V_{max}$			
Michaelis constant (a)	486.8 ± 9.1(c)	463.7 ± 4.6 (c)	–	-	–	–	–	–	–
Maximum reaction rate (в)	–	–	–	–	–	–	361.7	377.1	92 ± 3 (d)
Inhibition constant (a)	–	–	419.2 ± 48.8	457.7 ± 23.7	424.4 ± 38.6	445.0 ± 27.7	572.2	508.7	403.8 ± 3.4

Note. а – mg/dm^3; в – A.U./mg; consistent with V_{max} of the intact enzyme; с – statistically significant difference, t = 2.267; 0.02 <p <0.05; d – here, results are given as percentage. Anywhere, apart in case "c", p < 0.001.

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STUDY AND IDENTIFICATION OF MAIN PROTEINS AND PEPTIDES TO DETERMINE THE CONTENT OF MUSCLE PROTEIN IN STRUCTURELESS COOKED PRODUCTS BY THE METHOD OF TWO-DIMENSIONAL ELECTROPHORESIS FOLLOWED BY THE TIME-OF-FLIGHT MASS SPECTROMETRY IDENTIFICATION

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Abstract: Proteomic technologies in the modern laboratory practice proved to be very efficient to reveal biochemical changes in meat products, such as changes in heat-resistant and species-specific proteins that have the ability to become the relevant bio-markers. Several tissue-specific proteins were identified in the work under review using proteomic technologies in tested samples of meat and in specially manufactured sausage products that may be used as individual biomarkers to verify conformity of meat products to the alleged composition. Also, individual non-muscle proteins (soya and chicken protein) were determined in test samples of meat products apart from species-specific muscle proteins that may act as functional ingredients used in cooking process. Overall, total of more than 200 protein fractions were identified in the completed studies by the mass spectrometry method which are described in this review in part. The results obtained will be used to draft the procedure for quantitative evaluation of the meat component content in structureless cooked products (cooked sausages) as well as to draw proteomic protein charts of the native meat stock used to manufacture goods as per GOST (State Standard). Studies conducted in the range of this discipline will help to formulate and considerably develop approaches to identify and evaluate protein markers of quality, functionality and safety of meat for processing and processed meat products.

Keywords: proteomics, two-dimensional electrophoresis, bio-markers, mass spectrometry

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INTRODUCTION

All biosignatures and biosignature formation mechanisms are controlled including both the gene activity and proteins, the gene expression products.

Currently, the characteristics and gene functional properties are the area of great challenge for the postgenomic age. Postgenomic tools and technologies apply integral, variable experimental approaches that may be characterized as complex biosystems.

Proteomics is the essential keystone to describe the genom functionality as all other functional genom tools, including transcriptomics and metabolomics. The purpose of proteome study is the genom information conversion to the efficient conception of biological mechanisms for scholars to create and realize hypotheses to find solutions to various problems regarding safe and quality food production [2, 3, 6, 7].

As for agricultural sciences, as well as for all other biosciences, introduction of proteomics and other postgenomic tools is vital to understand processes that occur in the multicomponent matrix named "meat".

It is specified currently that all quality properties of meat are of quite complicated origin. Despite the fact that many properties are being studied intensely, molecular mechanisms to form these properties are still unclear up to date. Consequently, there is a demand in novel approaches to clarify the way the meat quality properties form.

The use of proteomic strategy in studies of molecular mechanisms to form quality properties of the meat stock is the vital stage to produce high-quality animal products and to stabilize the production process more efficiently [8, 9, 11].

Most proteomic works are performed using two-dimensional electrophoresis (2-DE). This is the method to keep significance for proteomic researches. However, the volume of work to be completed requires methods and the equipment with the known high

capacity, information content and sensitivity. Today, most scientists of world-wide reputation involved in proteomics sphere are assured that the combination of high performance liquid chromatography (HPLC) and tandem mass-spectrometry (MS/MS) may result in the quicker breakthrough in proteomics [1].

This work was aimed to create the integrated methodological approach to determine the content of the muscle protein in structureless cooked products by using two-dimensional electrophoresis followed by the time-of-flight mass spectrometry identification of confirmatory marker proteins.

OBJECTS AND METHODS OF STUDY

Experimental studies were conducted in the laboratory of "Scientific and Methodological Works, Biological and Analytical Researches" of "The Gorbatov's All-Russian Meat Research Institute" in collaboration with "Protein Research" Laboratory of Federal Research Center of Biotechnology, Russian Academy of Sciences.

The following methods were used in the work:

– Protein fractionation with 2-DE by O'Farrell using ampholytes with isoelectric points in a pH gradient.

Proper modification of two-dimensional electrophoresis by O'Farrell as the main proteomic technologies which, in particular, used isoelectric focusing of ampholyte pH gradient (IEF-PAGE) and nonequilibrium pH gel electrophoresis (NEPHGE).

– Protein fractionation with two-dimensional electrophoresis by O'Farrell using isoelectric focusing in the immobilized pH gradient (IPG- PAGE)

When using IPG-PAGE of two-dimentional electrophoresis modification, the fractionation in the first direction (isoelectric focusiong) was performed in so-called strips – manufactured strips of polyacrylamide, conjugated with immobilines that ensured pH gradient within the range from 3 to 10 (13 cm Immobiline^{TM} DryStrip pH 3–10, "GE Healthcare") on the Ettan IPGphor 3 [4, 8].

– Mass spectrometric methods to identify proteins

Protein fractions selected for identification were cut from gel plates obtained by the two-dimensional electrophoresis. Gel sections were grinded, the containing protein was hydrolyzed with the trypsin and tryptic peptides were extracted for identification using time-of-flight mass spectroscopy on the matrix (MALDI-TOF).

At MS/MS analysis, mass-spectrum of fragments was recorded using MALDI-TOF mass spectrometer Bruker Ultraflex in the tandem (TOF-TOF) mode to detect positive ions.

Proteins were identified by the Mascot Software, Peptide Fingerprint ("Matrix Science", USA) option, with the weighing accuracy rate MH^+ equal to 0.01% (assuming cysteine modification with acrylamide and methionine acidizing), as well as per database of the National Center of Biotechnological Information of USA (NCBI, address: http://www.ncbi.nlm.nih.gov) [8].

Samples of muscular tissue of pork and beef as well as test batches of cooked sausage goods were used for the analysis manufactured in compliance with the GOST and samples of sausage goods of similar products purchased in the retail network.

RESULTS AND DISCUSSION

It is known that the minimal and sometimes soft changes in the composition or structure of protein components may decisively affect the meat properties. Biological processes that result in delicacy changes that is the most vital property of the pork include proteolytic transformations that occur in the meat protein system during rigidity, aging and further storage as cooled or frozen [10].

The meat quality is closely related to biological characteristics of the animal. Thereat, it is borne in upon that the meat quality characteristics such as delicacy, water-binding capacity, fractional structure, autolytic changes and others are complicated and multi-component systems and thus, they would be characterized in detail based on experimental approaches and technologies aimed to simultaneous study of multiple genes and proteins in parallel [12].

The proteomics aims to identify molecular markers usually named bio-markers that allow earlier and more accurate diagnostics of diseases in medicine, for instance. Currently, bio-marker search and identification is vital since bio-markers may be used to improve the larger range of characteristics, including methods to be used for meat production and processing.

Nevertheless, identification of the muscle protein content in structureless cooked products upon thermal treatment is a great challenge.

General concepts have been formed throughout several decades that proteomic technologies allowing transition to qualitative improvement of results when protein cell extracts and tissue samples are tested. This general conclusion is proved by results of this work.

During this cycle of works, the comparative study was performed to evaluate the efficiency of 2-DE methods where isoelectric focusing in ampholyn (IEF-PAGE) or immobiline (IPG-PAGE) pH 3–10 gradients are used. As an example, the Fig. 1 shows results obtained from fractionation using two 2-DE modifications above of the pig muscle tissue samples.

When comparing Fig. 1a and Fig. 1b, it can benoted that both modifications revealed almost similar fraction arrangement belonging to actins, tropomyosins and myosin light chain At the same time, the qualitative abundance of actin on two-dimensional electropho-regrams obtained by IPG-PAGE, was considerably higher than that in fraction obtained by IEF-PAGE. Apparently, the reason of this difference is the origin aggregation of proteins with the trace seen on the left edge of IEF-PAGE two-dimensional electrophoregram (Fig. 1a).

It is meanwhile seen that in the central and the right sections of 2-DE IEF-PAGE, more fractions are registered than on IPG-PAGE 2-DE. Measurements made specially showed that isoelectric focusing performed in normal conditions on strips with immobiline with pH gradient 3–10 does not result in segregation (and further identification) of proteins with $pI \geq 6.2–6.5$. In this view, most proteomic tests

performed later used the proper 2-DE modification described above that included isoelectric focusing in ampholyn pH gradient.

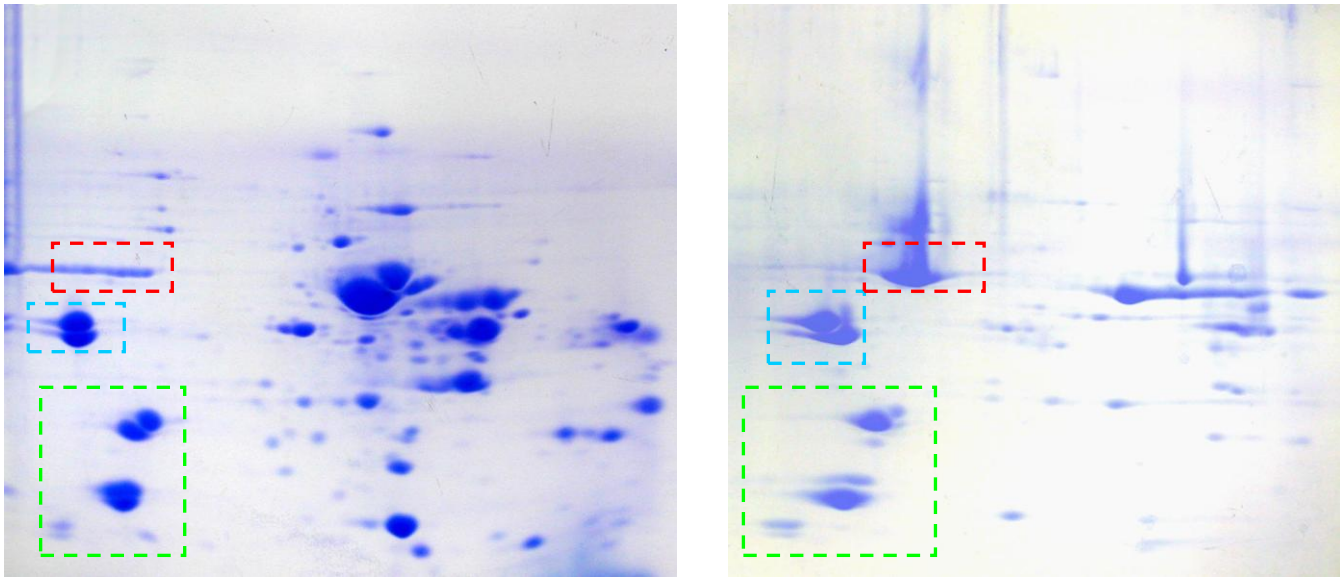
IPG-PAGE modification was use din special analysis to characterize the delicate electrophoretic features of proteins of pI < 6.0. The Fig. 2 below shows the test results of electrophoretic features of tropomyosin and myosin light chains in samples of various pig muscles.

As it is seen in Fig. 2, IPG-PAGE modification visually demonstrates tissue-specific features of this protein groups.

Essential for experimental validation of proteomic technologies is the potential of 2-DE along with mass spectrometry methods to reveal and identify chicken and vegetable protein in meat products (cooked meat products) manufactured as per reference documentation. The relevant results are shown below in Fig. 3 and Fig. 4.

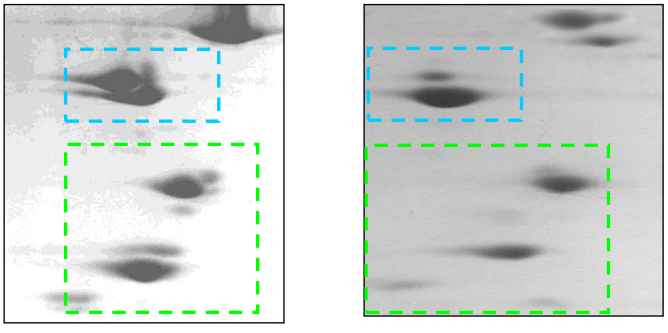
In particular, the Fig. 3a shows the fragment of 2-DE protein extract of "Lyubitelskaya" sausage where the fraction №1 was identified as the chicken pyruvate kinase by MALDI method. The reference fractions no. 2 and no. 3 for this case were earlier identified as pyruvate kinase and the isoform of M creatine kinase *Sus scrofa*. The mass spectrum of tryptic peptides for fraction no. 1 is given in Fig. 3b.

The fraction “Gcn” shown in Fig. 4 was similarly identified as the soya protein glycinin (4249566 Protein NCBI).



(a) (b)

Fig. 1. Two-dimensional electrophoregrams obtained by fractionation of the pig muscle tissue samples by 2-DE modifications with the help of isoelectric focusing in ampholyn (a) and immobiline (b) pH 3–10 gradients. Dashed rectangles show zones of muscle protein anatomic location: actin – red; tropomyosins – blue, myosin light chain – green.



(a) (b)

Fig. 2. Two-dimensional electrophoregram fragments obtained by fractionation of samples of various pig muscles (a – skeletal muscles, b – cardiac muscle) by 2-DE modification using the isoelectric focusing in the immobiline pH 3–10 gradient. Dashed rectangles show arrangement zones; tropomyosins – in blue and myosin light chains – in green.

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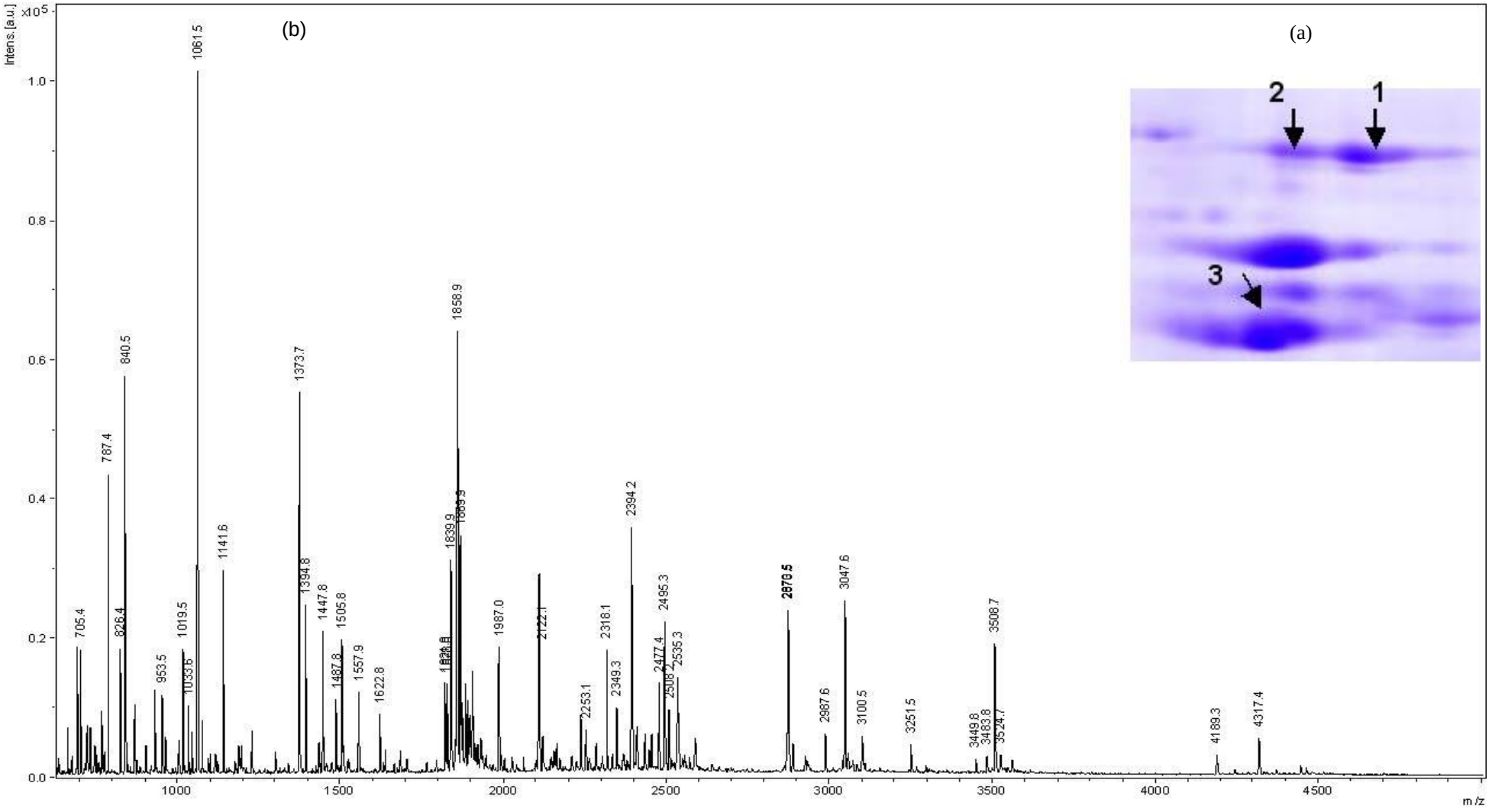

Fig. 3. The results of proteomic identification of chicken pyruvate kinase in the sample of "Lyubitelskaya" sausage: (a) fragment of two-dimensional electrophoregram with the identified fraction №1 and two reference fractions no. 2 (*Sus scrofa* pyruvate kinase) and no. 3 (*Sus scrofa* creatine kinase), (b) Mass spectrum of tryptic peptides obtained at MALDI-TOF MS identification of fraction № 1 as *Gallus gallus pyruvate kinase.*

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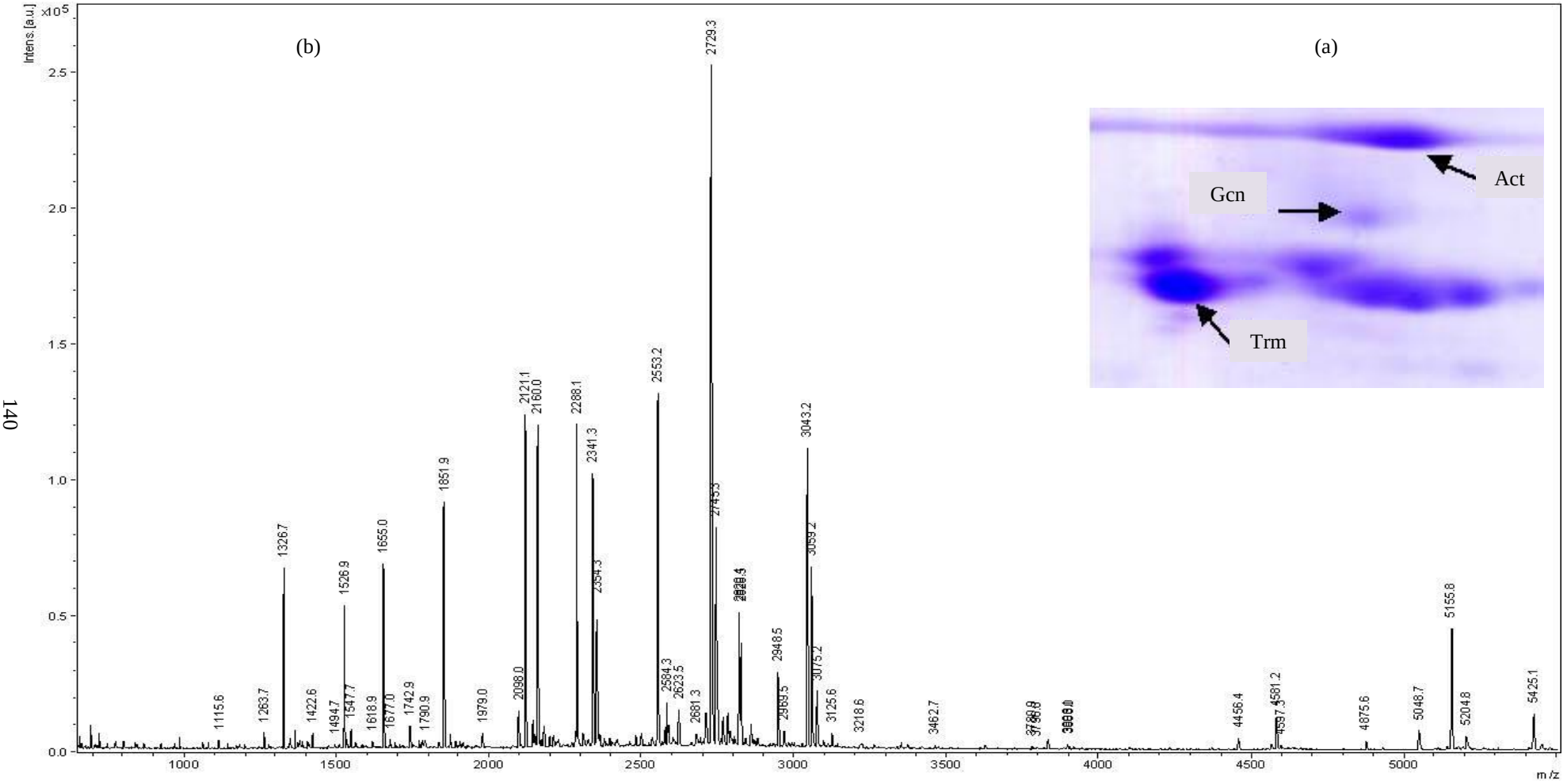

Fig. 4. The results of proteomic identification of soya protein glycinin (4249566 Protein NCBI) in the sample of "Lyubitelskaya" sausage: (a) fragment of two-dimensional electrophoregram with the identified fraction Gcn and two reference fractions Act (*Sus scrofa* actin) and Trm (*Sus scrofa* tropomyosin), (b) Mass spectrum of tryptic peptides obtained at MALDI-TOF MS identification of Gcn fraction as *Glycine max glycinin.*

Sum it up so far, it can be concluded that studies performed resulted in experimental validation of selected control methods for some proteolitic properties in the cooked sausage products using total biochemistry and proteomic techniques.

Example of identification and validation of some selected protein fractions by the time-of-flight mass spectroscopy followed by interpretation based on the protein molecule database (DB) available (Fig. 5).

In total, the results of proteomic analysis of the proteins contained in the muscle tissue of the Sus scrofa pig were used as the basis to form the new information module in the early created database "Proteomics of muscle organs" ("PMO" DB, http://mp.inbi.ras.ru) with the relevant title.

As it is seen from results shown in Table 1, the data on most identified proteins available in public databases are based on the relevant transcripts of analysis material only. Accordingly, the data of proteomic analysis of such proteins (marked with **** in Table 1) is then the vital conclusive proof of their availability obtained on the protein level. At the same time, some results of mass spectrometric identification (marked as ****** in the Table 1) of similar and individual proteins due to the lack of information required supplementary studies.

However, the identification results for a variety of main contractile proteins, such as α-tropomyosin, some myosin light chains, desmin and other happened to be highly valid and quite convincing.

Differences sometimes reported in standard and experimental values of Mm and pI might be associated with the calculation performed on the basis primary protein structure data that were extrapolated from information of the respective transcript. As the consequence, the post-synthetic modifications of the proposed protein were neglected, and in particular, extraction of the signal sequence. In individual cases, when the direct data on the same protein in another mammal species were available in public databases, the appropriate adjustment was possible. So, the data by the transcript (F1SGH5) only were provided for the pig protein "Mitochondrial beta sub-unit E1 of pyruvate dehydrogenase component" (no. 9 in Table 1) in the UniProt Database, while there were direct results of the male elk that evidenced on extraction of first 30 amino-acid residue off the sequence during the protein migration in the mitochondrion (P11966). Determination of standard values of Mm and pl for the fraction no. 9 subject to potential extraction of the same section of amine acide sequence resulted in 35.90 and 5.38 and these values were notably closer to experimental values (33.50/5.50) as compared with values initially measured by the transcript (39.00/6.20). Apparently, the differences in standard and experimental values of Mm sometimes reported on abnormal electrophoretic protein lability at SDS-electrophoresis or might result from "fragmentation" of the full-length protein product predicted by the relevant transcript (for example, for the fraction no. 24).

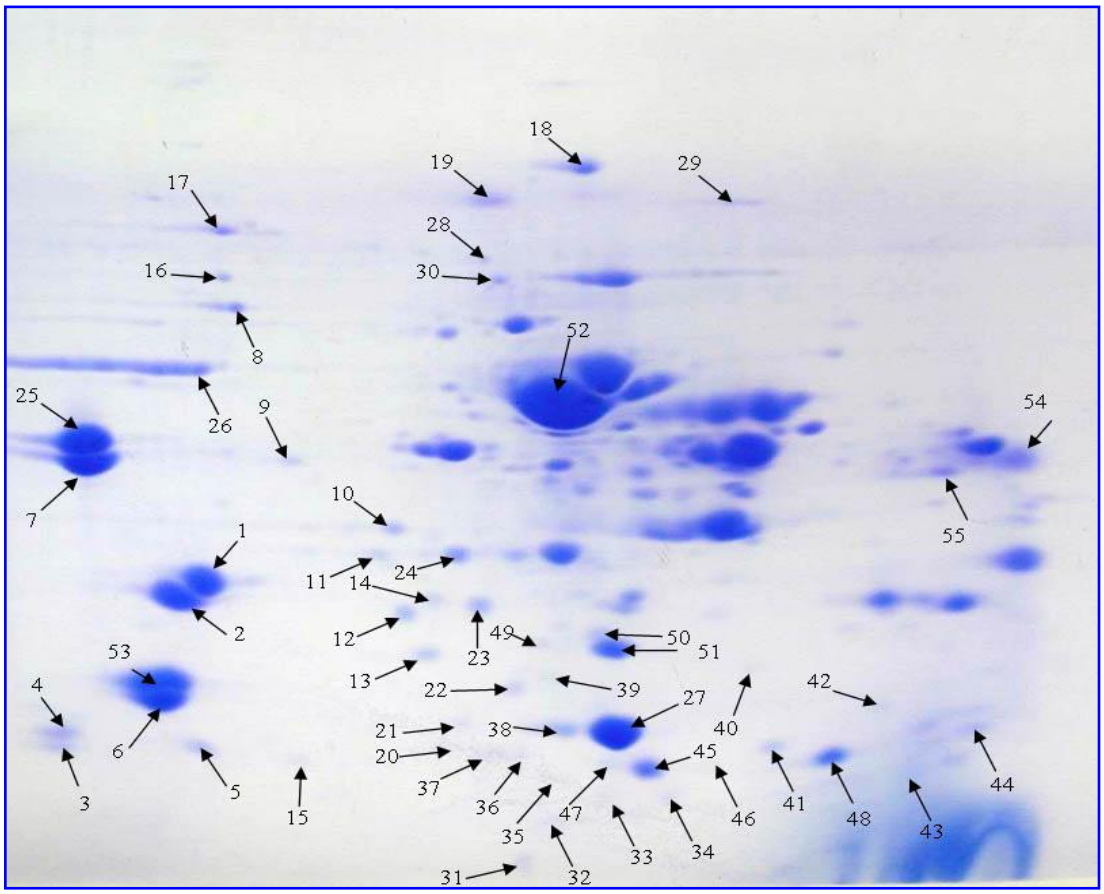

Fig. 5. Two-dimentional electrophoregram of proteins in the pig muscle tissue *Sus scrofa*. Arrows indicate the proteins identified. Identification results are given in the Table 1.

Table 1. *Beginning*. Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

No. in the 2D electroph oregram	Name of protein, some synonyms, including the English name, (*gene symbol*), **description in database "PMO"**	Numbers in Protein NCBI and/or *UniProt*	Score/No. match peptides*	% coincidence**	Mm/pI (exp.)***	Mm/pI (stand.)***
1	2	3	4	5	6	7
1	Myosin light chain 3, Myosin light chain 1, slow-twitch muscle B/ventricular isoform (*MYL3*), **MLC1s/v**	332656187, 311268794****	204/20	81	22.0/5.24	21.8/5.00
2	Myosin light chain, MLC1f, MLC1F/MLC3F (*MYL1*) **MLC1f**	157427687, 117660874****	211(273)/12	93	21.0/4.90	21.0/4.90
3	Light chain of myosin 1/3, sceleto-muscular shortcutelectrophoretic isoform, **MYL1 variant 3** (*MYL1*) **MLC3f-ei**	157427687 / **A1XQT8**	136/18	78	16.5/4.63	16.7/4.63
4	Light chain myosin 1/3, sceleto-muscular shortcut isoform, **MYL1 variant 3** (*MYL1*) **MLC3f**	157427687 / **A1XQT8**	268/32	92	16.8/4.63	16.7/4.63
5	Cytochrome C oxidase Va isoform 1-like, mitochondrial (***COX5A********) **COX5A-l**	350586831****	145/15	62	16.7/5.15	16.7/6.42
6	Myosin regulatory light chain 2, skeletal muscle isoform MLC2B (*MYLPF*) **MLC2**	54607195****	269/50	95	19.0/4.89	19.0/4.89
7	Tropomyosin isoform alpha 1, α-tropomyosin (*TPM1*) **TPM1**	148222268 / **P42639**	155/24	58	33.5/4.71	32.7/4.71
8	Desmin (*DES*) **DES**	2959454 / **P02540**	375/43	90	53.0/5.25	52.6/5.21
9	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (*PDHB*) **PDHB**	346986351 / **F1SGH5******	257/29	60	33.5/5.50	39.2/6.20 (35.9/5.38) ******
10	Light chain myosin 6B, PREDICTED: myosin light chain 6B [Sus scrofa] (MYL6B) MYL6B	194037529****	376/40	98	24.0/5.90	24.0/5.53
11	Heat-shock protein HSP27, beta 1 isoform, (HSP27) HSPB1	55926209 / Q5S1U1	470/20	98	23.0/5.85	23.0/6.23
12	Subunit d of mitochondrial F0 complex of ATP synthase (ATP5H) ATP5H	347658971****	639/35	97	18.5/6.00	18.5/5.99
13	Hypothetic protein containing the barred domain, gene product of locus LOC494560, Hp-cry	311257410****	371/12	77	19.0/6.05	17.4/5.35

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Table 1. *Continued*. Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

1	2	3	4	5	6	7
14	Protein DJ-1 (*PARK7*) **DJ-1**	118403904 / **Q0R678******	286/24	83	20.5/6.15	19.9/6.33
15	Protein binding fatty acids 3, cardiac/muscle isoform (***FABP3***) **FABP3**	374637318 / **O02772**	145/12	59	15.0/5.50	14.7/6.11
16	Chaperonin, heat-shock protein 60, HSP 60 mitochondrial (*HSPD1*) **HSPD1**	359811347 / **F1SMZ7******	339/47	69	61.0/5.30	61.0/5.70
17	Heat-shock protein 70 isoform 8, HSP70 (*HSPA8*) **HSPA8**	345441750 /	540/38	54	66.0/5.27	71.0/5.37
18	Aconitase mitochondrial (***ACO2***) **ACO2**	47522738 / **P16276**	268/52	61	86.0/6.80	85.7/8.24 ******
19	Serum transferrin, PREDICTED: serotransferrin isoform 1 (*TF*) **TF**	350591529****/ **P09571**	329/44	59	79.0/6.70 ******	78.8/7.57
20	Fatty acid-binding protein, epidermal (*FABP5*) **FABP5**	89886167 / **Q2EN74******	122/12	48	16.5/6.45	15.2/6.60
21	Superoxide dismutase 1 (SOD1) **SOD1**	15082144**** / **P04178**	86/25	72	17.0/6.40	15.8/6.04
22	Ubiquitin-conjugating enzyme E2 variant 2 [Sus scrofa] (*UBE2V2*)****** **UBE2V2**	343432604 / **I3L6T2******	63/8	40	19.5/6.75	16.4/7.79
23	PREDICTED: adenylate kinase isoenzyme 1 [Sus scrofa] (***AK1***) **AK1**	350579688**** / **P00571**	516/54	87	21.0/6.50	21.6/8.38
24	PREDICTED: heat shock protein beta-1-like isoform 1 [Sus scrofa] ******	335284210****	213/19	83	23.0/ 6.40	29.7/9.51 ******
25	Tropomyosin beta chain (*TPM2*) **TPM2**	194018702 / **A1X899******	105/14	29	34.0/4.80	33.4/4.62
26	Actin alfa skeletomuscular (***ACTA1***) **ACTA1**	**/ P68137**	209/22	49	43.0/5.23	42.5/5.23
27	Myoglobine (MB) **MB**	**47523546 / P02189**	157/13	77	17.0/7.00	17.1/6.76
28	PREDICTED: stress-induced-phosphoprotein 1-like [Sus scrofa] (STIP1), **STIP1-pig********	**335281609******	191/35	56	63.0/6.50	62.4/6.36
29	Alfa subunit of mitochondrial trifunctional enzyme (HADHA) **HADHA**	**47522754 / Q29554**	175/14	21	80.0/8.70	79.7/9.17

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Table 1. *Continued.* Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

1	2	3	4	5	6	7
30	Dihydrolipoyldehydrogenase plastosome (***DLD***), **DLD**	47522940 / **P09623**	235/30	50	60.0/6.60	50.2/6.31
31	Ubiquitin ****** **UBIQ**	229532******	339/24	97	8.5/6.65	8.4/6.56
32	Acyl-CoA-binding protein, Diazepam-binding inhibitor (***DBI***) **DBI**	47523046 / **P12026**	165/6	52	10.0/6.75	9.9/7.88
33	ATPase inhibitor, electrophoretic isoform (*ATPIF1*) **ATPIF1-ei**	148222591	330/27	62	12.0/7.00	12.1/9.34
34	ATPase inhibitor, plastosome. (*ATPIF1*) **ATPIF1**	148222591	313/26	62	12.0/7.40	12.1/9.34
35	PREDICTED: histidine triad nucleotide-binding protein 1-like [Sus scrofa] (*HINT1*) **HINT1-pig**	311250094****	172/17	94	13.7/6.90	13.7/6.36
36	Histidine triad nucleotide-binding protein 1-like (*HINT1*) **HINT1-ei**	311250094****	172/17	94	14.0/6.60	13.7/6.36
37	Cytochrome C oxidase subunit 5B mitochondrial precursor (*COX5B*) **COX5B**	55926217 / **Q5S3G4**	185/18	75	11.5/6.65	10.6/6.07
38	Myoglobine, electrophoretic isoform (*MB*) **MB-ei**	47523546 / **P02189**	357/29	99	17.0/6.75	17.1/6.75
39	**PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 7-like [Sus scrofa]** ?******	350580438**** /	122/12	43	20.0/6.80	12.0/7.71
40	NADH dehydrogenase 1 α-subcomplex subunit 8 (*NDUFA8*) **NDUFA8**	298104126 / **F1SLR1****	279/10	40	20.0/8.20	20.0/8.09
41	Ubiquinol cytochrome c reductase binding protein (***UQCRB***)	297747368 / **H0VKS2****	375/33	89	16.0/8.70	13.6/9.07
42	PREDICTED:Subunit 4-like beta subcomplex NADH dehydrogenase (ubiquinone) (*NDUFB4*) **NDUFB4-pig**	335310208**** / **I3LPW0****	227/15	69	20.4/9.00	15.1/9.66
43	ATP synthase subunit e, mitochondrial (*ATP5I*) **ATP5I**	148887343**** / **Q9MYT8.4**	425/17	98	8.8/9.10	8.2/9.30
44	PREDICTED: cytochrome c oxidase subunit 6A2, mitochondrial-like isoform 2 (***COX6A2***) **COX6A2**	311251232**** /	498/5	31	11.0/10.0	10.8/10.58
45	Hemoglobin β-chain (***HBB***) **HBB**	261245058 / **P02067**	334/25	89	16.2/7.10	16.2/7.10
46	**FKBPIA-like, FK506-binding protein (***FKBP1A***)** FKBP1A-pig	83921635****	170/8 + 1 msms	89	14.5/7.90	11.9/7.88

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Table 1. *Ending.* Proteins identified by mass spectrometric methods (MALDI-TOF MS and MS/MS) on two-dimensional electrophoregram of protein extracts from the pig muscle tissue (*Sus scrofa*)

1	2	3	4	5	6	7
47	Hemoglobin β-chain electrophoretic isoform (***HBB***) **HBB-ei**	**/по P02067**	157/18	87	16.5/6.95	16.2/7.10
48	Hemoglobin α-chain (*HBA*) **HBA**	/by **P01965**	324/27	80	15.5/8.76	15.0/8.76
49	Crystallin αB, electrophoretic isoform (*CRYAB*) **CRYAB-ei**	335294877	159/5	14	21.0/6.70	21.1/6.76
50	PREDICTED: phosphatidylethanolamine-binding protein 1-like [Sus scrofa] (***PEBP1***)****** **PEBP1-pig**	311270662**** /	553/31	99	20,8/6,90	21.0/6.96
51	αB-crystallin (*CRYAB*) **CRYAB**	335294877**** / **Q7M2W6**	260/19	86	20.6/6.95	20.1/6.76
52	Creatine kinase, subunit M (***CKM***) **CKM**	194018722 / **Q5XLD3**	356/40	80	43.0/6.60	43.0/6.61
53	Myosin, light chain myosin isoform 2V (*MLC2V*) **MLC2V**	47523262 / **Q8MHY0**	270/27	89	19.9/4.85	18.9/4.86
54	Four and a half LIM domains 1 protein, isoform C (*FHL1C*), **FHL1C**	47523806**** /******	175/22	57	34.0/10.50 ******	33.6/8.79
55	Four and a half LIM domains 1 protein, isoform C (*FHL1C*), **FHL1C-ei**	47523806**** /******	128/19	51	33.4/9.60 ******	33.6/8.79

Notes. * – Score / No. match peptides – characteristics widely used in the English publication for the mass spectrometry results (Score – suitability mark or "score record", No. match peptides - number of coincided peptides) Scores are given in view of MS/MS; ** – % coincidence of revealed mass tryptic peptides with the protein sequence; *** – Mm/pI – values of molecular masses (Mm) in kilodaltons and isoelectric points (pI); **** – Predicted by transcript; ***** – Defined by analogue with other relative genes of other mammals (human and/or bovine animals); ****** – Explanations are in the text above.

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Post-synthetic modifications might influence the results of *pl* determination. Thus, it was revealed in the work that the fraction no. 11 identified as "Heat-shock protein HSP27" is phosphorylated. This modification obviously produced the decreased pl value (5.83) obtained in the experiment as compared with the standard one (6.23). Still, it should be noted that in some other cases (for example, no. 18, no. 19), the decreased experimental pl values were apparently due to the fact that individual large proteins do not reach positions relevant to their real pl values when the method of isoelectric focusing is used.

Finally, it should be emphasized that, as said above, the results of mass spectrometric identification obtained for some fractions in two-dimensional electrophoregrams of the pig muscle proteins, were only based on information on nucleotide sequences of relevant transcripts (marked **** in the Table 1). In this case, electrophoretic characteristics measured in the experiment and theoretically calculated sometimes notably differentiated.

Hence, the performed proteomic analysis shown in this article of the pig muscle tissues resulted in identification of 55 muscle proteins, that included, among others, main muscle contraction members (myosins, actin, tropomyosins), enzymes of glycolysis and other metabolic processes (aldolase, dihydrolipoyldehydrogenase, NADH-dehydrogenase and other mitochondria enzymes), heat-shock proteins as well as the new protein (putative protein containing the barred domain, gene product from locus LOC494560) and several tissue-specific proteins - potential bio-markers (desmine, creatine kinase, myoglobine).

Thereat, the literary material and proprietary experimental data gave evidence that some of identified proteins are tissue-specific and may be considered as potential bio-markers. In particular, myosin light chains, desmine, creatine kinase, myoglobine and others may be classified as such proteins.

Nevertheless, in some cases considerable difficulties were faced to collect publications on the identified protein or the relevant transcript for the Sus scrofa species. For example, experimental data obtained for the fraction no. 31 had such characteristics.

Thus, the results shown in this work indicate that the compiled experimental and literature materials were useful to form the basis to develop the assay content test method for meat proteins in structureless meat products and, in particular, in cooked sausage products.

CONCLUSION

Application of proteomic strategy in the study of molecular mechanisms to create quality parameters of the meat raw stock is essential to produce high-quality animal products and to stabilize the production process more efficiently.

The works performed resulted in formation of the consolidated methodological approach to determine the content of the muscle protein in structureless cooked products by two-dimensional electrophoresis followed by the time-of-flight mass spectrometry identification of convincing protein markers.

It can be concluded from studies performed that the use of proteomic technologies along with new data acquisition on muscle proteins allows creating new effective control methods of meat products including, in particular, determination of the content of muscle proteins and their specificity in end products.

The experimental data obtained will be also used to generate proteomic maps of proteins in native meat stock.

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ECONOMICS

SOME TRENDS IN RUSSIAN FOOD PRODUCT EXPORT IN THE MEANING OF THE INTERNATIONAL TRADE DEVELOPMENT

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Abstract: Against the background of world economy globalization, the international trade, being the oldest form of international economic relations (IER), is the major decisive factor for the development and market environment of food and agricultural commodity. The hypothesis is suggested and proved in this study that the international trade may and should be considered not only as the driver for global and national economy, but also as the new effective tool to address numerous global problems as follow: sustainable development, environmental and comestibles problems. The results of the study to evaluate the impact of the current international trade on the growth of food product market of global and national level proved the crucial function of the international trade and its two-fold character to mitigate challenges of today as above. On the one hand, it was proved in terms of food and agricultural product market that the international trade is the reliable factor of global resource enhancement with regard to minimizing carbon dioxide emissions and cost-effective use of scarce water resources available. On the other hand, the negative impact of international trade results in the export growth that contributes to national resource depletion, flow of pollution-related industries to countries with milder environmental control and prevention to address environmental issues. When considered in view of national economy development and operation of national commodity markets, current trends in the movement of the Russian export of food products in the post-crisis period, established its role to support the import substitution policy and commodity self-sustainment in the Russian Federation have been highlighted. The practical value of identified consequences of the current international trade is the opportunity to minimize the negative global challenge consequences and to improve the strength of the import substitution policy in Russia.

Keywords: Export, international trade, commodity market, global economy globalization

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INTRODUCTION

The global economics of today faces obvious global challenges and problems to surge up such as the threat of biological, chemical and nuclear war, expansion of armaments and a third world war, exhaustion of natural resources, epidemic diseases, hunger, poverty, etc.

Global issues are conventionally classified for three groups:

(1) of natural climatic origin (ecological problem, global warming due to increasing ozone holes and the "greenhouse effect", space exploration and the World ocean learning, energy and raw material related problem);

(2) of socio-economic origin (global food scarcity or the food problem today (more than 2 million people are starving in the world), problem of uneven economic development and expanding the difference between the rich and poor in the world or the issue of poverty and underdevelopment elimination, demographic problem, the problem of sustainable development.

(3) of political origin (wars, revolutions and unstable global political situation leading to the slowdown in global economic growth; peace and demilitarization; international terrorism).

Global humanity challenges are impossible to address solely by a single country only: joint provisions on environmental protection, approved economic policy, assistance to underdeveloped countries, etc.

The international trade, among the effective tools to address these issues, is of high significance and role, in our opinion, that is one of the oldest forms of IER.

However, the concept of international commodity exchange is substantially transformed against the background of the increasing globalization of the world

economics that predetermines the change of the international trade significance on different stages of the global economic system evolution. Currently, numerous experts note that this role is depreciated in contrast with the dominant influence of the international capital flows against the increasing presence of the financial sector of the world economy. Nevertheless, acknowledgment of this factor does not diminish the share of international trade as the effective vehicle of modern international economic relations. Reserving major powers to optimize the use of production factors at the national level and in terms of the world economy, the international trade of the XXI century considerable changes in its structure and tools more serving to ensure the global value chains and strongly focusing on the sale of semi-finished products, substances, half-finished materials. Recently, as per certain data, the trade in semi-finished products on the stock as global value chains is more than 60% of all world trade. However, this is to prove that the international exchange of end products is still the important zone of this conventional IER system and, respectively, it is too early to be disregarded, although it requires permanent monitoring of the international trade development trends in this field.

In context of "new normal" reform in the world trade, new tools and mechanisms will inevitably emerge to introduce its traditional strengths and capacities [1].

This pre-determined the relevance of the Russian export evaluation in terms of national economy development by changing a range of features designed to mitigate foreign problems (sanctions) and existing rules of international regulation of the food and agricultural product market in conditions of the world economy glocalization.

The work is aimed to study the impact of the current international trade on the development of food market in the context of addressing a number of issues related to the Russian export movement.

OBJECTS AND METHODS OF STUDY

The study aims to review current trends in modern international trade and the Russian exports of food products when it comes to mitigation of global issues and the existing rules of international regulation on the food and agricultural market.

Dialectical, systemic, logical, historical methods and comparative and statistical analyses, holistic approaches and expertise were applied to achieve the goals.

RESULTS AND DISCUSSION

Today, the key issue of global significance greatly associated with the international trade development is the issue of sustainable development, environmental and food problems.

The works resulted in the impact evaluation of the current international trade on the food market development on the global and national scale to be able to claim on its important role to mitigate global challenges and to make fundamental conclusions.

International trade in food is the effective tool in addressing global challenges

Recently most scholars and politicians from different countries recognize the need in elaboration and integration of the new economic world model with the green economy as the base.

This term is the follow-up of the long chain of "new economy" definitions: "post-industrial economy", "information economy", "knowledge-based economy", "networked economy", "digital economy", "intellectual economy", "creative economy", etc. All these definitions arose to respond to quality changes in developed economies in the second half of XX – early XXI centuries.

The scope of "green" economy is focused on the "stable" economy with all its aspects in unity: economic, social and environmental. It was acknowledged following environmental constraints increased that the world needs a new model of economic development based on "green" principles.

Principles of "green economy" are shown in a number of policy documents as accepted by the global community. So, in 2007, the UN Climate Neutral Strategy was developed at an UN level as the aspect of the unified approach to manage environmental impacts by elaboration of general tools and more grounded decisions of the efficient energy and resource consumption. Among the recent achievements of international settlements in this sphere, the Paris Climate Agreement signed within the United Nations Framework Convention on Climate Change on April 22, 2016 should be noted that is of the huge significance to achieve sustainable development goals. The Agreement is the "roadmap" of measures to reduce wastes and consolidate the Mother Earth's tolerance to climate changes.

In addition to fundamental tools of interstate address of ecological problems, it requires the wide use and engagement of a variety of means not directly associated with this issue. The international trade in food may be considered as the tool that has an indirect impact on the efficiency of "green" economy mechanisms, in our opinion, as the effective wedge to reduce environmental risks and the ecology degradation.

As part of neo-classical economic paradigm, the international trade is recognized as the tool to optimize the use of global resources world, with water and air as the most essential. The water resource sufficiency influences the agriculture sector development in different countries, where the integrity of air balance and carbon dioxide emissions affect the eco-balance on the global scale.

Production costs of water-intensive products (and, as a consequence, and their relative price) in water-scarce areas are higher than in the water-riched zones. Food production is less expensive in countries with sufficient arable lands and labor force than in countries experiencing a shortage of them. When specifying a single price for greenhouse gas emissions, the carbon-efficient products should be produced in places where most clean technologies are used that ensure the minimum volume of emissions. Thus, the countries

where production are the least abnormal in terms of resource consumption and environmental damage, have a competitive advantage, and the international trade allows to take advantage of them. This, in turns, contributes to the well-being of all transaction parties.

The international trade in food products is the new effective tool to address environmental and sustainability issues that impacts not only the consequences of the human-based environmental impact due to industrial activity, but also solutions to environmental issues on the supranational level.

It is reasonable to consider the international trade as a tool to ensure access to scarce resources in terms of the so-called "virtual" water trade, that is the water as the ingredient of the finished product or that is required for its production. Water-scarce countries may save own water resources, provided that the import volume of water-intensive products is increased. Through the sale of water-efficient commodity, the global fresh water saving only in the agricultural sphere will make 5%. However, such saving is not systemic: only few states deliberately apply the strategy of virtual water importing and, basically, it is the countries of North Africa and the Middle East. Nevertheless, the figure is growing. The international trade in virtual water can mitigate the water shortage implicitly with no ensuring its saving to the absolute value. For example, almost 3 times more water is consumed to crop a ton of wheat in Russia than in China - 2300 and 820 cubic meters, respectively [2]. This means that the wheat of China manufacture substituted by the Russian import could result in an increase in global water consumption. However, this commodity flow would commit to manage the water deficit in the region in view that China is the water-stressed region, on whole, and Russia is the water-riched country.

In certain cases, however, the international trade may negatively impact the sustainable development of the Mother Earth, climatic condition, water and food reserves.

In an attempt to provide privileges to national manufactures to take advantage to foreign competitors, some countries often turn a blind eye on the environmental management systems applied by companies. The export promotion is one of race to the bottom results that commits to the depletion of resources within the country. So, in Uzbekistan, the government subsidizes water to cotton producers thus, exacerbating the challenging situation with water resources enough as it is. The similar situation is reported in terms of land acquisition by rich investors in poor countries with unstable environmental legislation to produce food commodities that On the one hand, engagement of idle or inefficiently used land plots for agricultural purposes is something to encourage. On the other hand, there is a risk to ensure poor monitoring of such lands and lack of incentives for investors faced with high political risks to guarantee the sustainable land use. Such land acquisition may result in the soil degradation and the whole range of related environmental and social problems may arise. The key factor to define the exposure of the trade on resources and environmental challenges in developing countries is the typical system of institutions. In countries where institutions normally run and property rights are protected, the export results in the well-being improvement (due to export earnings, job creation, etc.). In countries with low-level institutions, these positive effects may be completely offset by the natural capital degradation. The international trade may trigger the aggravation of these issues in unfavorable institutional environment that is still available in most developing countries. Therewith, its strength is quite considerable to become an important tool to address such issues. Though, this strength is so far not applied.

In some particular cases this results in the "over-inflow of pollution industries to countries with moderate environmental policy. For example, the enactment of the Kyoto Protocol binding developed countries and countries with transition economies to limit greenhouse gas emissions caused an increase in imported carbon-intensive products from developing countries not restricted yet. The Kyoto Protocol procedure on prevention of global climatic changes is a significant precedent to adequately assess the value of natural wealth for both economic theory and practical measures. Upon agreement to create a new global market for greenhouse emissions, the countries agreed, in fact, to trade in fresh air. Each ton of greenhouse emissions carries a value. It is critical that the mechanism evaluates economic benefits for the global community referenced to greenhouse gas emissions.

A number of countries implicitly use the international trade to latently obtain additional preferences under the Kyoto Protocol at the expense of efforts taken by other countries without reducing their emission reduction commitments. The growth of"virtual carbon" export caused by the Kyoto Protocol (i.e., emissions discharged to produce exported goods) from countries with transition economy amounted to about 8% that exceeds the value of their emission reduction commitments, additionally charging their own commitment to minimize emissions.

Thus, the negative impact of the international exchange on global issues of natural and climatic origin is seen and strengthened towards countries mainly with the mono-commodity economy that refer to the group of developing countries with no effective development institutions. The agrarian and raw material sector in these countries often play a key role to maintain the long-term economic advancement that pre-determines the need in more effective use of limited resources for industrialization and economic growth in developing countries [3].

In this view, the special relevance of this issue is currently related to conditions of post-crisis development of global and national economy of the Russian Federation.

Impact of the international trade on the food problem

The influence of the international trade on global problems of today largely results not only in consequences of the human-induced environmental

impact but also at the interstate institutional level when settling commercial disputes and disagreements.

Herewith, the international trade acts as the two-valued tool for export competition policy at the the food market, playing a role of the commercial and ecological expansion factor by the side of countries with developed industries using specific forms of "neoprotection", on the one hand, and, on the other hand, it is considered as the vehicle for the food problem constant settlement as part of the World Trade Organization and creating the "special protection mechanism" for the least developed countries. When functioning, this mechanism closely relates to food assistance programs at the international level by creation of food reserves and restriction of the state regulatory functions and reducing the agricultural export competition.

When considering the impact of the international trade on the food problem of the global size, it should be noted that liberalization of the global food market to be discussed at the WTO level depends on objectives of global food security.

In this day and age, the role and significance of the international trade as the factor that impacts the food market development considerably change. This is largely explained by the policy of "neoprotection", integration and regionalization processes and emergence of global supply chains of goods.

In our opinion, the industrialized countries apply a kind of commercial and ecological expansion or the latent "neo-protectional" policy to protect its national manufacturers and commit to promote exporters, often in an aggressive form, to foreign markets. Herewith, the international trade structure is optimized for their own benefits by applying the foreign economic policy to bargaining national benefits, and by minimizing certain country commitments when allocating quotas for emissions under the Kyoto Protocol. Thus, the new protectionism of global forefront countries is expressed in the parallel application of commercial and ecological expansion along with other discriminatory methods towards to the least developed countries.

Global companies that operate in food markets own specific influence characteristics and mechanisms, that require special consideration when elaborating control measures to prevent their negative impact on the population supply with in such a vital resource as the food.

A policy of "export competition" in agriculture vigorously discussed at the WTO level may be classified as the current method favorably used by leading countries to their own advantage. The core of the proposed policy is the withdrawal of previously widely used agricultural export subsidies in US and EC countries, limitation of regulatory function in respect of activities held by export-oriented trading enterprises in Australia and New Zealand, but the permit to use such measures for developing countries during emergencies.

WTO negotiations on food assistance matters were aimed at provision of opportunities to countries to optimize the emergency response measures, while ensuring that such assistance in no emergency circumstances will not actually be considered as the hidden export subsidies. This scheme is primarily expected to attract donor funds for the certain part of food assisstance in emergency circumstances.

China, India, Indonesia and some other countries as part of WTO insist on approval of the draft on the "special protection mechanism" that would allow raising rates for short-term period in the event of import surge or price fall. The followers of this approach have long argued that most developing countries cannot benefit from this scheme included in the end of the Uruguay Round for countries that had modified, by that time, the other types of the rate margin monitoring. However, many agricultural exporters say, that the new protection mechanism should become the part of the more comprehensive agreement to reduce rates and other barriers to the market access.

Such policy directly impacts the level of international trade growth causing discouraging and restricting effect on its dynamics and volume. The dominant impact is seen in food reserves creation as the "constant solution" to some problems related to the creation of food reserve programs in compliance with WTO regulations for agricultural subsidies. Today, if the developing countries purchase foodstuffs at prices set by the government as per these schemes, they should include such transactions in their total trade-distorting assistance. The new proposal by members to the WTO negotiation of 2015–2016 includes annulment of transactions included in estimations of the maximum trade-distorting assistance volume as part of such programs. Least developed countries propose excepting their own food and agricultural product purchases at rated prices within the framework of relevant programs based on the maximum volume of distortive state support [4]. Such a mechanism of institutional regulation of the international trade contributes to adequate consideration of its results at national and international levels and is aimed on the positive solution to the food problem on a global scale.

This impact is especially significant in the order of developing countries where the long-term economic progress is not sustained without a powerful agricultural sector, and a variety of structural, institutional and market reforms are followed by infrastructural transformations to stimulate the entrepreneurship, innovation and investments as well as for better and more efficient management in the food sector. In these countries, the use and further expansion of these programs for agribusiness support will facilitate more efficient use of limited resources to build thee strong, dynamic and sustainable agricultural sector for industrialization and economic development of peripheral countries of the world. The WTO platform can and should be used to elaborate a "special protection mechanism" for the least developed countries to slowdown the export competition in agriculture.

Thus, the role and importance of the international trade in recent days significantly vary as a factor affecting the development of the food commodity market. In our view, current approaches to settle trade disputes in the international food market more

effectively influence the growth rate and volume of the international trade volumes and commit to solve the food problem by creating favorable conditions for agricultural business and slowing down the competition between exporters.

Russian export trends against global market trends

The international trade has traditionally been the key factor for the national economy growth and performance of national commodity markets. However, against the post-crisis period, this factor has a growing impact on the higher pricing dependency in the Russian food market on the world market, and it facilitates aggressive import substitution, the most important trend of food self-sustainability in the Russian Federation.

Since the mid-2000s of XXI century a steady decline in the growth rate of international trade is reported. Since 2007 to 2015, its growth rate declined twice as much with the growth expectations to be quite pessimistic for the coming years.

According to the World Trade Organization, the global volume of merchandising increased by 2.8% in 2015, however, in dollar terms, the export figures decreased by 13.5% [5].

In relative terms, the world trade growth rates decreased up to 1.7% in 2015 as compared to 3% in 2014 [6]. As predicted by leading international organizations as the World Bank, IMF, OECD, the world trade growth is expected to decline again in 2016 to an average of 1.5%. Two factors refer to major causes of such decline: decline in world commodity prices and deceleration in the Chinese economy growth that transforms its economic model. The period of globalization known for growing supply of components and raw materials has already completed or is about to end, and the global growth rate is less dependent on the trade in these commodity.

In practice, it is most clearly seen in the context of solving the trade in food. Beside almost double decline in the global trade growth rate in 2015 as compared with that in the previous year, the overall world trade structure is noted with an increase in the share of food import, say, grains by 130%, and oilseeds – by 33% [7].

Most foreign countries are reported to experience a decline in the consumer demand against the economic slowdown as evidenced by the declining dynamics of purchases, especially of luxury goods. In terms of food staff, the experts often agree that people eat the same as before since the income impacts the consumption method not the volume. Figures contradict, though. The fewer obstacles are there to import food products competing with those by national manufacturers and suppliers, the higher is food accessibility at affordable prices all else being equal. For example, the Russian embargo of 2014 on the food import from the West became one of major reasons that resulted in the food price growth in Russia exceeding 20% per annum.

As compared with the global growth rate for the world economy that proportionally affect this global market segment, the Russian food market is diametrically differs from the global one. At the time when the RF domestic market prices jumped by more than 20%, cost parameters on the global food market changed to the same extent, but to the opposite: the aggregate value of the global import of food, as estimated by the Food and Agriculture Organization of the United Nations (FAO) in 2014, decreased from 1.35 trillion USD to 1.09 trillion USD in 2015, that is, the reduction almost makes 19% [8]. The FAO data states that the major factors to stimulate the decline were the trade in cereals, dairy products, meat and sugar, strongly influenced by the decline in freight rates.

The agricultural sector in most countries undergo significant deformations at the national level with the slowdown of its growth since such a fall in prices results in the reduction of farmers' income, which, in turn, results in the reduction of their investment volume in farming. This will require greater incentives to increase investments in agriculture and agricultural services, including loans, roads and warehouses.

By emphasizing the decline in sales and price volatility for major agricultural commodities reported recently, FAO identifies main reasons to this situation as follow: high level of food stocks, abrupt decline in oil prices and US dollar strengthening. With regard to exposure of these reasons, further decline in prices is predicted in the nearest future allowing for the global good crops over the past few years, as well as the breakthrough replenishment of food stocks (by the FAO forecast, the world grain reserves will amount to 638 million tons by the end of 2016, which is 4 million tons more than the volume as of the season beginning) [8].

Considering the trends of the Russian export in the context of global market trends, we note that recently the Russian foreign trade has demonstrated the remarkable decline rate. The overall Russian export in 2015 reduced by 31%, as compared with that of 2014, to 343.4 bln USD and the import reduced by 16% to 194.0 bln USD. [9].

It is due to two reasons, at least, as follow: one of them is the oil price decline as one of the major export commodity at the national level; the oil supply reduced by 40.1% to 174.3 bln USD and the other reason is the mutual economic sanctions and food import embargo response imposed by Russia in August 2014.

In the face of decline in turnover and volatility of world prices for major agricultural products in 2015 as compared to 2014, both domestic market prices and export of certain items of the Russian exports showed a rapid increase (vegetables export grew by 3418%, the export of cucumbers and cornichon increased by 2302.6%, and etc.) [9].

During that time period, legumes became one of the most popular Russian export crops. Due to the ruble devaluation, the crop export appeared even more profitable, and the legumes manufacturers have taken an advantage. Main export leguminous crops include peas, chickpeas, lentils, where the 70% of legumes export is covered by the pea. Major countries that import legumes from Russia include Turkey, India and Pakistan. In the group of oil and cereal crops, soy beans showed the highest growth – its export increased

by 401% up to 119.1 mln USD. The export growth was driven by considerable investments of Russian farmers in this crop production. Soybean has become a popular form of investment for Russian agricultural holdings and its production from 2010 to 2014 increased by 6 times [9].

So, in the late 2014, Rusagro, a leading Russian company in agribusiness, purchased 26.5 thousand hectares of the land plot in Primorye to crop soybean and maize along with 13.75% fat and oil plant "Primorskaya soya" in Ussuriysk. As a result, Rusagro planted more soybean than sugar-beet. Using perfect opportunities for agricultural activity in Primorye, one of the leading Russian food industry companies, Rusagro, intends to invest 25 billion rubles to the region and start-up a cycle of agricultural production, powerful and effective for Russia and the neighboring countries, as the cluster of crop production, pig breeding and soybean processing. The company is engaged in four profile areas: meat production and processing, crop production, sugar and vegetable oil production. As of June 2015, Rusagro managed 495 000 hectares of land, sugar production (716 600 t), pig breeding (186 800 t), fat and oil business (188 000 tons of vegetable oil, 47 100 tons of margarine and 57 700 tons of mayonnaise) [10].

When export positions of Russian commodity producers expand, new types of business management, including strategic alliances, are of great significance. So, by involving selected Japanese, Chinese and other Asian investors, Rusagro considers engagement of COFCO, the largest food holding company in China, in the pork project. China is concerned in such collaboration due to vast environmental problems in this country, increasing consumption of pork, and furthermore, and some time ago China counted on its own meat production. As a consequence, 50–70 mln tons of soy is purchased annually. It is stated that it would be far more effective for China to import the pork. The project in Primorye announced by Rusagro goes well with the willing by the Chinese leadership to start importing high-quality, eco-friendly pork meat from Russia at a competitive price. Moreover, the Russian pork meat is quite competitive in the Asian market and costs 20–30% lower than that in China.

In September 2015, Yug Rusi announced on the investment of 12 billion rubles in construction of the plant in the Far East to process soy and oilseeds.

Such focus on soy and soy food production in the Far East is not sporadic. Soy-eating countries like Japan, Korea and, especially, China neighbour this region. Production of soybean and soy-processed food (eg. soybean meal) may become one of trends to trigger trade relations in the field of food products. China restructuring its foreign trade, including its structure, pays more attention to food importing and soybean may be the significant resource for Russian producers.

Apart from legumes, the export of Russian potato rapidly increased in 2015 and foreign potato trade drastically increased by 124.6%, up to 17.3 mln USD. This is the considerable breakthrough as compared with the recent period when Russia used to import the great volume of potatoe. The main reason for export expansion was the record-breaking potato crop in 2015 amounted to 33.6 million tons that is 15.9% higher than the average crop over the past 5 years. As a result, the average retail domestic price for potato in 2015 fell by 25.3% up to 19.91 rub per kg [11]. In addition, the ruble devaluation made the price for the Russian potato very attractive for foreign purchasers. In 2015, the Ukraine appeared the most popular export destination for the Russian potato. However, export line, similar to legumes export line for Turkey in 2016, is unlikely to take a significant stand. However, Azerbaijan, Egypt and Central Asian countries concern in the Russian potato.

The vegetable export is noted among the list of distinct export items. The phenomenal growth of vegetable exports refers to carrots, turnips and beets (by 3418%) to 2.6 mln USD, export of cucumbers and gherkins to 2.4 mln USD (by 2302.6%. The Russian production of cucumbers grows due greenhouse development, in part by using such facilities by Russian retailers, in particular, "Magnit" that is specialized in this area in its own greenhouse in the Krasnodar region [9].

As per the report of the National Vegetable Producer Union, 682 thousand tons was cropped in 2015 in Russia (increase by 6.6% against 2014) in the protected ground. Cucumber is the most popular culture that covers three quarters of this volume. Despite the explosive growth of the cucumber production, the average retail price for 1 kg of cucumber increased by 26.8% to 159 rubles in 2015. The growth was certainly achieved due to the effect of the low base. But, on the other hand, it tells about prospects, opportunities to increase this export line in future, promotion of processed leguminous products and vegetables using the appropriate marketing policy with the required adaptation for food-importing countries, production localization of these products. This opens significant opportunities for the Ministry of Agriculture, Food Industry, and the Russian business to increase the contribution to the foreign trade turnover of Russia.

Import embargo to certain countries encouraged some Russian producers to closely consider the import substitution. In this regard, vegetable growing and production of processed plant products in Russia has become quite popular field of trade. So, in early 2016, AFK "Sistema" acquired the "Yuzhniy" Company, the largest agricultural plant in Russia to crop cucumbers and tomato in the Karachay-Cherkessia of 144 hectare in area. In its turn, "Rusagro" intends to to build 100 hectares of greenhouses to grow cucumbers, tomato and lettuce in one of the central regions of Russia with the possible extension of up to 300 hectares. Other Russian companies also focus on the development of this line of business. So, the "Parus Agro Group" has the intention to construct greenhouses in Krasnoyarsk Territory. "Cherkizovo", the well-known food company considers the plant production in the long-term perspective and the plant product processing as one of business areas. The "Fabrika ovoshey" Holding – intends to build a greenhouse complex in the

Stavropol Territory with the area of 75 hectares. The first line to grow 23.5 tons of vegetables per year is scheduled to start-up as early as 2017.

As per the estimate of the Ministry of Agriculture, the land plots for greenhouses of up to 8500 hectares in area should be arranged to be self-sustained by 2020 in Russia. However, cucumber production investments should related with the plant market for this very crop. In spring 2016, the green channel opened for vegetables and fruits from Iran. And the cucumber might dominate this channel since Iran is specialized in this crop.

Cereals and flour-grinding products rank the third in the quickly growing export line in 2015. The malt export mostly increased in this group amounted to 35.4 mln USD in terms of value. This growth was achieved through the active investment policy of the Russian malt manufacturers and the poor grain crop in Asia and the cheapened ruble, once again. Latvia was the main malt purchaser in 2015; the supply volume in this country grew by 27.7% or by 8 mln USD as well as Taiwan (growth by 16.4%; 5.8 mln USD) and Kazakhstan – growth by 16.2% and 5.7 mln USD [12].

The meat and meat by-product volumes increased as the export items from Russia. In 2015, these commodities were exported for 177 mln USD or 11.7% higher than in 2014 [12]. The major share of this increase covers the export of poultry meat – an increase made 16.9% up to 75 mln USD. Pork saes increased by 447% up to 9 mln USD.

These commodity group manufacturers intend to increase overseas shipments. So, "Cherkizovo" obtained a permit in February 2016 to export chicken meat to the United Arab Emirates. By 2019, "Cherkizovo" plants to increase the export of the chicken meat by 15–20% of the total sales volume. The company plans to achieve this result by expanding its export coverage. It intends to get access to markets of Egypt, Iran and Iraq, as well as China, and it holds active negotiations with the latter to enter the Chinese market by diversifying the mutual trade.

The Asian market more and more attracts the Russian food industry. So, the "Miratorg" company supplies meat products to Iran, Hong Kong and the UAE. The absence of veterinary access holds back the expansion of Russian export to major Asian markets – China, Japan, South Korea, Vietnam. By overcoming this obstacle, the Russian meat product producers will obtain the access to a huge market for efficient and long-term development. Turkey meat is the valuable export item. This meat production in 2015 was faster than other types of meat products by 34.9% to 205 thousand tons. Main lines of turkey export include African countries – Sierra Leone, Gabon, as well as Asian countries – Hong Kong, Vietnam. Turkey export is expected to be diversified. Thus, the "Damate" Company plans to supply turkey meat in Serbia and other European countries, agreement on meat supply to the UAE are reached, the export permit is expected to Saudi Arabia. In future, the company plans to export a quarter of its total production.

"Evrodon", the largest duck and turkey meat manufacturer in Russia, proceeded to supply duck tongues and feet to China. The Company concluded the contract with the Chinese partner to supply 40 t of thid product per month.

In the late 2015, The Minister for Agriculture A. Tkachyov stated that Russia would hold its prominent niche in the Asian market in three years. According to the Minister, beef, wine, grain processing products are potential food products. As per the Minister, in 2020 Russia plans to reach its full self-sustainable supply with milk, meat and vegetables [13].

The President of the Russian Federation, V.V. Putin in 2014 supposed that the Russian export of highly processed items should increase by one and a half in 3 years. [12] Currently, the raw material export in the total food export is about 40% and finished products of 45%.

The breakthrough of 2015 in the Russian food exports to foreign markets is still the first sign that, however, still does not evidence on occurrence of the export epoch to the full extent. However, on the one hand, the import substitution policy in this segment makes it possible to better meet the needs of Russian consumers of food products, and, on the other hand, by promoting their products abroad, Russian food industries reduce their dependence on the demand fluctuations in the Russian market and obtain more opportunities for maneuvering. Against the food sanctions and destruction of foreign food in Russia, the active import substitution remains the most important area for the food self-sufficiency.

It is noteworthy that the export breakthrough of foodstuffs occurred during reduction of employment in food industries. In 2015, the employment in this sector decreased by 0.9% (in 2014 it increased by 0.4%), investments decreased by 12%, while the output increased by only 2% [14]. And this occurred in conditions when households have chaged their choice in favor of domestic products. It should be noted that the Russian Federation owns quite large opportunities to reform the production structure and foreign trade of agricultural products though the export maneuver largely occurred due to the reduced consumer demand and emergence of surplus output.

These facts prove the particular edge of the crisis situation in our country that directly relates to issues of "new poverty" emergence and formation as a consequence of public income and consumption level. The crisis evidently describes the downfall related to the level of food consumption back to 8 years to the level of early 2008. By the study estimates conducted by the Center for Macroeconomic Analysis and Short-Term Forecast, it is notable that the consumption of food products declined much stronger as compared with the segment of non-food commodity consumption (totaling to the level of 2011) that is a sign of the abrupt rise in the population differentiation by income. The emergence of the "new poverty" issue is proved by opinion polls. As per the VCIOM (Russian Public Opinion Research Center) polls, just a share of the poor for the year total ("not enough money even for food") increased twice as much from 4% to 9%, the share of the lower-income population ("enough money for food, but clothes are not affordable yet) increased from 18%

to 30%. At the same time, a segment of the rich significantly increases (1% to 4%) within 2014–2015, namely those who, as per polls, "can afford almost everything" [14]. In conditions of highly limited solvency of the Russians, caused by the emergence and growth of the "new poverty", the export expansion and active promotion towards foreign markets is deemed as the promising way to develop the Russian food business.

Currently, Russia ranks 5th in the world in terms of agricultural crop production and the export volume. In 2009, Russia produced 2.1 mln tons of poultry meat and imported 910 thousand tons. As a result, the national food economy growth achieved: crop production increased by 40 % for 10 years. This growth rate is more evident when regarding certain items: the cereal export increased from 20 mln tons to 30 mln tons by 1.5 times since 2010. In 2015, Russia ranked first in the world to export the wheat. As a consequence, the particular capacity is formed for certain items to build the basis for the food export expansion. This made it possible for the President of the Russian Federatin, V. Putin, to state in his annual address to the Federal Assembly that "Russia is able to be the world's largest supplier of healthy, environmentally friendly, and high-quality food products". Nevertheless, for purpose to implement this, the stable food export strategy is required in view of varying realities in global economy and trade.

In particular, it is important to use the Russian food export capacity in EEU countries. Currently, the total volume of mutual trade in food commodity and agricultural raw material within the EEU zone amounts to approx. 7 bln. USD. In 2015, as part of total "export breakthrough" above, the growth rate if wheat supply to EEU countries made 30% to the 2014 year level and meat – over 50%. The food market of the Asia-Pacific region should be specifically mentioned. In particular, the Chinese food market is perspective in this regard, as mentioned above. Despite of specifics of the Chinese food commodity, as the living conditions improve, the Chinese consumer gets more concerned in Western food products. Russian food industries focused on the Chinese market of meat, fish, seafood, and some cereal supplies. However, supply of these products is constrained by restrictions by the Chinese side. In the meantime, in early 2016, Chine permitted supplies of wholesale batches of grain and rice. In addition, the joint Russian-Chinese online marketplace is arranged for the export of Russian goods, including food commodities. The fresh commodity segment will be of special focus. Concurrently, the food will be exported to China from any Russian regions.

Creation of the free trade zone with Vietnam and expansion of trade and economic relations with ASEAN countries assumes creation of the consolidated list of investment projects to develop the food export. Overall, the Asia-Pacific countries is quite the promising market for the food export of Russian manufacturers since recently, the consumption of foodstuff is notably increasing along with the improvingsolvency of the region. Russian food manufacturers proceed to gradually use their improving export opportunities. These opportunities are due to favorable rouble exchange rate and the production capacity. In particular, the Russian producers have reached the saturation of the domestic chicken meat market and are on their way in terms of the pork.

"Miratorg", one of the largest meat producers in Russia, intends to increase the share of exported products from 5 to 25% in three years. As per the company, China is the most promising market for the company upon the receipt of authorization from the veterinary service.

The expansion of the export routes for Russian meat producers is the chance to trade in the carcass parts undemanded in the domestic market. For example, 1 kg of chicken feet in China costs 2 USD whereas they are far cheaper in Russia.

Except "Miratorg", other large meat producers plan to increase their export volumes to various countries. Same "Miratorg" has commenced the delivery of small shipments of poultry meat in Serbia and Italy, and it is in process for its beef supply authorization to the EU countries and Iran. In June 2016, the Iranian Veterinary Service officially notified the Rosselkhoznadzor (Federal Service for Veterinary and Phytosanitary Surveillance) on approval of the statement on the supply of boneless frozen beef from Russia to Iran. "Damate", the other large meat producer delivers turkey meat in Sierra Leone, Gabon, Vietnam, Hong Kong, while the export to the UAE and Serbia is approved already. "Cherkizovo", another "grand" food market in Russia, declared on its intention to increase the export of chicken meat by 10–20% of the total sales volume.

To strengthen their niches in foreign markets, the Russian food producers should reduce the cost of raw material component and increase the volume and quality of end products.

Local producer-specific products and the local manufacturing content may become one of schemes to conquer foreign food markets. The possibility for Russian actors to take part in food production chains should not be disregarded where they may hold strategic positions.

For purpose of the food export expansion of the Russian Federation, a number of quite difficult tasks should be solved, including customs and tariff regulation, certification issues, export safety and supply management. At the same time, addressing issues to increase the food export, the best balance should be striken between the globalization of export process and the domestic food safety. Extension of the food embargo by the RF Government till 2017 is essential to define the consistency of the import substitution strategy and capacity building of food industries in the field of foreign trade. This approach is typical for the Export Food doctrine developed by the international independent Institute of agrarian policy.

In general, the current situation for Russian food producers in terms of import substitution strategy may be stated to allow chance to hold higher positions in the global food market.

CONCLUSION

By the outcomes of the study to assess the impact of foreign trade on the food market development, the role and current trends of modern international trade and the Russian export evolution are identified aimed to mitigate global problems and existing international regulation procedures in the food and agricultural product market, it has been proved that:

– The international trade in food is the effective tool to address environmental issues and sustainability problem that impact not only on consequences of the human environmental exposure resulting fro industrial activity, but also the measures to address environment protection issues at the supranational level;

– The international trade is a double function tool of the export competition policy, acting on the one hand, as the factor for trade and ecological expansion for industrialized countries and the form of "neoprotectionism" and, on the other hand, as the tool of very effective and "constant solution" to the food problem within the WTO, creation of the "special protection mechanism" for the least developed countries aimed to reduce the export competition in agriculture.

– The international trade acts as the major factor of the national economy growth and national commodity market functioning that impacts the increase in pricing depending on the Russian food market in the post-crisis period, and facilitates the vigorous import substitution as the strategic policy for food self-sustainability in the Russian Federation.

The appropriate assessment of identified consequences resulting from the impact of the current international trade on the food market growth will neutralize negative evidences of global problems, strengthen the role of international trade to improve the well-being of all trade transaction parties and increase the effectiveness of the RF import substitution policy.

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CLUSTER APPROACH TO THE DEVELOPMENT OF FOOD MARKET OF THE REGION: THEORETICAL AND APPLIED ASPECTS

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Abstract: The effective development of food market taking into account the interests of its participants predetermines a search of new approaches of their interaction. The cluster approach as the technology of management of development of food market of the region is the subject of this research. The aim of work is the justification of possibility of application of cluster approach to the development of food market as an organizational and economic mechanism allowing to compensate the problems noted in this work. For the justification of application of cluster approach to the development of food market of the region, its optimizing economic and mathematical model in the form of a multiple parameter problem of linear programming allowing to determine the optimum values of investment, production and financial parameters of functioning of food market of the region using the criterion of net present value is created and approved by the authors. It is revealed that the creation of agrofood cluster in the region will give an opportunity to reduce expenses in the scheme "production-processing-consumer" which will provide the growth of consolidated revenues by 2.25 times and will also allow to increase the product range and to strengthen the competitive positions of agriculture. As a result, the provision of population with food will improve, the rural economics of the region will be significantly strengthened, and the application of special tax regime (single agricultural tax) will allow to lower the tax burden, to simplify tax accounting and reporting for rural producers, to release a part of resources and to focus them on the development of agrofood cluster. The modeling of food market of the region with the use of cluster approach and the presented mathematical model also allows to support decision-making taking into account the requirements shown to the food supply of the population of the region in the quantitative and qualitative aspects.

Keywords: regional food market, cluster approach, mathematical modeling, expected economic effect, food resources (food)

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INTRODUCTION

In modern society an important value is the person and the satisfaction of his needs, the main of which is the satisfaction of needs for food. The changes in policy, economy, culture, social sphere are performed for the person. In this context the value of development of food market, both of the whole country and its separate regions, is the key element of domestic policy of the state [1].

The years of reforms testify that the state and functioning of the food market and its infrastructure in our country remain a poorly adjustable and insufficiently organized process. In the marketing system the organized channels of wholesale trade are not created, the operational information support of participants of the food market is poorly adjusted, there is practically no logistic system of control of commodity flows, the domestic and international standards are not brought to the unified system. This circumstance provides the abnormality of reproduction process in the agricultural sector and, as a result, sharpens the contradictions between subjects of the food market (agricultural producers, processing enterprises and food sellers). The aforesaid causes an increase in distribution costs, provides an increase in price and decreases the competitiveness of agricultural production, raw materials and food [2, 3].

In this regard, the food market holds a special place in the system of local markets [4] as the market forming the level of satisfaction of necessary (physiological) vital needs of population which, in many respects, determine its social sentiment. Characterizing the level of food supply of the Kemerovo region, it is possible to note that following the results of 2014 the output of agricultural production in all the categories of producers was 49652 million rubles or 145.1%, as compared to the level of 2010, according to the effective prices. At the same time, the main volume of production is made in the households of the population and equals to 51%. 40.3% of volume fall to the share of agricultural organizations and 8.7% – to the share of country farms in 2014 [5].

Estimating the level of food supply of the population of the region for a five-year period (2010–2014 inclusive), it should be noted that there was an increase in consumption per capita: by 8 kg of meat and meat products (12.3%); by 12 pieces of eggs (4.6%) and by 7 kg of vegetables. Besides, there was an insignificant increase in the consumption of vegetable oil, potatoes, fruit and berries. The consumption of milk and milk products decreased by 12 kg (5.3%) and the consumption of bread and bakery products decreased by 12 kg (9.1%) during the analyzed period [6].

At the same time, in comparison with the recommendations for the rational norms of consumption of foodstuffs the imbalance of food of the population is noted. Thus, the consumption of bread exceeds the norm by 20 kg; potatoes – by 31 kg and eggs – by 10 pieces in 2014. The consumption of milk and dairy products is lower than normal by 105 kg and vegetables – by 60 kg. At the same time, the content of animal protein is lower than normal by 30.2 grams, and its total daily consumption, according to the physiological norms, was 76.5 grams in the food structure of the population in 2014 [7].

The provided data testify that the insufficient consumption of animal products, vegetables and fruit provides the unbalance of food, low content of vitamins and minerals, iodine and animal protein in it. The unbalanced, insufficient food can provide the nutritional restrictions and emergence of imbalance in the food ration of population of the region and the discrepancy of volumes of caloric content concerning the vital human need. It can also result in the discrepancy of established level of the cost of living with its authentic parameters [8].

An important point in the solution of the problem of food supply of the population of the region is the prediction of interaction of such processes as production and consumption. The essence of production is the satisfaction of consumers with food. The analysis of structure of average per capita consumption and its dynamics are a basis for calculations of expected indicators in prospects various in terms. Various methods of mathematical modeling allow to reveal adequately the arising regularities and to predict various socio-economic indices, and also to estimate the probability of their achievement.

For providing the population with food according to the recommended standards, it is necessary to reach the following outputs by 2020, thousands of tons: bread and bakery products – 277; meat and meat products – 198; milk and milk products – 898; potatoes – 264; vegetables – 370; eggs – 687 million pieces [8].

The next moment is the solution of the problem of realization of food products and the reliable quantitative assessment of the efforts put in them. The food market of the region which, logically, would have to consider the interests of producers, processors, sellers and consumers of food products can solve this problem. In practice, the food market does not bind all the entitled potential subjects together, and each of them functions separately [9, 10, 11].

Thus, the producers of food products can be presented by regional and external participants, as such, as the suppliers of finished goods and the suppliers of raw materials and semi-finished products. The regional producers processing their production also supply the market with it both in the finished form and in the form of semi-finished products via wholesale and retail. Personal subsidiary farms which, due to their limited access to the regional market, cannot fully influence its commoditization, should be allocated in a separate group of producers [12]. Thereof, personal subsidiary farms often act as the consumers of food products, as well.

Considerable volumes of deliveries of food from external suppliers of products both via intermediaries and directly from producers have a significant effect on the modern functioning of food market of the region [12]. Thereby, there is another subject in the food market of the region – the supplier, i.e. the intermediary, as is.

Let us note that the specifics of food market of the region is that agriculture, due to its spatial extent and temporary period of production of some types of products, demands a developed infrastructure (roads and warehouses). However, it is not capable to create and support this infrastructure because of the low profitability of production. As a result of developed adverse macroeconomic conditions, there is discrepancy in profitability of agricultural enterprises and other branches of economy. The low profitability of agriculture limits its opportunities to perform not only expanded, but also simple reproduction [13, 14].

An important aspect for development of food market of the region is the regulation of its activity by the state by the creation of necessary conditions of providing the poor segments of the population with food. Besides, the state regulates the food market by the establishment of quotas, duties, the organization of government procurement, and also by means of the organization of control of import of food.

Thus, the food market of the region is a complex structure which includes its above-named participants, on the one hand, which solves their general problem – the provision of population with food; on the other hand, all the participants are in unequal conditions for their effective activity as they act separately in the market space and often ignore the interests of each other.

The provision of effective development of food market and combination of interests of its participants on a parity basis predetermines the search of the new organizational and economic mechanisms capable to provide the population with food according to the scientifically based norms of consumption, and also the forms of interaction and mutually beneficial cooperation of its participants. In our opinion, the agrofood cluster can become such an organizational and economic mechanism.

The cluster, as an organizational and economic mechanism, does not only increase productivity at the level of the country, but, interacting in the market environment, can effect the activity of other clusters [15, 16, 17]. This circumstance predetermines the same attention to food clusters as to traditional ones, the development of which must improve. A specific feature of a cluster is an opportunity to put together the efforts of all the participants of food market in the territory of the region for the purpose of increase in its competitiveness, decrease in risks in the redistribution of commodity streams, fast adaptation of the pursued policy to the constantly changing market condition that fully meets the expectations of development of food market of the region.

Clusters have a unique potential to gain a synergetic effect on the basis of cooperation of various economic entities with the participation of public authorities and different infrastructures in the form of science and technology institutions and technoparks [18, 19].

In our opinion, the application of cluster approach to the development of food market of the region will provide the development of agriculture and processing industry, directly the food market itself and, as a result, will provide the food supply of the population with the products of high quality and in sufficient volumes.

On the basis of the performed analysis, it is possible to draw a conclusion that the knowledge of problems of functioning and development, assessment and regulation of the regional food markets is adequately presented in scientific literature and, at the same time, the attention is insufficiently paid to such aspects as the development of food markets in industrially developed regions taking into account their specifics and benefit of participants. This circumstance has determined the main directions of the presented research.

OBJECTS AND METHODS OF STUDY

The food market of the region acts as a research object in this work. The object of research is the assessment of efficiency of functioning of the food market and its development with the application of cluster approach. The aim of research is the modeling of development of food market of the region within the cluster approach for the identification of indicators of food supply of the population.

The following methods are used as the research methods in the work:

(1) The economic and mathematical modeling of development of food market;

(2) The numerical research of constructed model with the use of financial and analytical package of application programs which is described in detail in the work [20];

(3) The method of comparative analysis.

For the solution of the problem of assessment of efficiency of functioning and development of food market of the region we use the method of mathematical modeling.

The food market of the region is an example of large social and economic system, therefore during the assessment of efficiency of its functioning the problems of mutual coordination of operational, investment and financial streams, circulating in this system, arise. The description of processes in difficult systems demands the use of large number of data, which necessitates their automated processing. In turn, the efficiency of application of automated means of analysis is determined by the balance of mathematical models describing substantial processes in a difficult system.

Let us consider the following **substantial statement of the problem** of assessment of efficiency of functioning of food market of the region. Let there be n agricultural productions in the food market of the region using n various fixed business assets (FBA) for the production and sale (realization) of n types of products (meat, grain, milk, vegetables and so forth). At the same time, the technical and economic characteristics of FBA – the cost, service life and productivity of unit of FBA, and also the cost of unit of output of each type – are set. It is supposed that agricultural producers can use borrowed financial means in the form of credits or donations. It is required to determine the sums of investments, the volumes of production in total and of each production separately, and also the amounts of financing of food market of the region with which the discounted balance of strategic income (profit and estimation of cost of property) and strategic expenses (total investments), reflecting the added value of the regional production market for a certain period of time (the planning horizon), will be the maximum. We will treat in the problem stated above the functioning of food market of the region as the functioning of food cluster.

Mathematical statement of the problem.

Let us introduce the following symbols:

– y_k is the output of agricultural products of type k, thousand tons;

– m_k is the quantity of acquired fixed business assets for the output of agricultural products of type k, units;

– q_k is the expected demand for the products of type k in value terms, rubles;

– V_k is the design productivity of unit of fixed business assets of type k;

– T_k is the service life of unit of the main fixed business assets of type k, years;

– P_k is the cost of unit of products of type k, rubles;

– c_k is the average annual cost of unit of FBA of agricultural products of type k, rubles;

– $x_k = c_k m_k (k = 1, \ldots, n)$ is the investments (the cost of acquired FBA) of type *k*, rubles;

– $x_{n+k} = P_k m_k y_k \ (k = 1,...,n)$ is the proceeds from the sales of products of type *k*, rubles;

– $x_{2n+1}=Cr$, $x_{2n+2}=Dot$ is respectively a sum of credit and donations for the producer of agricultural products, rubles;

– $y_k = \dfrac{x_{n+k}}{P_k m_k} = \dfrac{c_k x_{n+k}}{P_k x_k} (k = 1,...,n)$ is the output of products of type *k*, tons;

– $R = \sum_{k=1}^{n} P_k m_k y_k$ is the total sales proceeds of all the types of products, rubles;

– $F = \sum_{k=1}^{n} \beta_k R$ is the total salary fund defined as the expertly set percent β of proceeds *R* from the sale of all the products (a labour-output ratio), rubles;

– $I = \sum_{k=1}^{n} x_k$ is the total investments of food cluster, rubles;

– $Am = T \sum_{k=1}^{n} \dfrac{c_k m_k}{T_k}$ is the total depreciation charges for all the planning horizon *T* of all types of FBA, rubles;

– z_k is the material inputs for the agricultural products of type *k*, rubles, defined in the form of the set percent p_k of the total expenses with the functioning of producer of agricultural products of type *k*;

– $\delta_k = P_k V_k / c_k$ is a relative indicator of efficiency (capital productivity) of type *k* of FBA;

– *r* is the annual discount rate,

– *T* is the planning horizon, years;

– r_0 is the annual rate of credit,

– T_0 is the term of credit in years,

– I_{max}, Cr_{max}, Dot_{max} is respectively the maximum sums of investments, credits and donations into the food cluster.

The net profit (after taxation) earned by each producer is described by the following expression:

$$W_k = \frac{1-\alpha_3}{1-p_k}\left(x_{n+k}\left(1-\beta_k\left(1+\alpha_4\right)\right) - \frac{T}{T_k} x_k \right) \quad (k=1,\ldots,n), \qquad (1)$$

where α_3 is the rate of single agricultural tax (SAT), α_4 is the rate of assignments from the salary fund for obligatory insurance. Then the net profit *W* (after taxation) of the whole agrofood cluster is presented in the following form:

$$W = \sum_{k=1}^{n} W_k \cdot$$

Let us assume that in the course of their functioning all the enterprises of food cluster get the identical funds and are solvent, that is the following conditions are met:

$$Ds_k = \left(1-\alpha_3\right)\left(\frac{\left(1-\beta_k\left(1+\alpha_4\right)\right)}{1-p_k} x_{n+k} - \frac{1}{1-p_k}\frac{T}{T_k} x_k \right) + \frac{x_{2n+1}}{n} + \frac{x_{2n+2}}{n} \geq 0 \left(k=1,\ldots,n\right), \qquad (2)$$

where Ds_k is the current means of producer of products of type *k*. Then the current means *Ds* of the whole food cluster and the condition of his solvency can be presented in the following form:

$$Ds = \sum_{k=1}^{n} Ds_k \geq 0 \cdot \qquad (3)$$

The main factors influencing the stable development and effective functioning of any economic system are the following:1) the demand for the output as the main market factor that allows to avoid the inefficient development of economic system because of overproduction; 2) The scientific and technical progress limiting the production capabilities of economic system with the characteristics and the level of development of fixed assets which are directly involved in the process of production and influence the volume and quality of products. Therefore, due to the functioning of food market of the region it is reasonable to bring in the following restrictions:

(1) $0 \leq P_k m_k y_k \leq q_k$ – the volume of sales of products of type *k* (in value terms) does not exceed the demand for it;

(2) $0 \leq y_k(t) \leq V_k$ – the production of type*k* in units of FBA does not exceed the volumes determined by the productivity of FBA (or by the level of scientific and technical progress).

We will consider the net present value *J* of agrofood cluster reflecting the added value of the below integrated structure as the criterion of efficiency (optimization) of agrofood cluster in the model of food market of the region:

$$J = T \cdot \frac{W + Am}{1+r_e} - I - \left(1 + \frac{r_0\left(12T_0+1\right)}{24}\right) Cr, \qquad (4)$$

where $r_e = \dfrac{rT}{1-\left(1+r\right)^{-T}} - 1$ is the discount rate of the project on all the planning horizon considering the rate of inflation, requirements of profitability of investor and other risks for the entire period of functioning of agrofood cluster.

Considering the introduced symbols and formulae (1)–(4), the mathematical model of agrofood cluster of the region will take the following formalized form of static problem of linear optimum control:

$$J = T \cdot \frac{\sum_{k=1}^{n} \sigma_k x_k + \sum_{k=1}^{n} \gamma_k x_{n+k}}{1+r_e} - \left(1 + \frac{r_0 (12T_0 + 1)}{24}\right) x_{2n+1} \to max \quad (5)$$

$$\begin{cases} -\theta_k x_k - \gamma_k x_{n+k} - \frac{1}{n}\left(x_{2n+1} + x_{2n+2}\right) \le 0, \\ x_{n+k} \le q_k, \\ x_{n+k} \le \delta_k x_k \quad (k = 1,...,n), \\ \sum_{k=1}^{n} x_k \le I_{max}; \quad x_{2n+1} \le Cr_{max}; \quad x_{2n+2} \le Dot_{max}, \\ x_k \ge 0 \quad (k = 1,...,2n+2). \end{cases}$$

$$\theta_k = -\frac{1-\alpha_3}{1-p_k}\frac{T}{T_k}; \ \sigma_k = \theta_k - \frac{1+r_e}{T}; \ \gamma_k = \frac{(1-\alpha_3)(1-\beta_k(1+\alpha_4))}{1-p_k} \ (k = 1,...,n).$$

The model (5) is a multiple parameter problem of linear programming after solution of which the optimum level of investments, production and, if necessary, credit resource for the financing of the current activity is determined. Tax flows, labor input, the material capacity of agricultural production, the characteristics of the fixed business assets (average annual cost, useful service), the characteristics of products (the cost and consumer demand for each type of agricultural production), the indicators of internal and external environment of functioning of agrofood cluster (the discount rate considering the rate of inflation and requirements of profitability of the investor and the planning horizon are considered in this model. The model (5) considers the main restrictions of activity of food cluster (solvency, the demand for production, the scientific and technical progress, restrictions of financing and so forth) taking into account the specifics of agricultural production of the region. This model can be the basis for the determination of expected economic effect of functioning of regional food cluster as:

(1) They consider high material cost intensity, characteristic of agricultural production (the specific weight of material inputs fluctuates at the level of 60–70 percent in the structure of costs of agricultural products);

(2) They consider the specifics of agricultural production in the form of increased wage (specific weight of wages of employees of the branch in the total proceeds from the sales of production of agroenterprises), which determines the low labor productivity and limits the competitiveness of the branch;

(3) They consider the increased service life of fixed business assets in the agrarian and industrial complex, significantly different from other branches of economy. More than 80 percent of machinery and equipment in agriculture used in production are outside the rated service life;

(4) They consider a special taxation scheme, characteristic of the agrofood specifics, in the form of single agricultural tax for agricultural enterprises significantly reducing the tax burden of agricultural producers and primary processors of agricultural raw materials;

(5) They consider the features of demand for production which is characterized by the relative constancy that distinguishes the agricultural branch from other branches of economy.

Let us note that linearity of the specified model allows to use the specialized package of investment analysis described in detail in the work [20].

RESULTS AND DISCUSSION

The scientifically based rational norms of consumption of food are developed based on ten main types of crop and livestock production. The agrofood cluster of the Kemerovo region will be focused on the production, processing and realization of the following types of products made in its territory: meat and meat products, milk, egg, grain, potatoes, vegetables.

According to the data of Department of agriculture and processing industry of the Kemerovo region, as a result of the financial and economic activity following the results of 2015, the following values of indicators were obtained by agricultural producers (Table 1).

According to the statistical data, [6] Table 2 gives the necessary indicators for entering the input information to the package and the approbation of created optimizing mathematical model of the agrofood cluster of the Kemerovo region.

The calculations performed by means of the optimizing package of application programs "Karma" became the result of our research [20]. The use of input data of the model for the main types of production (Table 2) allowed to determine the expected economic effect of activity of agrofood cluster of the Kemerovo region for the period till 2020 (Table 3).

Table 1. Basic data for creation of economic-mathematical model (2015)

Type ofproduction	Capital productivity	Salesproceeds, millionrubles	Average annual cost of fixed assets, million rubles
Meat and meat products k_1	1.30	9 031.18	6 935.7
Milk k_2	0.64	2 923.04	4 508.4
Egg, k_3	1.59	3 382.15	2 128.4
Grain k_4	2.16	2 543.21	1 178.6
Potatoes k_5	2.25	557.91	247.3
Vegetables k_6	0.63	1 019.05	1 612.9

Table 2. Key input parameters of the agrofood cluster of the region

Contentof	Name	Referencevalue	Measurement units
Quantity of types of production	n	6	–
Planninghorizon	t_0	10	years
Maximumsumofinvestments	I_{max}	1 200 000	thousandrubles
Maximumsumofcredits	Cr_{max}	0	thousandrubles
Maximumsumofdonations	Dot_{max}	800 000	thousandrubles
Rate of the unified agricultural tax	α_3	0.06	–
Rate of assignments for insurance premiums	α_4	0.2	–
Specific weight of material inputs in total of expenses	p_1 p_2 p_3 p_4 p_5 p_6	0.72 0.72 0.72 0.72 0.72 0.72	–
Percent of sales proceeds for contributions to the payment fund	β_1 β_2 β_3 β_4 β_5 β_6	0.137 0.137 0.137 0.137 0.137 0.137	–
Annual cost of business assets of k type	c_k	1. 6 935.7 2. 4 508.4 3. 2 128.4 4. 1 178.6 5. 247.3 6. 1 612.9	millionrubles
Annual proceeds from the sales of production of k type	R_k	1. 9 031.18 2. 2923.04 3. 3 382.15 4. 2 543.21 5. 557.91 6. 1 019.05	millionrubles
Annualdiscountrate	r	0.25	–
Annualcreditrate	r_0	0.16	–
Creditterm	T_0	5	years
Service life of the fixed business assets of k type	T_k	40	years

Table 3. Expected economic effect of activity of agrofood cluster of the region by 2020

Typeof production	Proceeds from the sales of agricultural producers (before the creation of agrofood cluster), million rubles	Proceeds from the sales of agricultural producers (after the creation of agrofood cluster), million rubles	Economiceffect	
			million rubles	times
Meat and meat products k_1	9 031.18	13 590.3	4 559.1	2.17
Milk k_2	2 923.04	13 473.2	10 550.2	4.61
Egg k_3	3 382.15	7 848.8	4 466.7	2.32
Grain k_4	2 543.21	3 531.9	988.7	1.39
Potatoes k_5	557.91	719.2	161.2	1.29
Vegetables k_6	1 019.05	4 614.3	3 595.3	4.52
Total	19 456.54	43 777.7	24 321.2	2.25

Let us note that the statistical data of agricultural production for the period of 2015 and the production of the main types of products for the period till 2020, taking into account the rational norms of consumption and expected population of the region are taken as the basis of predicted calculation in Table 3 [6].

The total economic impact of agricultural producers of the Kemerovo region during the production of six types of crop and livestock products can be 24 321.2 million rubles by 2020. After the creation of agrofood cluster the highest growth of proceeds is possible due to the realization of milk and vegetables by 4 times and more, from the realization of meat, meat products and egg twice and more. The proceeds from the production and sale of grain and potatoes will increase to a lesser extent (by 30–40 percent).

In the agro-industrial complex of the region (before the introduction of cluster approach) the lowest capital productivity of producers of milk and vegetables is noted, which indicates a low level of proceeds with a high cost of fixed assets for this production. The creation of agrofood cluster will allow to determine the need for credit resources, to optimize investment flows and material capacity of agricultural production. It will also allow producers of milk and vegetables to maximize their proceeds.

When forming an agrofood cluster it is very important to make the right choice of tax regime. The existing tax law allows to apply such a tax regime that will allow to perform the activity of agricultural enterprise most effectively.

In this research the account of tax component is based on the application of one of the special tax regimes for agricultural producers – the single agricultural tax (SAT). One of the terms of application of SAT is the self-determination of agricultural producers. According to Chapter 26.1 of the Tax Code of the Russian Federation, the single agricultural tax can be applied by agricultural producers if the share of agriculture in their total income from the realization of their products is not less than 70 percent [5]. The single agricultural tax is characterized by the fact that the tax reporting is quite simple and gives the opportunity to reduce the tax base by the sum of losses which can be received following the results of the previous tax periods. Besides, the low rate of 6 percent of the income reduced by the sum of expenses together with only 20 percent of assignments for compulsory social insurance of labor of employees make this special tax regime quite attractive for rural producers.

The creation of agrofood cluster will provide the changes in the tax system both of the organization and its members. The changes of tax regimes for basic farms and processing enterprises will be performed centrally, according to the scheme (Fig. 1).

This circumstance will cause essential changes in tax accounting and tax reporting which were as follows before the formation of agrofoodcluster (Fig. 2).

On the basis of primary documents of accounting, registers of primary tax accounting are formed (if there is no difference between the tax and accounting, then the accounting documents are at the same time the registers of tax accounting) from which tax declarations, provided to tax authorities, are formed.

The agrofood cluster will have a vertically arranged consolidated account, including tax accounting. Only the primary registration documentation will be formed at the level of each division, and the summary reporting, which will be reported to tax authorities via electronic means of communication according to the tax law, will be formed at the level of the head company.

As a result, the system of tax accounting and reporting of the agrofood cluster and the institutes, which are part of it, will be as follows (Fig. 3). At the same time, it does not mean that there will be not any synthetic reporting at the level of units, it will be reported from the head company to its production units, besides, internal reporting will be formed.

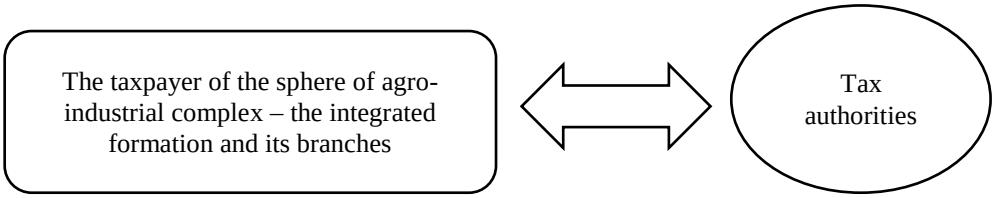

Fig. 1. Tax relations in the agrofood cluster.

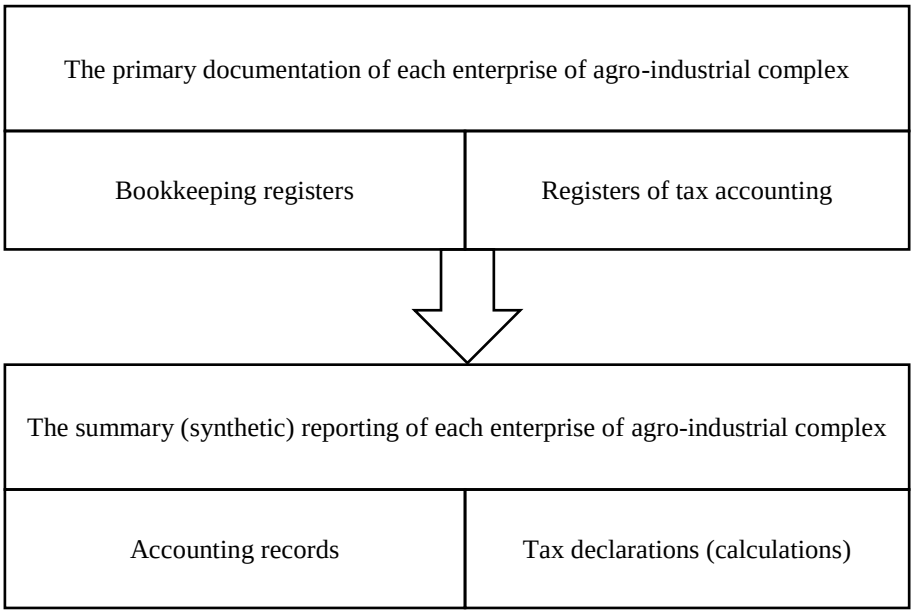

Fig. 2. Existing tax accounting and reporting in the agro-industrial complex of the region

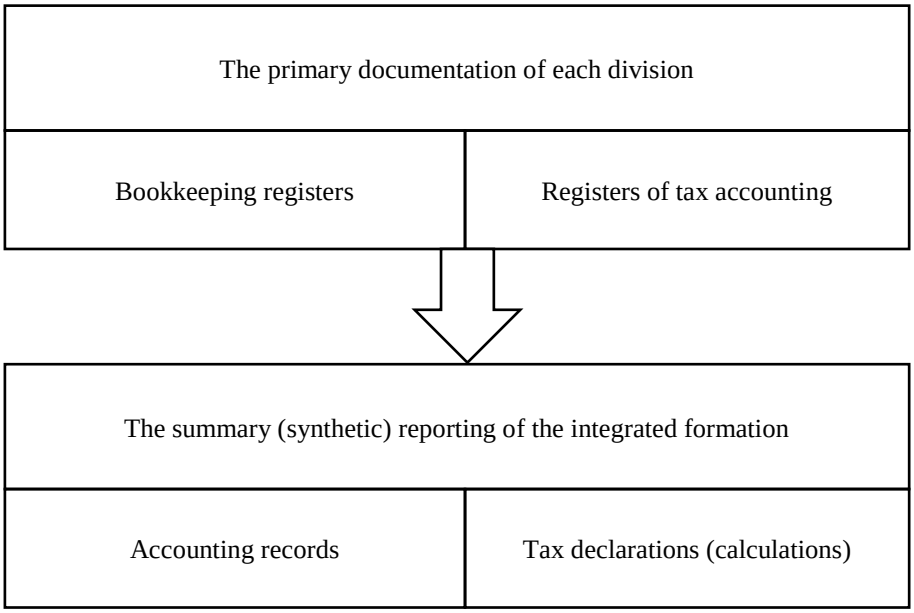

Fig. 3. Formation of tax accounting and reporting in the agrofood cluster

There is the centralization and distribution of tax duties in this system of relations. Besides, the existence of centralized account solves the personnel problem concerning the qualified financial personnel. The tax relations in the sphere of tax control will change in the agrofood cluster, too: firstly, the internal tax control necessary for the formation of summary reporting will be introduced from the head company; secondly, the approach to the performance of tax audits will change as the requirements for the companies having branches will cover them.

Along with other advantages, the tax relations which will allow to release a part of financial assets which can be aimed at the general development of agrofood cluster and, as a result, at the increase in food supply of the population of the region will be improved in the agrofood cluster.

It should be noted that the social security of the population with various income, especially that of lower-income strata, substantially depends on food supply and, as a result, will provide an increase in the quality of its life.

Thus, an agrofood cluster including the production, storage and realization of agricultural products characteristic of such an industrial region as Kuzbassin is described in the work on the basis of economic and mathematical modeling. The created model is quite universal and allows to make a predicted assessment of economic efficiency of functioning of agricultural producers, to reveal an economic capacity of agricultural branch of any region, and also to develop scenarios of development of regional agrofood cluster according to the recommended scientifically based norms of food supply of the population of the region taking into account the interests of agricultural producers. It is reasonable, for the immediate consideration of interests of society (social efficiency), to add one or several criteria to model (5). Now authors consider the following options of criteria: 1) the maximization of income of consumers of products of the cluster (with the purpose to increase a solvent demand for production); 2) the minimization of damage to the environment in case of use of ecologically dirty production technologies.

This circumstance gives the grounds to claim that an agrofood cluster in the region will give the chance: to considerably reduce expenses in the scheme "production-processing-consumer"; to increase the range of foodstuffs and to strengthen the competitive positions of agriculture; the application of single agricultural tax will allow to lower a tax burden, to simplify tax accounting and reporting for rural producers. It will improve the provision of population with food and will significantly strengthen the regional rural economics. The creation of regional integrated formations in the form of clusters will provide an increase in the sustainable economic development of not only the agrarian sphere, but the region in general, and also to be one of the tools for the solution of strategic tasks facing the agrofood sphere.

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PROBLEMS AND PROSPECTS OF DEVELOPMENT OF HUMAN CAPITAL AS THE IMMANENT BASIS OF QUALITY OF LIFE OF THE RURAL POPULATION OF THE RUSSIAN FEDERATION

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Abstract. The agrarian sector plays an important role in ensuring the food security of the state and the improvement of quality of life of each person and all the society. The increase in the infrastructure, economic and social problems of development of rural territories against the background of the transition of Russia from the state planned economy to market relations caused the formation of migratory outflow and a decrease in the rural population, the reproduction of human capital of the individuals living in the rural area on a narrow basis. The problems and prospects of development of human capital acting as the main factor of sustainable development of social and economic systems in the rural territories are considered in the research.The paradigm reflecting new a vision of sustainable development of social and economic systems is proved: human capital is the quality of life the implementation of which will allow to provide the balance of social and economic processes of rural territories, and, therefore, their sustainable development. The trends of institutional transformations oriented to the provision of reproduction of human capital in the rural area on a wide basis are considered.

Keywords: Agrarian sector, rural territories, human capital, quality of life, sustainable development

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INTRODUCTION

The transition of Russia from the planned economics to the development of market relations caused not only the initiation of transformation reforms related to the formation of institutional bases of interaction of economic entities in the market conditions, creation and development of institutes of state regulation of economy and social sphere but also caused the origin and development of a number of stagnant processes, including that in the agrarian sector. The sustainable development of agrarian sector plays an essential role in ensuring food security of the state. The import substitution policy pursued now in Russia in the conditions of economic sanctions from the USA and the European Union countries related to the prohibition of import of a number of food products to the territory of the Russian Federation, and, first of all, food, is oriented to the support and stimulation of domestic producers, ensuring food self-sufficiency of the regions and their sustainable development. At the same time, the official data of a state statistical observation, and also the researches of Russian scientists confirm the availability of a number of problems in the development of rural territories which are related, first of all, to the restrictions in the field of ensuring reproduction of the human capital on a wide basis and the unsatisfactory level of indicators of quality of life of the citizens living in rural areas. The migration outflow has caused the revision and development of new trends of the state policy in the agrarian sector since 2001 focused on ensuring the sustainable development of rural territories and the improvement of quality of life of the citizens living in rural areas.

Thus, the Concept of Sustainable Development of Rural Territories of the Russian Federation for the period until 2020 was approved by Order of the Government of the Russian Federation dated November 30, 2010 No. 2136-r. The institutional basis for the adoption of this document was the Concept of Long-Term Social and Economic Development of the Russian Federation for the period until 2020 (approved by Order of the Government of the Russian Federation dated November 17, 2008 No. 1662-r) in which the main objectives of the state agrarian policy in the long term, and also the Food Security Doctrine of the Russian Federation were determined (approved by Decree of the President of the Russian Federation dated January 30, 2010 No. 120) in which the close attention was paid to the development of the main trends of the state economic policy in the field of sustainable development of rural territories. About 38 million people (27% of total number of the population of the Russian Federation) live in rural territories, at the same time the economically active population constitutes about 23.6 million people (62% of total number of

people living in rural areas). The rural territories are the basic element of agrarian sector of Russia and perform a number of major national functions among which is a production function (the satisfaction of public needs for food), a demographic function (the country population reproduction is provided thanks to the population of rural territories, as well), labor resource (providing with a manpower of both the agrarian sector, and other industries of the national economy), a housing function (the importance of ecological factor for the population living both in urban and rural areas significantly increased due to the shift of accents in the perception of quality of life and the deterioration in the ecological situation), a spatial and communication function (the placement of various infrastructure facilities – highways, power lines, communication lines, etc. which require servicing in rural territories), a social control function (the rural territories are part of the Russian Federation, and it means that the tasks of observance of the legislative legal regime, safety and protection of law and order lie down on the administration of rural settlements). Thus, the importance of contribution of rural territories to ensuring sustainable development at the meso- and macrolevels is obvious.

One of the tasks formulated in the Concept of sustainable development of rural territories is the assurance of quality of life of the citizens living there. However the problem is that the concept of quality of life [1] are not considered both by the scientific community and bodies of authority and management in interrelation with the quality of the human capital which provided the formation of a consumer approach to the perception of quality of life and the concealing of the sources of its increase.

The social and economic nature of the consumer approach to the perception of quality of life originates from the Soviet command system of public administration, one of the proclaimed principles in which was "… the more and more complete satisfaction of the growing material and cultural needs of the people by the continuous development and improvement of social production" [2]. Even at that time, the blurring of the formulation concealed *the role of each person* in this process and provided the strengthening of the tradition of paternalism in the Russian society which is shown in the consumer behavior of citizens in relation to the state – the expectation of firm social guarantees and the needs for "fatherlike government and care" [3].

This circumstance requires forming an essentially other paradigm of quality of life, bringing the person to the foreground as the carrier of the human capital the quality of which acts as an immanent basis of quality of life of each specific individual and community living in a certain territory (the state, a region, municipalities presented by municipal districts, city districts, urban and rural settlements).

The research purpose is the consideration of the existing theoretical and methodological approaches to the determination of quality of the human capital and quality of life, the analysis of the current state and problems of social and economic development of rural territories in the context of the possibility of reproduction of the human capital on a wide basis and the substantiation on this basis of a new paradigm of provision of quality of life.

OBJECTS AND METHODS OF STUDY

The processes proceeding in the agrarian sector of Russia in the context of their influence on the formation of the human capital as the immanent basis of quality of life act as an object of research. The research is based on the dialectic approach. In the course of research a system, process, institutional, evolutionary and other methodological approaches were also used which is caused by the complexity of categories of the human capital and quality of life and the need of justification of their interrelation and interconditionality.

The emergence of concepts of the human capital in the second half of the 20th century is related with the recognition of the role of science and education in the progressive development of society and ensuring the economic growth of states. The founders of the modern theory of the human capital are T. Schulz [4] and G. Becker [5]. At the same time, it should be noted that the ideas about the human capital were laid at the end of the 17th century. Thus, V. Petty in his work "Political Arithmetics" (1676) advanced an idea that a small country and even a small people can become equivalent to larger ones as a result of their location, trade and policy. The main merit of V. Petty in the formation of bases of theory of the human capital was that he approached the justification of the need of assessment of monetary value of productive properties of the human person and he was the first to assign the concept "equity" to them [6].

A. Smith in his "Research about the Nature and the Reasons of Wealth of the People" (1776) emphasized the difference between productive and unproductive work and the role of productive work in the increase in the annual product of any nation. A. Smith was convinced that the increase in the productivity of useful work depends, first of all, on the increase in the dexterity and skill of the worker, and already then on the improvement of machines and tools using which he worked [7]. Continuing to develop A. Smith's ideas, D. Ricardo introduces a concept of "the natural price of work", i.e. that payment for work which will allow to provide the reproduction of the worker without an increase or decrease in the number of his family [8].

K. Marx operated with the category "human capital", considering the savings of working hours in the production process as the production of fixed capital the person actually is [9].

Thus, the classics of political economy laid the foundation for the scientific analysis of human capabilities to work and their interrelation with the results of this work. However in these researches the human capabilities to work, including that to intellectual work, were not considered in interrelation with the emergence of new equipment and technologies. The sources of formation of mental abilities of the person remained neglected, too.

The founders of the theory of the human capital considered the specified concept in relation to the individual as an integral property immanent to him, and the source of his income. Thus, T. Schulz believed that the human capabilities are either inborn or learned, allocating at the same time such concepts as the inborn human potential and the human capital. The first, according to T. Schulz, is determined by an individual complex of genes of the person at the moment of his birth, the second is determined by the valuable qualities learned by the person which can be increased by the corresponding investments [4]. Schulz considered the human capital as a component of the person and associated it with a special form of capital as it is the source of future earnings or future satisfactions [4].

In turn, S. Fischer determines the human capital as the measure of the capability realized in the person to bring income which is determined by inborn abilities, talent, education and the gained qualification [10].

Thus, a value approach prevails in the theory of the individual human capital, and the capabilities of the individual, his knowledge, mental abilities, skills and experience are an integral condition of formation of his income.

The supporters of the theory of the individual human capital tried to explain the differentiation of capabilities of individuals. Some of them assigned it to a heritable and a biological factors, others – to the learned capabilities of the person and the differentiation of income caused by various levels of investments into their production [11].

In the course of understanding of the paramount role of the person in social development, a wider and wider range of components (civic consciousness, political and legal culture, good breeding and education, etc.) enters the human capital.

Subsequently the researchers begin to consider the human capital not only in relation to the individual, but also at the level of an organization which served as the beginning of development of the corporate human capital theory the representatives of which are L. Edvinson, M. Malone, F. Fukuyama and others within which the category of the human capital began to be used for the designation of one of production factors acting as the source of income and competitiveness of the company, its philosophy and moral values. Thus, F. Fukuyama notes that "… in modern conditions not only the ground, plants, tools and machines are the capital, but also the knowledge and qualification of people are, the value of which is constantly growing" [12].

In the conditions of globalization and increase in the differentiation at the level of social and economic development of individual territories the approaches to the research of the specified category change. The researchers begin to consider the human capital not only at the individual, corporate, but also at the national level [13], in relation to a specific social and economic system (the state, a region, a municipality).

Noskova K.A. and Noskova S.V. fairly believe that at the individual, organizational and regional levels the human capital can have interconnected factors influencing the regional economy and business processes of the region [15].

The authors of this article hold the view that the results of functioning and the stability of social and economic system are directly related with the quality of its aggregate human capital – an integrated indicator accumulating the human capitals of individuals who are the active elements of social and economic system of this or that level of hierarchy [12].

Today the human capital is generally recognized by the scientific community as the main factor of social production acting as a basis of work, capital and economic growth (see S. Shojai [16], O. Galor and O. Moav [17] Podshivalenko D. V. [18] and others).

There are also some essentially other points of view of the category of the human capital. For example, professor A.I. Rofe is convinced that only those economic categories that can be measured are of importance for economic comparisons and calculations which is not the case for the human capital [19]. We beg to differ with the point of view of the scientist as the level of development of the human capital and its quality can be measured by means of the results of functioning of social and economic system of this or that hierarchical level.

The results of the social and economic development of certain countries and territories testify to the differentiation in the level and quality of the human capital. Already Simon Kuznets attached essential significance to the starting positions of the human capital, considering them as a limiter for the developing countries from the point of view of a possibility of use of the best practices of the countries with a high level of social and economic development [20].

In the process of development of economic science, researchers begin to consider the human capital in interrelation with the category of quality of life. However the performed analysis of scientific publications allowed to draw a conclusion about the essential differentiation of scientific points of view in the understanding of interconditionality of the specified categories.

The historical prerequisite of emergence of the concept of quality of life was the theory of wealth a bright representative of which was T. Mun (Thomas Mun) and which gained further development in the works by L. Walras (Marie-Ésprit-Léon Walras) – the founder of the concept of general economic balance, V. Pareto (Vilfredo Pareto) – one of the founders of the theory of elites, J.St. Mill (John Stuart Mill) – a supporter of the ethical doctrine of utilitarianism, A. Pigou (Arthur Cecil Pigou) – a representative of the Cambridge neoclassical school, the founder of the theory of economic welfare. In the West, the concept of quality of life entered circulation in the middle of the 20th century [21]. During the same period of time domestic scientists proceeded a research of various aspects of quality of life [22].

The closer attention to the problem provided the study of laws of economic growth. The West German researcher E. Eppler in his work "Die Qualität des Lebens" specified the formation, in fact, of a new paradigm of social development according to which the

quality of life of citizens is the key factor of economic growth of the state, and not vice versa [23].

Such a formulation of the question turned out to be possible as the negative influence of the rapid economic growth on the major components of quality of life – income and its distribution, the national priorities, the environment, etc., became obvious. An especially deep alarm concerning a possible catastrophic crash for mankind because of the growth of the population, scales of production, irrational use of natural resources was heard in the work by Dennis L. Meadows «Dynamics of Growth in a Finite World» (1974) [24].

The considerable attention to quality of life was paid in the Fifth Report to the Club of Rome "The Purposes for Mankind" prepared under the supervision of E. László and published in 1977 [25].

Various aspects of quality of life were researched abroad by J.K. Galbraith (John Kenneth Galbraith), U. Rostow (Walt Whitman Rostow), D. Bell (Daniel Bell), J. Fourastié (Jean Fourastié), E. Eppler, R. Inglehart (Ronald Franklin Inglehart), A. Maslow (Abraham Maslow) and others. The works of such Russian scientists as V.A. Baburin, S.A. Bazhenov, I.V. Bestuzhev-Lada, N.A. Gorelov, O.V Glushakova, T.I. Zaslavskaya, N.V. Zubarevich, E.A. Morozova, V.V. Mikhaylov, N.M. Rimashevskaya, M.V. Udaltsova, V.A. Shabashev, L.N. Shcherbakova and others are devoted to the problematics of quality of life.

Despite the variety of points of view in the determination of essence and content of quality of life, a lot of researchers share one view that quality of life is a difficult social and economic category integrating such derivative categories as welfare, a mode of life, a lifestyle and the cost of life within itself (see O.V. Glushakova [1], E.V. Mukhacheva [26] and others).

Now the unanimity of views is generally reached in the scientific environment that quality of life is revealed through the needs of individuals and the levels of their satisfaction.

The authors of this article consider quality of life as a dynamic category and consider it to be the developed (in case of a certain level of development of social and economic system) life environment of individuals determining the degree of satisfaction of their requirements taking into account the subjective perception of their role and place in the surrounding reality. The dynamic nature of quality of life is shown in the continuous renewal (development) of life environment of individuals and society in general which is caused by the processes proceeding in the social and economic system, resulting from the movement of elements of the specified system.

The researchers treat the measurement of quality of life through the prism of an objective and a subjective component. To the objective components of quality of life the indicators are referred that characterize the state of the social, economic and ecological processes proceeding in the social and economic system of this or that hierarchical level, and the assessment is performed, as a rule, with the use of official indicators of state statistical observation (see, for example, N.V. Zubarevich [27], O.V. Glushakova, V.V. Mikhaylov [28], A.V. Mukhacheva [26] and others). Lack of the specified approach consists in ignoring the satisfaction of individuals with the quality of their life. At the same time, in a lot of countries researches aimed at the accounting of the subjective component of quality of life are performed (scientific centers of the USA, the EU, International organizations). Thus, Economist Intelligence Unit allocates objective and subjective factors as the determinants of quality of life – material welfare, health, political stability and safety, family and public life, climatic and geographical life conditions, safe work, political freedom and gender equality. At the same time, sociological surveys are performed during the assessment of quality of life to detect the satisfaction of the interviewed respondents with the quality of life [29].

In spite of the fact that quality of life is considered by a lot of researchers in interrelation with the category of the human capital, their interconditionality is understood differently by scientists.

Quality of life is considered by a lot of scientists as the integral condition of development of the human capital determining its quality. This view is held by I.V. Gruzkov, V.N. Gruzkov [30], Yu.A. Korchagin [13] and others.

However, in our opinion, it is the quality of the human capital at the individual, regional or national level that determines the quality of life. Let us rationalize this position.

Scientists tried to prove the interrelation of efficiency and labor quality with individual qualities of the person even before the emergence of the theory of human capital. Thus, J.S. Mill noted "The moral lines of workers as deeply influence the efficiency and quality of labor, as their intellectual development does" [31]. At the same time, he paid great attention to the role of family in preparation of the person for labor activity.

S.G. Strumilin considers the interrelation of the formation of skill and the quality of the performed works in his researches. He notes that "for the measurement of labor quality the skill needed for each of these degrees of qualification is such a correlative value. The worker's labor quality and skill are closely interrelated, just as the result and the reason are" [32]. The worker does not only pay back his earnings in full by means of the product of his labor, but, moreover, he also designs a product for society – an additional product increasing together with an increase in the productivity of work and the qualification of the worker [32].

Thus, scientists relate the quality of work with the individual properties of individuals, such as their skill, the moral lines of workers, etc.

The characteristic of human capital, P.A. Shvetsov notes, is its personification which is expressed in the impossibility of its transfer from individual to individual and the disproportion of gain in the course of investment [33] which emphasizes once again the diversity of quality of human capital of individuals and its uniqueness.

Let us note that the personification of the human capital predetermines the unbalance of development of its components and, therefore, it's any quality in relation to each specific individual. It also results in the differentiation of social and economic systems (the state, a region, a municipality, an industry, an organization) from the point of view of the available aggregate human capital and, therefore, various opportunities of the specified systems in providing the adequate quality of life.

The quality of human capital is directly related to the possibility of individuals to perform the enhanced reproduction which is determined by the salary level adequate to labor results, the quality of housing conditions and medical care, the opportunities (first of all, financial) for providing balanced nutrition, sports activities, the organization of good rest, travel, the level of development of mental abilities of the individual, his professional competence, etc.

The most essential value from the point of view of providing the adequate quality of life in the structure of human capital, in our opinion, just belongs to the intellectual component. Let us rationalize this position.

The researchers pay attention to the interrelation between the quality of human capital and the knowledge assets of the individual. For the individual as an active element of social and economic system the unique properties which are absent in its other elements are inherent. *First of all, it is the capability to create the cost exceeding the expenditures of its reproduction. Secondly, it is the capability to accumulate knowledge and to generate new knowledge. The higher the level of development of intellectual component in the structure of human capital of the individual the higher the capability of the individual to generate essentially new knowledge, and, therefore, his capability to produce products (works, services) with a higher percentage of added value.*

In our opinion, the education system, thanks to which there is a process of transfer to the individual and his accumulation of already existing knowledge (the processes of preschool, basic general, secondary professional and higher education) and the generation of new knowledge, plays the primary role in ensuring the enhanced reproduction of intellectual component of the human capital.

The quality of the human capital as an immanent property of each specific individual acting as an active element of social and economic system creates a quality of the aggregate human capital of the system in general. At the same time the amount of the added value is in many respects determined by the level of development of intellectual component in the structure of human capital of each specific individual which finally causes the results of functioning of social and economic systems (the state, a region, a municipality, an organization) and consequently – the amount of available resources specified by the systems, and, first of all, that of financial ones. It also results in the availability of opportunities for the satisfaction of constantly growing needs of each specific individual and all the society, and, therefore, of opportunities of improvement of quality of their life.

The aforesaid allows to formulate *a new paradigm of sustainable development of the social and economic systems "human capital – quality of life"*. Bringing the person to the foreground as the carrier of the human capital in the structure of which the most significant, according to the authors, is the intellectual component and allows to provide higher innovative, financial, investment, technical and technological, ecological and other results of functioning of social and economic systems of various hierarchical levels under the conditions of high variability of factors of internal and, in particular, the external environment, the all-round meeting of the requirements and ensuring the adequate quality of life of each specific individual and all the society.

RESULTS AND DISCUSSION

The stability and results of functioning of the agrarian sector as the components of social and economic system of the state are shown through its percentage and dynamics in the total amount of GDP. Despite the positive dynamics of results of functioning of the agrarian sector in GDP in current prices, its percentage in the structure of GDP has decreased in comparison with 2004 (–1.1%) (Table 1).

The index of agricultural production is characterized by high variability. The most essential fall of growth rates of the agrarian sector was observed in 1994 at the beginning of the transformational transition of Russia from planned economy to market relations, and also during the periods of crises of 1998 and 2009 and in 2012 against the background of Russia's accession to the WTO (Fig. 1).

Table 1. The dynamics of GDP and agrarian sector of Russia in 2004–2014, billion rubles [34]

Parameters	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
GDP of Russia, in current prices, billion rubles.	13964.3	18034.4	22492.1	27964.0	33908.8	32007.2	37687.8	45392.3	49926.1	54103.0	58745.0
Percentage of agrarian sector in GDP, billion rubles.	837.9	937.8	1102.1	1230.4	1559.8	1568.4	1620.6	2133.4	2096.9	2272.3	2819.8
Percentage of agrarian sector in GDP, %	5.9	5.2	4.9	4.4	4.6	4.9	4.3	4.7	4.2	4.2	4.8

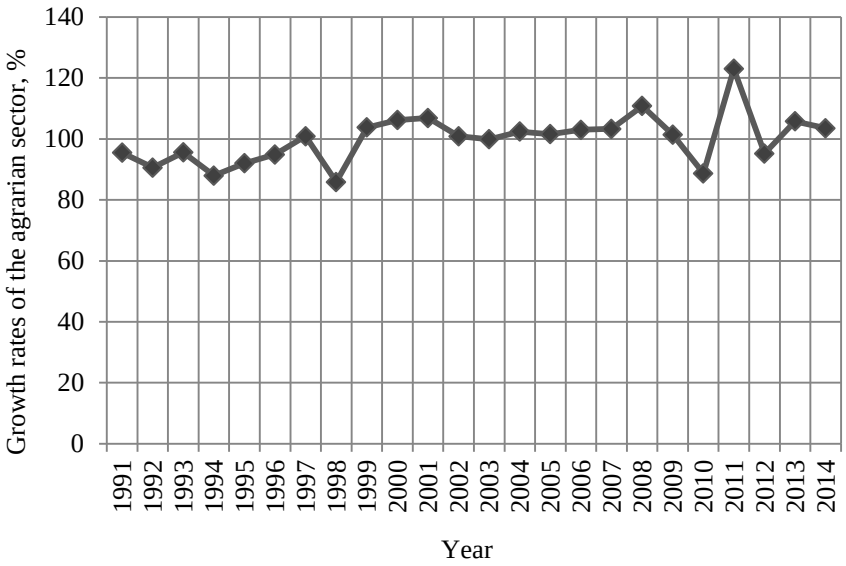

Fig. 1. Growth rates of the agrarian sector of Russia in 2005–2014 [35].

The agrarian sector plays a significant role in ensuring food security of the state, however the official statistics testifies to an essential decrease in the level of the main indicators of activity of agricultural organizations. Thus, acreages in comparison with 1992 decreased in 2015 by more than 1.9 times (108.7 million hectares and 55.1 million hectares), cattle livestock – by 4.7 times (40.2 and 8.5 million heads), agricultural production – by 1.4 times (104.1 million tons and 76.2 million tons, respectively) [36].

The sustainable development of agrarian sector under the conditions of high variability of factors of the external environment, the possibility of deployment and use of innovative technologies are in many respects determined by the level of development and the quality of human capital which finally specify the quality of life of the citizens living in rural areas. In this regard, let us consider the conditions of forming and development of human capital as the main factor of production and immanent basis of quality of life of the population living in rural areas.

There was a considerable decrease in the urban and an increase in the rural population in Russia in 1990–1992 under the conditions of transformational transition from planned economy to market relations. The similar tendency was caused by an increase in the crisis processes in the country, closing of a number of entities in urban areas, the growth of unemployment and an essential decrease in the average per capita income of the urban population. However, since 1993 the picture has been changing. There had been a sharp migratory gain of urban population and a decrease in the migratory gain of rural population up to 1996. The turning point - the excess of the number of people who moved from rural areas over the number of those who arrived to rural areas – was in 2001, and, since this period of time, the negative migratory gain of rural population continues to remain (Fig. 2).

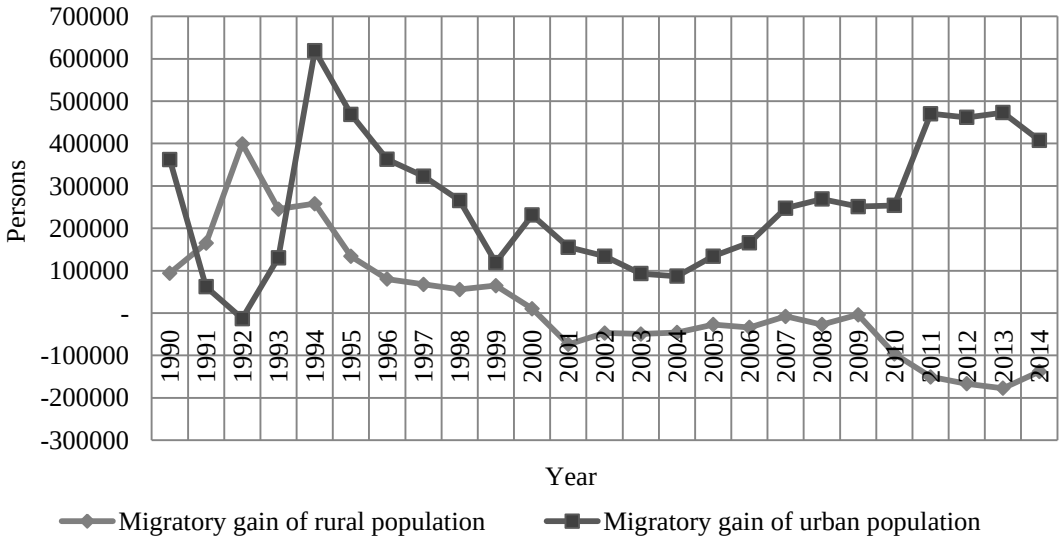

Fig. 2. Migratory gain of urban and rural population in the Russian Federation in 1990–2014 [37].

The basic reasons of migratory outflow from rural territories are the existing infrastructure problems, lack of opportunity to get quality education and medical care, low income, unemployment interfering the reproduction of human capital on a wide basis. It should be noted that already S. Kuznets paid attention to the enhancement of negative migratory processes in rural territories because of the specified factors [20].

It is obvious that migratory processes provide a change in the ratio of the number of urban and rural people. According to the official data of Rosstat, there had been a decrease in the number (-1321.16 thousand people) and the percentage (-0.8%) of rural people up to 2013 and positive dynamics (Table 2) was outlined only in 2014.

The income of rural population is the most important condition of ensuring the reproduction of human capital on a wide basis. It is obvious that the obtaining of them is provided with the availability of possibility of employment of economically active population. The main financial source for the unemployed population is pension provision. At the same time, the average duration of unemployment for the persons living in rural areas despite the tendency for decrease in comparison with 2009 was 5.5 months in 2014 (Table 3).

The salary level of agricultural workers is much lower than in other industries of national economy. Thus, in 2014 the average nominal accrued payroll in this industry was 17 724 rub which is 1.65 times lower than in processing productions and construction, 3.3 times lower than in the extracting industries and 1.4 times lower than in wholesale and retail trade [38].

The available resources of rural households are 1.6 times lower in comparison with urban households (15 802.3 rub and 25 347.5 rub in 2014, respectively). There is the similar picture of gain of savings which are 1.5 times lower in rural households than that in urban ones (Table 4). Lack of the available resources also causes a lower level of cash expenditures of rural households (in 1.71 times in 2014). The compensation of lower expense level is provided due to the self-produced food the influx amounts of which in rural households is three times higher in comparison with urban ones. However, despite the remaining essential difference in the available resources, their steady positive dynamics is noted in 2009–2014. For urban households the available resources increased by 1.83 times, for rural ones – by 1.87 times (Table 4).

Table 2. The number and ratio of urban and rural people in the Russian Federation [37]

Parameters	2005	2010	2011	2012	2013	2014	2015
Population, thousands of persons	143236	142865	143056	143347	143667	146267	146406
including							
– urban	104848.75	105434.37	105718.38	106076.78	106600.91	108237.58	108469.82
– rural	38387.25	37430.63	37337.62	37270.22	37066.09	38029.42	37936.18
Percentage of urban population, %	73.2	73.8	73.9	74.0	74.2	74.0	74.1
Percentage of rural population, %	26.8	26.2	26.1	26.0	25.8	26.0	25.9

Table 3. Distribution of the unemployed persons living in rural areas according to unemployment duration [38]

Parameters	2009	2010	2011	2012	2013	2014
Number of unemployed persons living in rural areas, thousands of persons	846.6	699.4	867.0	493.9	424.9	382.2
including that with the following period of unemployment: no more than 1 month	121.1	94.5	75.7	61.5	56.1	56.1
from 1 to 4 months	284.4	244.5	201.5	187.7	153.1	145.6
from 4 to 8 months	186.3	155.9	121.4	113.5	91.5	82.0
from 8 to 12 months	127.4	97.5	80.8	86.4	62.6	48.4
12 months and more	126.3	106.9	87.6	64.8	81.7	50.1
Average period of unemployment, months	6.0	6.0	6.0	5.7	5.9	5.5

Table 4. Structure of the available resources of urban and rural households per month, rub [38]

Parameters	2009	2010	2011	2012	2013	2014
Households living in rural areas						
Total of available resources	8416.9	10128.7	11745.8	13320.3	14191.7	15802.3
including:						
– cash expenditures	6639.0	8079.0	9424.8	10733.7	11383.7	12693.1
– cost of natural receipts of foodstuffs	780.4	840.9	886.4	912.1	944.1	974.7
– cost of natural receipts of non-foods and services	65.4	77.2	95.0	103.7	99.6	106.8
– increase in savings	932.1	1130.9	1339.5	1570.8	1764.2	2027.7
Households living in urban areas						
Total of available resources	13869.4	16265.0	18291.1	20405.0	23645.0	25347.5
including:						
– cash expenditures	12222.3	14357.5	16180.5	17908.5	20431.5	21788.8
– cost of natural receipts of foodstuffs	233.5	270.7	293.7	293.2	302.5	322.3
– cost of natural receipts of non-foods and services	105.1	114.1	153.7	159.8	165.9	164.3
– increase in savings	1308.5	1522.7	1663.3	2043.5	2745.1	3072.0

In the Russian Federation about 27.7% of the total number of pensioners live in rural areas (as of 12/31/2014) [38, p. 144]. It should be noted that the level of their granted pensions is much lower than this indicator for urban pensioners which is caused by lower income of the rural population in the period of labor activity (Table 5). The amount of pension provision of the average pensioner both in urban and rural areas does not allow to provide not only a worthy life for persons of pension age, but also the reproduction of human capital on a wide basis as, contrary, for example, to Switzerland where the coefficient of replacement of the lost income by pension is about 80%, in Russia pension does not provide the insurance function of loss of earnings (The long-term development strategy of the pension system of the Russian Federation: Approved by the Decree of the RF Government of December 25, 2012 no. 2524-r.). The current situation is related to the permanent deficiency of the Pension Fund of the Russian Federation and the need of its constant "feed" at the expense of transfers from the federal budget and the budgets of constituent entities of the Russian Federation, the aging of the population, the availability of practice of payment of illegal salaries to personnel for the purpose to avoid paying taxes for the budgets of the budget system of the Russian Federation and for state non-budgetary funds, the high tax load on persons occupied in economy (Table 5).

The low level of available resources determines also the amount of expenses for final consumption – the acquisition of foodstuffs, non-foods, the payment of various services, etc. In 2009–2014 the expenses for final consumption in rural households were much lower in comparison with urban ones through all the expense groups. In 2009 the difference was 1.67 times, in 2014 there was an insignificant decrease in the gap – 1.57 times. In rural households the expenses for food are on average 1.2 times lower than that in urban ones. The most essential gap in the expenses for the acquisition of non-foods was in the crisis year of 2009 – by 1.8 times, it was the minimum in 2012 – by 1.53 times. Of note is the growth of expenses for the consumption of alcoholic beverages both in urban and rural areas (by 1.76 times in 2014 in comparison with 2009) which provides cardiovascular diseases, a decrease in reproductive health, etc., and, thus, has a negative effect on the reproduction and quality of human capital. Let us note, however, that, despite the total growth of expenses for the acquisition of alcoholic beverages, this indicator in rural households is on average 1.6 times lower than that in urban ones (Table 6).

The analysis of consumption, nutrition and energy value of foodstuffs of households allows to draw a conclusion that the nutritious diet is more balanced in urban households. In rural households, due to the insufficiency of available resources, the consumption of bread products, potatoes, sugar and confectionery prevails while the consumption of fruit and berries, meat and meat products, milk and dairy products and eggs prevails in urban households. For this reason the energy value of consumed products in urban households is lower than the similar indicator of households of rural areas (2545 and 2766 kcal a day in 2014, respectively) (Table 7).

Table 5. Some indicators characterizing the state of the pension system of the Russian Federation

Parameters	2010	2011	2012	2013	2014
Income/expenses of the budget of the Pension Fund of the Russian Federation, billion rubles *	4610.1	5255.6	5890.4	6388.4	6075.5
	4249.2	4922.1	5451.2	6378.5	6190.1
Deficit (–), surplus (+), billion rubles *	360.9	333.5	439.2	9.9	-114.6
Transfers to the budget of the Pension fund:					
– from the federal budget, billion rubles *	2643.8	2379.8	2815.6	2843.2	2410.2
– from the budgets of constituent entities of the Russian Federation, billion rubles *	4.6	4.4	3.9	3.4	2.8
Number of pensioners in the Russian Federation, thousands of persons [38]	39090	39706	40162	40573	41019
Percentage of pensioners living:					
in rural areas, % [calculated by the author regarding Reference 38]	30.04	29.65	29.49	29.10	28.93
in urban areas, % [calculated by the author regarding Reference 38]	69.96	70.35	70.51	70.90	71.07
Number of persons employed in economy per one pensioner, persons [38]	1.63	1.61	1.60	1.58	1.56
Average size of granted pensions of pensioners living:					
– in rural areas, rub [38]	5609.5	6885.1	7436.7	8243.4	9008.1
– in urban areas, rub [38]	6421.4	7892.6	8622.4	9527.3	10445.5

Note. * – Laws on implementation of the budget of the Pension Fund of the Russian Federation: for 2010 dated 10/6/2011 No. 268-FZ; for 2011 dated 10/2/2012 No. 152-FZ; for 2012 dated 9/30/2013 No. 255-FZ; for 2013 dated 10/14/2014 No. 298-FZ; for 2014 dated 10/5/2015 No. 279-FZ.

Table 6. The structure of expenses for the final consumption of urban and rural households (on average per one member of household per month, rub) [38]

Parameters	2009	2010	2011	2012	2013	2014
Households living in rural areas						
Expenses for the total final consumption	6074.8	7256.8	8156.6	9305.4	9739.5	10611.9
including						
– expenses for food	2880.3	3222.7	3579.3	3842.8	4073.8	4457.0
– expenses on non-foods	2054.7	2609.3	2972.1	3700.2	3737.2	3967.0
– expenses on alcoholic beverages	101.1	118.7	131.7	150.8	157.5	178.0
– fees	1035.1	1295.0	1464.6	1604.8	1765.0	2001.1
– cost of the services rendered by the employer free of charge or at preferential prices	3.6	11.1	9.1	6.9	6.1	8.8
Households living in urban areas						
Expenses for the total final consumption	10133.4	11693.3	12957.7	14369.0	15695.0	16648.4
including						
– expenses for food	3419.6	3892.9	4252.3	4559.5	4911.8	5337.8
– expenses on non-foods	3733.9	4376.4	4958.3	5691.5	6250.9	6516.4
– expenses on alcoholic beverages	162.6	191.4	215.3	238.1	259.7	285.5
– fees	2798.0	3216.8	3515.3	3861.1	4249.0	4489.6
– cost of the services rendered by the employer free of charge or at preferential prices	19.3	15.8	16.6	18.8	23.7	19.0

Table 7. Consumption, nutrition and energy value and cost of foodstuffs in the households of urban and rural areas [38]

Parameters	2009	2010	2011	2012	2013	2014
Households living in rural areas						
Consumption of staple foodstuffs, kg per year:						
– breadproducts	121	122	117	116	113	112
– potatoes	80	76	72	75	73	70
– vegetables and melons	95	97	97	100	98	98
– fruit and berries	52	60	59	62	65	65
– meat and meat products	67	71	75	76	78	79
– milk and dairy products	243	245	245	249	249	249
– eggs, pcs	205	207	209	212	207	209
– fish and fish products	20	21	21	22	22	22
– sugar and confectionery	35	36	35	35	34	35
– vegetable oil and other fats	12	12	12	12	12	12
Nutrition value, g per day						
– proteins	76	78	78	79	79	79
– fats	99	103	104	104	105	105
– carbohydrates	390	395	382	382	375	373
Caloric value, kcal per day	2767	2831	2794	2792	2766	2766
Households living in urban areas						
Consumption of staple foodstuffs, kg per year:						
– bread products	91	94	92	92	90	89
– potatoes	62	63	60	60	56	55
– vegetables and melons	95	96	98	100	96	98
– fruit and berries	68	74	75	79	81	80
– meat and meat products	76	82	83	85	87	87
– milk and dairy products	261	269	269	274	278	271
– eggs, pcs	213	226	220	223	221	218
– fish and fish products	21	21	21	22	22	22
– sugar and confectionery	30	31	31	31	31	30
– vegetable oil and other fats	11	11	11	11	11	11
Nutrition value, g per day						
– proteins	73	76	76	77	78	77
– fats	99	105	105	106	107	105
– carbohydrates	319	331	326	326	323	319
Caloric value, kcal per day	2472	2587	2563	2577	2577	2545

Ensuring the enhanced reproduction of human capital from the point of view of the organization of leisure and rest, education, etc. implies the availability of comfortable housing conditions. However, in spite of the fact that the floor area per inhabitant in rural areas is larger than that in urban areas, the indicators of improvement of housing stock is at a low level and requires its improvement (Table 8).

The performed surveys of the citizens living in rural areas for the assessment of satisfaction with the quality of life conditions allowed to reveal that 16.4% of the respondents note lack of heat, 15.5% – excess humidity and dampness, 8.8% – lack of sunlight, 13.1% – problems because of a bad noise isolation. In general, only 5.3% of the respondents estimated the quality of their life conditions as "excellent", 41.3% – as "good", 46.2% – as satisfactory, 7.4% – as "bad" and 0.9% – as "very bad" [38]. According to the data of selective inspections of budgets only 24.9% of rural households are going to purchase other housing (or to exchange for other housing), 13.9% stand in a queue for the improvement of housing conditions, 4.2% expect to inherit housing, 50.3% build a new house or an extension to the existing house [38].

The availability and quality of medical services is one of the conditions of ensuring the reproduction of human capital on a wide basis and the formation of possibility of future earnings. The network of out-patient and polyclinic organizations in rural areas develops generally at positive rates though the dynamics of their input in action is very volatile. On commissioning of the capacities of hospital organizations mainly negative growth rates (Table 9) are noted. It is not possible to receive the highly technological types of medical care as well as the consultations of highly qualified specialists in rural territories which means that it is rather difficult to provide the adequate reproduction of such a component of human capital as health. This fact confirms the high level of invalidization of persons living in rural areas which was 1.18 times higher in 2014 than the similar indicator for the urban population [38]. The higher level of invalidization is also provided by the remaining severe conditions of work of rural people. The results of research of working conditions in rural areas showed that 13.2% of the respondents consider their working conditions as arduous, 55.4% – as moderately arduous, and only 26% of the respondents consider their working conditions as easy [38].

Table 8. Providing with housing and the improvement of housing stock in urban and rural areas [38]

Parameters	2009	2010	2011	2012	2013	2014
Households living in rural areas						
Total area of the premises on average per one inhabitant, sq.m.	23.4	24.0	24.5	24.8	24.7	25.0
Housing quality improvement – the percentage of the total area equipped with:						
– a watersupply system	47	48	49	49	52	54
– wastewater disposal (a sewerage system)	38	39	39	40	41	43
– a heating system	59	60	61	61	64	65
– baths (showers)	28	29	29	29	31	33
– a gas system (a heating network)	74	75	74	74	73	73
– hot water supply	25	25	26	27	28	30
– floor electric stoves	3	4	4	4	5	6
– a water supply system, water disposal (a sewerage system), a heating system, hot water supply, a gas system or floor electric stoves together	23	24	25	25	26	28
Households living in urban areas						
Total area of the premises on average per one inhabitant, sq.m.	21.8	22.1	22.5	22.9	22.9	23.3
Housing quality improvement – the percentage of the total area equipped with:						
– a watersupply system	89	89	90	90	90	86
– wastewater disposal (a sewerage system)	87	87	88	88	88	84
– a heating system	92	92	92	92	92	88
– baths (showers)	81	81	81	81	82	78
– a gas system (a heating network)	67	67	67	66	65	62
– hot water supply	80	80	80	80	81	77
– floor electric stoves	24	25	25	26	26	25
– a water supply system, water disposal (a sewerage system), a heating system, hot water supply, a gas system or floor electric stoves together	77	77	78	77	78	74

Table 9. Commissioning of health care capacities in rural areas [38]

Parameters	2009	2010	2011	2012	2013	2014
Commissioning of hospital organizations, beds	1219	886	1625	1120	394	911
Growth rate, %	–	72.68	183.4	68.92	35.18	2.31
Commissioning of out-patient and polyclinic organizations, visits per shift	3305	2587	5896	5971	5121	8429
Growth rate, %	–	78.27	227.91	101.27	85.76	164.59

The formation of intellectual component of human capital begins from the first days of life of the individual. The essential role in this process belongs to the education system. G. Psacharopoulos and H.A. Patrinos pay attention to the enhancement of importance of education in the developed countries which is testified to by the its acceptance as one of the key indicators of development in the countries of OECD according to annual reviews of "Formation" of the Glance series and other program documents of OECD. Besides, the governments recently began to finance the researches devoted to the assessment of macroeconomic effects of investments in education [39].

According to official statistics, the number of organizations which perform educational activities according to preschool educational programs, supervision and care of children in rural areas was 23.8 thousand as of the end of 2014 (by 12.17% less than in urban areas). However, in spite of the fact that the number of pupils in the specified organizations is considerably lower (about 20.51% of their total number), the problem of provision with places for children in the specified organizations remains. The number of places per 1000 children aged from 1 up to 6 in rural areas is less by 167 than that in urban areas (660 and 493 places, respectively). The coverage of children with preschool education is much lower, as well. Thus, in urban areas and urban-type settlements this indicator is about 72.1%, and in rural areas it is only 46.1%) [38].

Of note is also the fact that if the commissioning of capacities of preschool educational organizations is characterized by steady positive dynamics, then for general education organizations this indicator is quite volatile and in 2012 it was at the lowest level (Table 10). The realization of model of continuous professional education in rural areas is not possible due to lack of educational organizations of higher education. In this regard, there is a migration of youth to urban areas after the completion of education, however young specialists, as a rule, do not come back to rural areas which is caused by the existence of a number of infrastructure and other problems described above. For this reason the percentage of qualified specialists in rural areas remains extremely low. In 2014 this indicator was 3.2% of total of skilled workers of all branches – 71 539 thousand persons.

The insufficient level of available resources of households of rural territories also acts as a restraining factor of forming and development of human capital as the average consumer prices of educational services in the education system grew on average by 1.6 times in comparison with 2009. The maximum increase in prices is fixed in relation to such a type of service as education in general educational institutions of secondary professional education (by 1.8 times), the minimum – in the courses of professional education (by 1.4 times) (Table 11).

The formation of cultural component of human capital in rural areas is provided by means of public libraries and organizations of cultural and leisure type. The professional theaters the number of which, according to the Ministry of Culture of the Russian Federation, was 661 by the end of 2014 (+60 in comparison with 2009), and museums – 2731 (+192 in comparison with 2009) are located generally in large cities which limits the possibility of visiting them by villagers. As for public libraries and the library stock, these indicators are characterized by steady negative dynamics. The same situation with regard to the number of library copies per 1000 people of the rural population had been noted till 2013, inclusively (Table 12).

Table 10. Commissioning of health care capacities in rural areas, thousands of places [38]

Parameter	2009	2010	2011	2012	2013	2014
Preschool educational organizations, thousands of places	1.7	3.5	6.4	6.8	15.6	26.3
General education organizations, thousands of pupils' places	23.5	20.2	21.9	14.4	24.2	16.8

Table 11. Average consumer prices of certain services in the education system, by the end of the period, rub [38]

Parameter	2009	2010	2011	2012	2013	2014
Visit to a children's nursery and kindergarden, per day	53.44	54.86	56.97	61.66	67.30	76.56
Education in non-state general education organizations, per month	8134.97	9420.77	10002.34	11431.92	12008.43	14037.35
Education in secondary professional education institutions, per semester	13148.96	13981.86	16616.96	17639.59	19943.13	23731.83
Education in non-state institutions of higher education, per semester	22389.11	22983.61	24793.75	27358.44	28840.34	33030.85
Education in state and municipal institutions of higher education, per semester	24556.03	25520.38	28211.22	35273.32	38813.35	42331.74
Classes within the courses of foreign languages, per class period	135.80	148.21	181.84	206.34	221.36	236.85
Classes within the vocational training courses, per class period	76.99	82.21	79.70	90.33	97.26	107.07

Table 12. Some indicators characterizing the development of cultural institutions in rural areas [38]

Parameters	2009	2010	2011	2012	2013	2014
Total of libraries, thousands	46.7	46.1	43.2	40.8	39.8	40.1
including that in rural areas, thousands	36.2	35.8	33.2	31.1	30.3	30.6
Library stock, millions of copies	934	923	888	864	851	854
including that in rural areas, millions of copies	330	324	300	281	274	282
Number of copies of library stock on average per 1000 persons of the population in rural areas	8739	8660	8034	7554	7382	7429

An important role in ensuring the reproduction of human capital is played by ensuring information availability. Providing access to the INTERNET and the availability of the ICT equipment necessary for these purposes in households is quite essential under the conditions of formation of information society and transition to essentially new ways of obtaining information and knowledge in Russia.

However, according to the official data of Rosstat, only 60.8% of households have access to the Internet, and 62.8% – to ICT equipment in rural areas [38].

It is obvious that the specified problems stimulate the migratory outflow from rural territories. In particular it concerns the young specialists for whom both the possibilities of future earnings and infrastructural satisfaction are unsatisfactory.

The performed analysis allows to draw a conclusion that, despite the positive dynamics, there are still some essential gaps concerning a number of the indicators reflecting the conditions of development of human capital of urban and rural population. It follows from this that the problem of providing the quality of life for the rural population still remains open and requires its solution.

In Russia there is an active formation of the corresponding institutional environment now, first of all, legislative and legal in nature, focused on the solution of problems of sustainable development of rural territories.

Thus, in July, 2013 Resolution of the Government of the Russian Federation No. 598 approved the federal target program "Sustainable Development of Rural Territories for 2014–2017 and for the Period till 2020" as a logical continuation of provisions of the Concept of Sustainable Development of Rural Territories and the Food Security Doctrine of the Russian Federation accepted in 2010. This program provides the recommendations about the adoption of similar programs by the entities of the Russian Federation. Total funding of the program is 252 589.6 million rubles (in the prices of the corresponding years), including: federal funds – 139 206.5 million rubles (55%); consolidated budget resources of the entities of the Russian Federation – 74 562.7 million rubles (30%); non-budget resources – 38 820.4 million rubles (15%).

In the Strategy of Sustainable Development of Rural Areas of the Russian Federation for the period till 2030 (approved by Resolution of the Government of the Russian Federation dated 2/2/2015 No. 151-r) (hereinafter the Strategy) the basic provisions embodied in the Concept of Sustainable Development of Rural Territories and the Food Security Doctrine of the Russian Federation were also further developed. Lack of the adequate state support of development of agrarian sector which is much higher in the developed countries is noted as one of the main problems in the Strategy. This circumstance constrains the modernization and innovative development of the industry, has a negative effect on the payroll rate and the forming of tax base of the local budgets. The shift of accents in the goal-setting of development of the major infrastructure of health care and education in favor of cost efficiency, but not of ensuring access of the population to the major social services, in fact, provides the violation of implementation of constitutional rights of rural people for education and medical care. It is noted in the Strategy that the solution of the existing infrastructure problems in rural areas, in particular, that of the development of road network and modern means of communication, proceeds at such a rate that does not allow to overcome in the nearest future the existing spatial and communication gap between urban and rural areas.

Among the purposes of state policy formulated in the Strategy and oriented to provide the sustainable development of rural territories there is employment, the increase in the level and quality of life of rural population taking into account modern requirements and standards. The instrument of control of achievement of stated purposes is the system of the indicators allowing to estimate finally the efficiency of strategy implementation. These include the achievement of stabilization of rural population at the level of 35 million people by 2030; the increase in life expectancy of rural population up to 75.6 years; the decrease in the migratory outflow of rural population up to 74.1 thousand people; providing the annual average rate of increase in agricultural production in the amount of 5.5 percent, etc. However nothing is stipulated in the Strategy about the need of development of human capital.

The existing budget restrictions the enhancement of which occured against the background of the imposition of economic sanctions from the USA and the European Union countries, and also of the fall of the prices of energy resources in foreign markets create risks of non-achievement of the indicators-purposes stated in the Strategy. This circumstance is caused by the fact that state and federal target programs as the instrument of achievement of strategic objectives of development provide several sources of financing. In particular, more than half of the total of financial

resources for the purpose of implementation of the federal target program "Sustainable Development of Rural Territories for 2014–2017 and for the Period till 2020" is provided at the expense of the federal budget which is essentially (about 50% of total of the income of the federal budget) dependent on oil and gas income.

The authors of the article are convinced that social and economic systems of all levels of hierarchy taking into account the limitation of all types of resources, shall have the internal sources of development allowing to provide the satisfaction of resource requirements of the system, including the financial ones which is just embodied in a new paradigm of sustainable development of social and economic systems – "human capital – quality of life".

The closer attention from the state to the processes of science and education will allow to provide development in the structure of human capital of the intellectual component and to increase the quality of human capital both at the level of a certain individual, and at the level of the state, region, municipality and industry which just predetermines the new opportunities of social and economic development of territories due to the activation of process of generation of new knowledge, the creation of innovative products with a high percent of added value, and, therefore, the solutions of the problems of reducing gaps under the conditions of development of human capital of the urban and rural population as an immanent basis of quality of life of each person and all the society.

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FACTOR ANALYSIS AND GROWTH PROSPECTS OF POTABLE WATER LOCAL MARKET

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Abstract: Currently, the clean potable water is globally the restricted economic benefit. In highly urbanized and environmentally unfavorable regions, including the Kemerovo region, development of food plants to fill drinking water is the most promising way to solve the problem of potable water availability. Factors and conditions of the drinking water market formation are studied by integral evaluation of drinking water availability in all municipal districts of the region, using the criteria of availability in terms of geographic location, management, technological process, economic value and quality. The volume of supply of bottled drinking water is also analyzed in view of its availability. As a result, the data on the level of availability of drinking water is first obtained for residents of all municipal districts of the Kemerovo region, on the potential of the population to pay for the pure water delivery and on prospects to expand the bottled water production market. The most population was identified to live in conditions with low technological, economic and environmental access to drinking water. The residents of big and medium-sized cities live in conditions of low environmental availability and high potential to pay for the drinking water delivery. The residents of peripheral municipalities live in conditions with low access to potable water due to management, technology and economic restriction but within the high geographic availability. Thus, the analysis of the drinking water availability and volume of its production suggest the possibility of the local market considerable capacity and its growth in future.

Keywords: drinking water availability, bottled water, local market, potential to pay, market capacity

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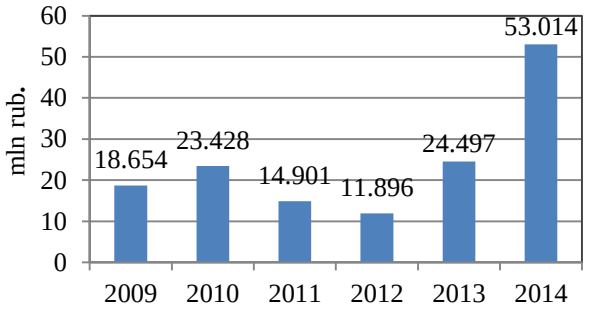
INTRODUCTION

Bottled water market formation is quite new for the Russian economics. Previously, it was common to assume that the country with the largest fresh water reserves cannot be short in drinking water. However, the geographic access of fresh water does not absolutely mean that the population has permanent supply of drinking water. In Russia and in the other world, the problem of clean drinking water supply is due to extremely irregular distribution and the extent of considerable water source contamination. Drinking water availability and supply mechanisms have long been the global public problem under discussion at all major global forums for the last 20 years: at the Environment and Development Conference in Rio de Janeiro in 1992, at the United Nations General Assembly session "Rio+5" (1997) dedicated to outcomes of Rio conference resolutions, in the Millenium Summit (2000), in the The World Summit on Sustainable Development "Рио+10" Johannesburg in 2002, in Rio de Janeiro in 2012, etc. The global water supply problem caused events performed every three years, namely: World Water Forum in 1997 in Marrakech, in the Hague in 2000, in Kyoto, Osaka, Shiga in 2003, in Mexico City in 2006, in Istanbul in 2009, in Marcel in 2012, in Korea in 2015. 40–50 years ago, on initial stages, the discussion mainly focused on irregular distribution of freshwater sources, that was followed by the priority of water source contamination. In the last 20–30 years only, including forums held by major international organizations, the problem to create various mechanisms to ensure free access to safe drinking water [1] became the top topic. The International Water Management Institute established in Europe introduced the strategy to supply clean drinking water around the world until 2025 using a variety of organizational and economic mechanisms and technologies [2]. Since then, the problem of drinking water availability has become the the domain of interests of economists, lawyers and policy makers.

Low accessibility of drinking water does not result from the source limits. This tells on the market "failure" to properly manage the water sector and use the shared resources. The peculiarity of drinking water as the irreplaceable product of high priority, unlike other food products, is that any country is held liable to ensure free access to it for the entire population. Since the market "failure" is caused by ineffective management of water resources by a country, the latter should be, thus, liable to ensure availability of drinking water for the population. The foreign and Russian practices of drinking water supply management were

analyzed to identify that three mechanisms are used to ensure water availability, that is, the administrative, economic and market mechanisms. The purpose of this policy is to achieve the high level of quality water supply for different segments of population by creating the effective institutional policy to apply and timely improve regulatory mechanisms. However, for Russia, it is difficult to address the issue of drinking water access for different population segments against the low level of of institutional environment and economic activity players. Russia and most countries are known to apply the administrative management policy to ensure drinking water access since it defines water pricing conditions, cost recovery and internalisation of environmental and resource costs. Developed countries proceeded to take measures for free access to pure water for the entire population by increasing the number of water-supply networks and their partial transfer for private ownership. However, it was found to be quite expensive to address the problem of drinking water access even for the leading European and Asian countries. The fact is that the clean water supplied to residential houses is not only used for drinking but also for domestic needs and it results in significant increases in the size of utility bills. Therefore, there was a need to offer options to the population to meet their demand in potable water, based on their willingness to pay for this economic wealth while improving their life quality [3, 4]. Application of economic and market mechanism convergence resulted in the potential to expand the drinking water availability in some regions of Russia, including the Kemerovo region, based on the public and private partnership. When placing the government order to supply drinking water to health and education facilities, the government encourages the development of bottled water production which drastically changes the population attitude to this product and improves the quality of life. The public-private partnership in the production of drinking water was first practiced in Russia as part of the Federal target program "Clean Water". The Kemerovo region is still a member of this program. It successfully implemented a range of projects for construction of plants for drinking water bottling whereas stimulating the local market formation and changing the behavior pattern of bottled water consumers. The experience to improve the water is successful for the Kemerovo region, either, through events as part of sub-program “Use of water resources” and “Development of Water Management Complex” of the state program “Ecology and Natural Resources of Kuzbass” for the Kemerovo region in the 2014–2017s. The funding of such events is growing from year to year which results in the direct impact on improvement of drinking water access (Fig. 1).

This is indirectly proved by the upward trend of main water resource ratio for the last 10 years (Table 1).



Source. Statistical Digest "Kuzbass Environment" of the Federal Statistics Service Department. Kemerovo, 2009–2015.

Fig. 1. Scope of finance of events to use and protect water bodies within the framework of the long-term governmental program “Ecology and Natural Resources of Kuzbass”.

Table 1. Ain water resource ratio for the Kemerovo region (1995–2014)

No.	Parameter	1995	2014	Changes for 20 years
1	Number of utility and drinking water supply lines	788	825	increase for 37
2	Share of water samples from the distribution network that do not meet requirements in terms of health and chenical parameters as per microbiological values	 16.7 21.9	 8.6 2.3	 decrease twice as less 9.5 times reduction
3	Fresh water intake from natural water bodies (mln m^3)	2624	2047	reduction for 577 mln m^3
4	Water intake from underground water bodies (mln m^3)	566	427.01	reduction for 139 mln m^3
5	Fresh water used (mln m^3) for: household and domestic needs industrial needs	2155 278 1705	1726.37 210.04 1449.9	reduction for 429 mln m^3 reduction for 68 mln m^3 reduction for 255 mln m^3
6	Waste water drained to water bodies (mln $м^3$): contaminated waste water purified as per standard partially clean (without purification)	2178 839 132 1207	1703.97 588.1 109.52 1116.19	reduction for 474 mln m^3 1.4 times reduction 1.2 times reduction reduction for 91 mln m^3
7	Losses during water transportation (mln m^3)	67	47.47	1.4 times reduction
8	Water flow in recycling water supply, water recycling and consistent water supply (mln m^3)	4983	4765.56	reduction in 217 mln m^3
9	Capacity of treatment plants (mln m^3)	1814	1044.37	1.7 times reduction
10	Wear-out of basic production assets, %	43	59	increase for 16%
11	Specific weight of water lines need to be replaced, %	45	47	insignificant 2% increase

Source. Governmental reports on “Environment protection in Kemerovo region”, 1995–2015.

The data supplied was reviewed to prove the effectiveness of the administrative policy to improve the quality and availability of drinking water.

As the economic wealth and the functional food product, water is complicated to exclude from everyday life and its competitive nature of its use is known, so the water should not be in private ownership. This means that the access to water cannot be limited. However, the water consumers are quite competetive. Not authorized consumers are willing to pay for the access to this wealth, thus creating the industry demand. Therefore, the concept that the water is a market commodity has been practiced for the last decade. Bottled water production has been acknowledged as one of the most reliable and fastest ways to meet the population needs in drinking water. Delivery of bottled water home or work places (Home & Office Delivery) has become one of self-sustained and actively developing segment of the services market. Private multinational companies in the field of botlled drinking water sale entered the market, like Perier, Evian, BonAqua (Coca-Cola Company), Aqua Minerale (Pepsi Bottling Group), etc. As a consequence, the entire economic sector emerged to produce the relevant equipment (filters, coolers, bottling lines and packaging of water, etc.).

The Russian market of bottled water is one of the extended and progressing one. In Europe, the bottled water consumption is 110 to 150 liters per year, while in Russia it is 30–40 liters. In view of that, the potential to increase the capacity of this market is quite high. Use of coolers in offices and the public urge to healthy lifestyle contribute to the above. The current situation resulted in that the purchase of mineral and drinking water for many Russians has turned to be a daily item of expenditure. The market of drinking water is represented by both national and international manufacturers. The regional product, although offered in the low price segment, significantly competes for the recent years with imported goods. However, local producers highly compete between each other whereas the average price segment goods are produced by larger market players. The share of imported products remains stable at 10–12% and is mainly represented by premium class goods. [1] Currently, plants in Russia tend to extend and upgrade by buying-up small regional plants by larger ones. The production concentrated region by region is reported. North Caucasus, Southern, Far Eastern and Siberian federal districts produce a total of about 75% of waster in Russia. Every year, more than 11 000 million liters of bottled mineral and drinking water is produced in Russia with the increase of up to 16–20% per year. In view of such prospects, the Russian market may become one of the most intensive markets in the world [5]. The global nature of the issue to supply pure water to population involves the development of local mechanisms to address the problem. In this view, this study seeks to analyze and assess the market prospects of drinking water in the industrial region in terms of drinking water access as per geographic, organizational, technological, economic and qualitative criteria, as well as the willingness by the population to pay for the quality improvement and level of availability through creating the local plant market and water delivery services.

OBJECTS AND METHODS OF STUDY

The local market of bottled drinking water is determined as the object of study in the Kemerovo region, the old industrial area with the high anthropogenic load on water bodies and relatively low population income. To clarify the term "study object", the definition of the bottled water should be explained. The water is considered bottled if it meets state standards, hygienic requirements to drinking water, is packed in the hygienic tare and is sold for human consumption. However, it should not contain artificial sweeteners or additives; flavors, essences and extracts of natural origin may be added to the bottled water in the amount not exceeding one weight percent. In case of using a greater percentage of additives, the water is considered as the soft drink. This is how the International Bottled Water Association (IBWA) defines the bottled water. The bottled water is classified for water for personal and household use, and it falls into three categories, either, as follow: ineral, artificial and potable. This study focuses on the bottled drinking water that is classified for two groups: first category drinking water (table water) and high category drinking water.

The market of the Kemerovo region mostly offers the natural water of the first category. As per WHO international standard requirements, the natural water should be bottled in containers directly from the well. in this case the bottles should be labeled with "Water from the artesian well" mark. The water category should be also considered. Drinking water of the first category may be developed from any source, but the drinking water of the highest category, which is in scarce at our market, may be only the artesian or spring water that complies with specified requirements. Water of both categories is safe to drink. They only differ in that the first category water should have the maximum permissible concentration of trace elements, whereas the requirements to the highest category water are strict – the standard defines the best concentration of substances in it. This is not of great significance for the consumer. This is rather the matter of prestige for the manufacturer and the so-called corporate competition. The highest category water should contain the certain amount of iodine and fluorine.

The Kemerovo region is known for the high urbanization which is 90% with the dense population and intense industries. As a result, more than 70% of the population was in the "high risk zone" associated with low access to drinking water due to the high level of technogenesis in water catchment areas of water bodies with the most population communities along them. When monitoring the drinking water market, it is important to consider specifics of human distribution on the territory of the region. As per the population pooling, three groups of areas may be emphasized:

[1] Investigation of the Russian market of drinking and mineral waters. Group of Companies Step by Step - http://www.step-by-step.ru/example11/swsw.pdf.

– Areas with dense population primarily engaged in industrial production (Kemerovovskiy, Prokopyevskiy, Leninsk-Kuznetskiy, Belovskiy, Novokuznetskiy, Yurginskiy regions);
– Areas with the average density of population that tends to industrial production employment (Yayskiy, Guryevskiy, Mezhdurechenskiy, Mariinskiy, Promyshlenniy, Yashkinskiy, Topkinskiy regions);
– Areas with low population density, tending to agricultural production (Chebulinskiy, Izhmorskiy, Krapivinskiy, Tisulskiy, Tyazhinskiy regions).

As per the data by the Federal Public Service for Supervision of Consumer Rights Protection and Human Welfare for the Kemerovo region, the share of population supplied with safe drinking water in urban districts was 87.2% in 2014 (81.4% in 2013), in rural areas – 28.7% in 2014. In general, the water quality as per the water pollution index for the Kemerovo region is as follows:
– Tom' River Basin – "contaminated-dirty";
– Inya River Basin – "dirty";
– Chulym River Basin – "highly contaminated";
– Chumysh River Basin – "dirty".

Another equally important factor driving this market development is the high level of morbidity due to the use of poor-quality drinking water. The population of urban and rural areas of all ages suffer from kidney diseases. [6] Earlier, the macro-economic assessment studies revealed significant economics losses due to the environment-induced morbidity in the Kemerovo region. Such losses amount to 7% to 10% of the Gross Regional Product cost. For regions similar to the Kemerovo region, the drinking water quality is one of life quality and sustainable development evidence [7, 8]. The state of water resources and high morbidity due to poor water quality determine the demand for clean drinking water.

In this view, it is also important to assess the state of water infrastructure that directly affects the quality of water and creation of the demand for drinking water. The location of the technological water supply infrastructure in the cross-sectional area of the Kemerovo region is uneven: higher density in urban districts and smaller – in municipal areas, which leads to differentiation in improvement of housing with supply services. The housing industry insufficiently provided with water supply services in terms of municipalities also indicates on the low level of access to drinking water. In relatively prosperous situation in case of water supply mains and the networks capacity, the insufficient number of wastewater treatment plants is reported in Belovskiy, Izhmorskiy, Kemerovskiy, Tisulskiy, Topkinskiy, Tyazhinskiy, Chebulinskiy and Yurginskiy areas. Berezovskiy, Kaltanskiy, Polysaevskiy, Taiginskiy municipal districts do not have the centralized water treatment facilities; in most municipal areas the water pass level through treatment plants does not exceed the average regional level (57.8%). The average rate of water treatment for urban districts amounts to 87%. Depreciation of the fixed capital stock of water supply system for the Kemerovo region is 59%. The percentage of the water supply system extension required to be replaced makes 47% in the total length of water supply networks average for the region. Financial needs in repair of water supply networks are estimated at over 10.5 billion rubles. High level of water body contamination in the Kemerovo region and lack of hydroeconomic infrastructure have been the very cause to open first plants in the region to manufacture bottled water and to render delivery services about twenty years ago.

Low population incomes and willingness to pay for the higher quality water were factors of equal significance that alternatively restricted the market of drinking water development in the Kemerovo region. It should be emphasized that the wage remains the main source of income for the majority of working population of the Kemerovo region. The wage share in the income of Kuzbass population is 43%, and among the Siberian Federal District (SFD) subjects, the region ranks the 6th when evaluated by the index above. The average monthly salary of the employed in the region is only 86% against the national rate and 98% as compared with the average rate for SFD. Despite the fact that the cash revenue of the Kemerovo region population, both nominal and actual, increase over the years, the region is ranked the 71st in the Russian Federation in terms of the minimum living wage (see Table 2) [9].

The average monthly income per capita is not the indicative parameter. This data may not be used to state that the most population in the region has the positive cash flow. The income in excess of 35000 rubles was reported in 8.5% of the population in the Kemerovo region in 2014, which is 1.5% higher than that in 2013.

Table 2. Income of the Kemerovo region population (2001–2014)

Parameter	2001	2005	2009	2011	2013	2014
Gross Regional Product per capita, rub.	39702	103.8	181624	272564	244064	254199
Consumer Price Index (December against December of previous year), %	118.0	110.5	107.7	106.5	106.7	111.9
Average earnings per capita: nominal, rub, actual (in % against previous year)	3058 112	7813 128	13470 84.4	16666 100.1	19697 99.5	19795 100.5
Average monthly wage paid, total for region, rub.	3313	8654	15995	20478	25326	26809
Average monthly pension size, rub.	1154	2554	6204.9	8250.9	10008	10891

The method to assess the readiness to pay for the better quality water may be used to identify that regional residents are concerned in the quality of drinking water. Sociological researches were first conducted in Russia in terms of the Kemerovo region with the involvement of authors and partners from the University of Hiroshima (Japan) to assess the readiness of the population to pay for the better quality drinking water. Researches were carried out in terms of settlements specific for the Kemerovo region, the big Kemerovo city and middle-sized Belovo and Belovo municipal district. More than a thousand households were surveyed in total. It was further found that almost all surveyed households in Kemerovo, Belovo cities and Belovo district take measures to improve the quality of drinking water (92.3%) by boiling, settling, water filtration or purchasing bottled drinking water. Boiling is the most popular way to decontaminate personal water reserves at home. 69.7% respondents were reported to use boiling. Settling is the second popular measure used by 51% respondents, whereas 49% filter the water and 23.3% regularly buy bottled water. Only 15% respondents drink the tap water and 4% use water from natural sources. Popularity of boiling and settling can be explained by reliability, decontamination speed and the relative cost-effectiveness. Installation of filters and bottled water purchase are more expensive ways to reduce the risk of the poor-quality drinking water use at home. On average, the cost of bottled drinking water is 150 rubles per 18.9 L in the Kemerovo region. Nine plants are constructed and run in Kemerovo, Novokuznetsk, Mezhdurechensk, Yurga, Belovo, Topka, and Mariinsk to produce and deliver the bottled drinking water. 172 cubic meters of water is produced every day, so the Kemerovo Region people pay more than 500 million rubles per annum to buy bottled water. The cost of water filters (flowing water purifiers) is 2500–6000 rub on average with the filter element service of up to 10 thousand L, and the cost of filter jugs is 500 to 1000 rubles with the replaceable cartridge service life of up 350 L. The costs of preventive measures to improve the quality of drinking water make 207.02 rubles per family monthly, i.e. 1.15% of the average monthly household income. However, only 2% respondents were willing to change the preventive charges upon the water quality is improved. This is due to some distrust of citizens in the ability of local authorities to improve the system of drinking water supply. Over 30% respondents were willing to pay extra funds to reduce the risk of poor-quality water consumption. On average, it costs 315.4 rub/mth for a family which makes 1.75% of the average household income in addition to the actual water rate in the amount of 210.6 rub/mth per person or 631.8 rub/mth for a family of three members, which makes 3.5% of the household income on average. However, most respondents tend to make the decisions above since they were aware of the problem significance (75.5%), some others found more convenient to pay at the proposed rate (11.3%), whereas individuals were greatly concerned in the personal income that might be lost during any illness, that is, the so-called loss of profit (1.7%). As a whole, the total commitment to pay for the quality water in the region amounted to about 336.5 million rubles per year [10, 11]. Since everything remains the same in terms of quality and availability of drinking water for the Kemerovo region population, the previously obtained data is used as the actual data in this study. The population size of the Kemerovo region has not changed significantly. For comparison purposes, let's see the assessment results for the European population to pay for higher quality drinking water at the time when the study was conducted in the Kemerovo region. In the European Union countries and namely in Germany, France, England, Spain and Slovenia and other countries people are available to bear expenses at a rate of 0.42 to 1.4% of the household income to increase the level of drinking water availability (Source: EEA, 2012, Eurostat household budget survey based estimates of the annual costs of water supply and sewage in EEA Member Countries, EEA staff data, Copenhagen).

The data obtained during the study of the population commitment to pay for the higher quality drinking water throughout the Kemerovo region and in European countries was analyzed to conclude that the regional public is available to bear expenses of the family budget at the same level, or even higher than that in the developed countries. It should be clarified that the drinking water market in these states is far maturer than that in Russia. However, we face the intentions of the Kemerovo region people to bear expenses above for the drinking water while the European population pays for the water quality improvement in view of the fact that the quality of tap water in those countries is much higher than that in Russian regions.

Thus, the analysis of factors that influence the creation of the bottled drinking water market in the Kemerovo region, allows, based on the methodical approach proposed by A.V. Antonova [12], identifying four criteria types to assess the availability of drinking water, namely, geographic, organizational and technological, economic and qualitative (Table 3).

As per authors, all these factors and criteria are extremely important to take into account when assessing the potential formation of the drinking water market and primarily, in terms of territorial coverage. When analyzing the market trend developments, the demand for particular product should be identified in terms of geographic point and the volume along with the population availability and potential to purchase it. This opinion is shared by other authors who study the drinking water market both in Russia, on whole, and in certain regions. They also conclude that the demand is formed in view of low availability of drinking water because of water source contamination and unfavorable condition of water supply networks, especially in old industrial regions of the Urals and Siberia. Also, most authors state that these regions tend to purchase the low-cost bottled water by local manufacturers which is more accessible as compared with the expensive water by international brands [13, 14].

Table 3. Criteria and indicators of the drinking water availability assessed [12]

Criteria to assess the drinking water availability	Indicators of the drinking water availability assessment
Geographic	Availability of water bodies Population density Urbanization extent Enterprise organization
Qualitative	Waste water volume Anthropogenic impact on water bodies Water body pollution degree (as per SCWPI, Specific combination water pollution index); Sample specific weight not consistent with hygienic standards; Excess level of quality standards
Organizational and technological	Number of utility infrastructure objects Specific weight of water supply networks in the need to be replaced Number of accidents per 100 km of networks Density of water supply lines Wear out of fixed capital stock Level of the housing total area coverage by water supply Level of water supply capacity available
Economic	Population income Population's committment to pay

RESULTS AND DISCUSSION

To evaluate the bottled water market in the Kemerovo region, materials provided by manufacturers, companies that specialize only on water delivery and retail networks have been analyzed. Specialists of the “Chistaya voda” LLC, the largest regional company to produce the bottled drinking water were invited as experts. Currently, 9 companies run throughout the Kemerovo region for production and delivery of bottled drinking water in bottles of 0.5, 1.5, 5.0, 10.0 and 18.9 L in volume (Table 4). Most of them are on the market for at least 5 years, except for “Chistaya voda”, “Noringi” and “Talinka”. In addition, often they do not own their plants but resell other manufacturers' products. First listed three companies own large productions at the Kemerovo region territory. They are perspective to increase sales since they run for the less capacity. The annual sales growth makes 10% on average. For example, the equipment capacity owned by the “Chistaya voda” LLC is 1200 bottles per hour. Currently, they have more than 30 thousand customers since they are for over 15 years on the market.

“Chistaya voda” LLC is the only company in the region that bottles the natural water taken from deep-water wells upon four-stage purification. Drinking Artesian water packaged in bottles and labeled with the “Berdovskaya tayozhnaya” trademark (“Chistaya voda” LLC) is also the only product in the Kemerovo region that suxxessfully validated by the ecological expertise committee and certified as the product of the highest ecological purity. The management system of the “Chistaya voda” LLC is certified in compliance with requirements of GOST R ISO 90001:2008 and the Food Industry Standard HASP [2]. In addition, other companies proved to be reliable suppliers of carefully purified drinking water. All companies that are engaged in water production and delivery possess their own regional client base, though 60–70% customers reside in larger cities like Kemerovo, Novokuznetsk and Prokopyevsk. The competitive environment of bottled water manufacturers is significantly mixed with numerous suppliers of drinking water from retail chains who sell it in small bottles of 0.5 to 2.0 L and hold their steady niche in the market space. In our opinion, all these companies and retail chains are prospective to increase sales in bottled water, since more than 70% of water consumers in the Kemerovo region are their potential clients today.

To assess the geographic discrimination in drinking water availability for the population and the growth prospects of the bottled water regional market, the area groups have been used in our study by the variety of accessibility as offered by A.V. Antonova. In particular, all municipal districts of the Kemerovo region were grouped and ranked as per geography, quality, management and technology and economic accessibility (Table 5) [15]. Arithmetic mean of the scoring results was used to assess the status of regional territories in terms of criteria and the minimum of these values.

As a result, a group of Tyazhinskiy, Yurginskiy, Leninsk-Kuznetskiy, Novokuznetskiy, Yaiskiy districts is classified as the category with "too low" at almost the critical level of availability by the aggregate parameter. The second group of districts with "low" accessibility of drinking water includes Belovskiy, Izhmorskiy, Kemerovskiy, Mariinskiy, Prokopyevskiy, Tisulskiy, Topkinskiy and Chebulinskiy districts. Guryevskiy, Krapivinskiy, Promyshlennovskiy, Tashtagolskiy, Yashkinskiy districts refer to areas of "medium" level of accessibility and only the Mezhdurechenskiy district refers to the category of "high" level of availability.

[2] “Chistaya voda” LLC [Electronic resource] – URL: http://www.vodakem.ru/feedback/.

Table 4. Production and delivery of drinking water in the Kemerovo region in bottles of 18.9 L in volume, 2015

No.	Company name	Sales volume, bottl/mth.	Delivery to population
1	Clean water	60000	+
2	Rodniki Kuzbassa	35000	+
3	Talinka	11000	+
4	Noringa (Novosibirsk)	10000	+
5	Osobaya (Special)	4500	+
6	Luchshaya (Best)	–	+
7	Vodovozov	–	+
8	Dalniy kluch	–	+
9	Yugus	–	+

Table 5. Rating of the Kemerovo region districts* in terms of drinking water availability

№	Territory	k_1**	k_2**	k_3**	k_4**
1	Belovskiy	1	2	3	4
2	Guryevskiy	1	4	4	2
3	Izhmorskiy	4	3	2	1
4	Kemerovskiy	1	1	4	4
5	Krapivinskiy	4	3	3	1
6	Leninsk-Kuznetskiy	1	1	4	3
7	Mariinskiy	2	4	2	2
8	Mezhdurechenskiy	2	3	3	4
9	Novokuznetskiy	1	1	3	4
10	Prokopyevskiy	1	1	4	4
11	Promyshlennovskiy	4	2	3	2
12	Tashtagolskiy	2	2	4	3
13	Tisulskiy	4	3	1	2
14	Topkinskiy	2	3	2	3
15	Tyazhinskiy	3	2	2	1
16	Chebulinskiy	4	4	1	1
17	Yurginskiy	2	1	4	1
18	Yaiskiy	2	2	3	2
19	Yashkinskiy	3	3	3	2

Note. * Here, a district is location of a municipal district of the same name and the city district; ** k_1 – criterion of the drinking water geographic availability, k_2 – criterion of quality water availability, k_3 – criterion of organizational and technological water accessibility, k_4 – criterion of economic water accessibility.

The study showed that 1110.7 thousand persons live in areas with "too low" total availability of drinking water (40.5% of the Kemerovo region population), and 1300.7 thousand persons live in areas with "low" accessibility (47.4% of the Kemerovo region population). It should be noted that these two groups include all towns in the Kemerovo region, where the enormous human-made anthropogenic load is imposed on water bodies. The highest rate of morbidity is reported in Novokuznetsk, Kemerovo, Prokopyevsk, Belovo, and other towns due to poor-quality water. Currently, bottled water sales by all manufacturers are concentrated in these areas. However, it should be noted that peripheral rural areas, namely Tisulskiy, Yaiskiy, Izhmorskiy and other areas, are assigned to this group though due to the scarce water supply networks or due to absolute absence of the latter. The sellers of bottled water in these areas are not so much active since the price for water will be higher than that in larger cities due to the small market size – poorly populated areas and high transport costs. Unlike other Russian regions, there are no areas in the Kemerovo region where water is supplied as per the schedule or just imported.

More than 300 thousand people (12% of the total population) reside in areas with the medium to high levels of water availability. These are area with the high level of geographical and quality accessibility. The human-made impact on water bodies is far less in here as well as the free access to a greater volume of pure natural water. Currently, the bottled water sale is these areas, as well as in rural areas of previous groups, is less and they represent a potential market for sellers. Thus, by the results of analysis it was found that 88% of the Kemerovo region population live in conditions of "too low" and "low" accessibility to drinking water and it proved that the potential capacity of the local bottled water market in the Kemerovo region is hypothetically quite great totaling more than 1500 thousand people.

However, the demand for drinking water by the territorial difference will be insufficiently described, unless it is complemented with the comparative evaluation of ranking resultants for economic

accessibility indicators of drinking water for people residing in different areas of the Kemerovo region. Analysis of the regional statistics results and assessment of the population commitment to pay for the higher water quality allowed classification of all districts for four groups:

– A group with "too low" level of economic accessibility, that is, Izhmorskiy, Krapivinskiy, Tyazhinskiy, Chebulinskiy districts;

– A group with "low" level of economic accessibility, that is, Guryevskiy, Mariinskiy, Promyshlennovskiy, Tisulskiy, Yaiskiy, Yashkinskiy districts;

– A group with "medium" level of economic accessibility, that is, Leninsk-Kuznetskiy, Tashtagolskiy, Topkinskiy districts;

– A group with "high" level of economic accessibility, that is, Kemerovskiy, Mezhdurechenskiy, Novokuznetskiy, Prokopyevskiy districts.

The resultants are of significance for suppliers of bottled drinking water and commercial networks, since they give a good indication on the spatial perspectives of market development. It should be noted that the expansion of bottled water sales in areas with "low" and "too low" economic accessibility will be significantly slow-paced than that in larger cities with "high" level of accessibility. For long years, the low population income will limit the market expansion towards poorly populated rural areas. In this case it is important to realize that understanding of the term "drinking water accessibility" and the understanding of other economic benefit accessibility is different from area to area with the varied socio-economic background. This is again to prove that there are no universal methods to create the bottled drinking water market to be used in each and every district and region. In this given case, is necessary to understand that the rarity of drinking water is the permanent imbalance of supply and demand due to contamination of drinking water sources or over-intensive exploitation thereof. The drinking water scarcity relates to the water distribution mechanism. Rarity, as compared with the scarcity, indicates on imbalance of demand and possibilities of the population, opportunities. The most difficult is to provide free access to this wealth. However, the best development practices of the society of today shows that the effective mechanism to regulate the access to water is infeasible without the pulic active intervention. Each party, whether business or community shall have significant advantages in case of free access to the welfare and influence, whether directly or indirectly the other party. The society may fail when confining the business within the strict limits. As per the authors, further on, the bottled water manufacturers and their customers will achieve the agreement by improving the life quality and adjusting the extent of socio-economic development in the Russian regions, as a whole.

CONCLUSIONS

Formation of global and local markets of drinking water requires the analysis and assessment of the demand volume for this product essential for human functionality and also the causes and factors decisive for the demand formation. Most activities in the drinking water market relate to the performance and production volumes, formation of delivery services, product branding and promotion, indeed. Activities to assess accessibility ratio of drinking water are in short and, in our opinion, it adds a greater complexity to the assessment of prospects for the drinking water market development.

The current situation known for rapid changes in scientific, technical, financial, economic and social industries contributes to support the tendency to convergent administrative, economic and market mechanisms of drinking water accessibility. Currently, the Russian policy of the drinking water accessibility is the aggregate of administrative and economic tools where market-based tools are rarely applied due to non-enforceability. The mechanism in place ensures current needs in water resources for economic industries and the population. However, the prospects for the national economics development require the volume of water resources of the proper quality guaranteed to meet drinking and household needs, as well as to be used for industrial, agricultural, energy and recreational purposes. Thus, it is required to develop the efficient combination of applicable tools to improve the current mechanism of drinking water accessibility in view of regional peculiarities (geographic, organizational and technological, qualitative and economic availability of the drinking water in the region).

The market of drinking water is created in old-industry regions at the background of low economic, qualitative (environmental) and technologically managed accessibility. Drinking water manufacturers and sellers are most concerned in areas of high population density and high economic availability; the availability of population to pay for the drinking water quality also makes sense. In other regions with low economic, organizational and technologically managed accessibility the governmental involvement is required along with integration of various types of public-private partnerships to encourage investors and increase the public access to drinking water. The governmental procurement for drinking water, among these tools, is beneficial for public and bottled water manufacturers, in conditions of low geographic, technologically managed accessibility, since the construction of new water facilities and infrastructure is not economically feasible due to the scarcity of consumers in these regions. This is also due to the fact that the government, for example, is held liable for the environmental safety, including the safety of water sources.

The market of bottled water in the Kemerovo region may be considered as one of the most potentially intensive markets among Siberian regions because of the total low availability of drinking water. Almost all the regional residents are the potential consumers of bottled water. Improvement of the life quality and increase in the public income will definitely result in the increase in the actual capacity of the drinking water market.

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STRENGTHENING OF THE ECONOMIC POWER OF THE DOMINATING ENTITIES IN THE FOOD INDUSTRY

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Abstract: The majority of food markets comes under the priority and socially important ones. Promptly growing global companies actively get on the Russian food markets under the conditions of globalization. The revelation of specifics of processes of strengthening the economic power of the dominating entities in the food industry is the base for the development of decisions on the state regulation of development of food production and food price formation. The article considers the essence of the concept of oligopolization that allows to prove the essence of strengthening the economic power in the market (in the industry) of several largest companies, to show the variability inherent for oligopoly, to uncover the reasons that cause these changes, to reveal the orientation of high-quality changes and to determine the historical regularity of development of oligopolistic markets on its basis; to analyse the consequences of this phenomenon for society. Within the research, the concept of oligopolization is applied to the problematics of development of the dominating entities that function in food markets. The work analyses the factors that influence the growth of intensity of processes of oligopolization (the change of scopes and structure of consumer demand; the discrepancy of investment policy of oligopolists; the advancing development of network of foreign subsidiaries, the acceleration of growth of transnational capital in the developed countries and the growth of activity of oligopolists from the developing countries) and designates the peculiarities of influence of these factors on the development of food markets. The peculiarities of activity of the factors functioning in the global food markets are considered in the aspects of sales revenues; the positions held by these companies in the top ratings; the investment and innovative activity of companies. Special attention is paid to the issues of penetration and functioning of foreign companies in the Russian food markets.

Keywords: dominating subjects, the food industry, the global food companies, oligopolization

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INTRODUCTION

The processes of strengthening the economic power of the dominating entities draw attention of researchers as this process exerts an ambiguous effect on the subjects of economic relations. On the one hand, there is a growth of competitiveness of these companies in the global markets, advanced technologies are introduced, labor productivity grows, the best practices in the field of production organization, management and marketing are adopted, on the other hand, – there is a growth of dependence of small producers and their replacement from the market. The strengthening of economic power of the largest companies can provide the unreasonable overpricing, the reduction of assortment and the quality degradation of products. These problems are especially significant are for food markets, the majority of which come under the priority and socially important markets. The food security of the country, the level and quality of life of the population in many respects depends on the functioning of food markets and food industry.

The governments of economically developed countries treat the functioning of food markets with special attention, and the level of providing with food is considered by them as one of the indicators of homeland security. For this reason, the food producing enterprises and also the competitive situation and the price development in food markets come into the view of the regulatory authorities.

At the beginning of its origin the food industry and food markets were characterized as competitive ones. In the process of development of processes of capital concentration and centralization, and also of economy globalization and the development of information technologies, the situation changed. According to the data of the company Oxfam International, 10 largest transnational corporations can create a food basket of the most of the population of the planet, influence the

conditions of their work, and also the environment [1]. It causes the relevance of research of processes of strengthening the economic power of the dominating entities and the changes happening in the structure of the food industry.

The aim of the work is the revelation of peculiarities of processes of strengthening the economic power of the dominating entities that function in the food industry.

OBJECTS AND METHODS OF STUDY

The object of research is the dominating entities that function in the food industry. The research is performed on the basis of the microsystem and institutional approaches.

The concept of oligopolization offered by the author which allows to prove the essence of strengthening the economic power in the market (in the industry) of several largest companies, to show variability inherent for oligopoly, to open the reasons causing these changes, to reveal the orientation of high-quality changes and to determine on its basis the historical regularity of development of oligopolistic markets and to analyse the consequences of this phenomenon for society is used as the basic concept. The concept ofoligopolization in this work is adapted for the problematics of development of the dominating entities that function in food markets.

The factual base of research are the data of rating agencies Forbes-2000, RAEX Ekspert RA, the Boston Consulting Group, the data of the Federal State Statistics Service, the Federal Antimonopoly Service of the Russian Federation and the data provided at the official sites of the analyzed companies.

The theoretical base of research are the works by S. Avdasheva, I. Vallerstayn, V. Kondratyev, N. Osokina and others.

RESULTS AND DISCUSSION

In modern conditions there is the strengthening of the economic power of the largest companies by means of the redistribution of capital, property and imperious relations with the purpose of profit maximization. This process provides the formation of the oligopolistic structure of the market or the formation of a tendency for oligopolization when the quality (the nature of interaction between the participants of the market) and quantitative indicators (first of all, concentration indicators) change, but have not yet reached the characteristics and values allowing to refer the market structure to a structure of oligopolistic type.

Oligopolization as the economic phenomenon

Oligopolization is the steady change of relations between the participants of the market of certain goods (industry) causing the allocation of key constituents (oligopolists) by the redistribution of objects of property, capital, imperious relations and the consolidation of their positions with their transformation into industry and market actors. It acts as the characteristic of process of transformation of big business.

Oligopolization is both the factor and the product of globalization, it exerts a considerable effect on the development of states, synchronizing their activities; it acts as the characteristic of process of transformation of big business, the reflection of megaprocesses, including that of cyclic nature. The aspiration to oligopolization of the market can be blocked by a number of internal (lack of financial resources, a low profit margin and so forth) and external factors (the antimonopoly legislation, the occurrence of competitors in this market, state regulation).

Characterizing oligopolization, it must be kept in mind that it is a process. If oligopoly, to a certain extent, is a static concept which reflects the profile of structure of the market at a certain timepoint, then oligopolization is a concept which is, to a greater degree, dynamic and reflects the availability of variability and fluidity of the market structure. The process of oligopolization is not linear and develops unevenly in time and space. For the last decades the processes of oligopolization have become characteristic of food markets and the food industry.

Oligopolization can have an intra-branch and inter-branch character. The intra-branch oligopolization is related to the strengthening of the economic power of oligopolists within the industry. The inter-branch oligopolization is related to the penetration of oligopolists into other branches.

Oligopolization has an essential effect on the national development, determining the place of the specific country in the world economic system. The flows of products, services, labor power, capitals, technologies, information, etc. circulating in oligopolistic companies has an effect on the export of certain countries, determining their international specialization. Oligopolization provides the development of new types of economic relations penetrating the national economic systems, strengthening their interrelations and synchronizing their activities. The external form of oligopolization is the allocation of the dominating firms, the actors controlling a considerable part of industry production and/or market sales in the structure of industries and goods markets.

Global food companies in the Russian market

Let us consider the peculiarities of activities of the actors functioning in global food markets in the Russian economy. The largest companies which are engaged in food production are allocated according to the rating of Forbes-2000 (2015) including the assessment of the companies by sales volume, profit, assets and market value [2]. The data on the first ten companies of the rating are listed below. The data provided at the official sites of these companies were also used to characterize them. Let us pay attention to the fact that most of the largest companies have settled down quite sufficiently in Russian food markets.

Nestle (Switzerland). Ranks 30th in the rating, the sales volume is 100.1 billion dollars. The company produces instant coffee, mineral water, chocolate, confectionery products, dairy products, baby food, dry breakfasts and so forth. It has more than 8 thousand

trade marks of foodstuffs among which are KitKat, Nescafé, Nescafé Cappuccino, Nespresso, Nestlé Pure Life, Nesquik Cereal, Cerelac, Nestea, etc. Operates in 86 countries. Nestle is the leader of the Russian market for instant coffee, cocoa, the market of products of baby food, cookery, the markets of dry breakfasts and instant porridges, and it also takes the leading positions in the market of packed chocolate, ice cream and forages for pets.

Mondelēz International (the USA). Ranks 188th in the rating, the sales volume is 34.2 billion dollars. The company produces chocolate, biscuits, lollipops and it is the second large producer of chewing gum in the world. Owns the brands Milka, Oreo, Cadbury, LU, Nabisco, Tang and Trident, Dirol, etc. Operates in more than 80 countries. The investments of the company into the Russian economy have reached 1 billion dollars since 1994.

Archer Daniels Midland Company (the USA). Ranks 220th in the rating, the sales volume is 81.2 billion dollars. The production of the company is divided into three main segments: the production of vegetable oils, the production of corn, the production of ingredients for food production, operational service. Owns the brands CardioAid, Coroli, VegeFull, Oilio, Nutrisoy, etc. Sells its products in 160 countries of the world. In Russia there is a department of the company that sales food ingredients and components of compound feeds, and also the regional sales office "WILD Flavors & Specialty Ingredients".

Danone (France). Ranks 285th in the rating, the sales volume is 81.2 billion dollars. The company produces fermented milk products, mineral water, drinks, baby and clinical nutrition. Operates in 140 countries of the world. The volume of investment of Danone has reached 2 billion dollars from the beginning of its activities in 1992 in Russia. The Danone Group of companies in Russia includes 18 plants which produces products of such brands as Danone, Aktivia, Actimel, Rastishka, Danissimo, Prostokvashino, Bio Balans, Aktual', Smeshariki, Tema and others.

Wilmar International (Singapore). Ranks 369th in the rating, the sales volume is 43.1 billion dollars. The company produces palm-oil, oil-bearing crops, vegetable oils, sugar and sweeteners, flour, rice and so forth. It operates in more than 50 countries.

General Mills (the USA). Ranks 407th in the rating, the sales volume is 17.6 billion dollars. It produces meat paste, flakes for breakfast, products for baking, muesli bars, tinned corn, etc. Owns the brands Häagen-Dazs, Old El Paso, Green Giant, Betty Crocker, Pillsbury, Cheerios, etc. Operates in more than 30 countries of the world.

Kraft Foods (the USA). Ranks 410th in the rating, the sales volume is 18.2 billion dollars. The list of products includes: chocolate Milka and AplenGold, Vozdushnyy, Côte d'Or, the brands of coffee Jacobs, Cartenoire and MaxwellHouse, cream cheese, chewing gum, staple foodstuffs (macaroni, cheese, meat, desserts, sauces and drinks). Has been operating In Russia since 1994, ranks 2nd in the chocolate market.

JBS (Brazil). Ranks 453rd in the rating, the sales volume is 51.2 billion dollars. Is the world leader in the field of production of beef, mutton and poultry, and also pork. Has access to consumer markets in more than 150 countries. Russia annually imports more than 500 thousand tons of chicken meat, about 1 million tons of pork, about 1.1 million tons of beef.

Associated British Foods (Great Britain). Ranks 462nd in the rating, the sales volume is 21.4 billion dollars. It produces sugar, vegetable oils, food ingredients, etc. Operates in 48 countries.

Tyson Foods (the USA). Ranks 499th in the rating, the sales volume is 39.6 billion dollars. It produces beef, pork and poultry.

Such companies as Mars, PepsiCo, Coca Cola, Unilever, etc. actively function in the Russian food market, as well. The processes of oligopolization are especially clear in the beer market. The tendency of the last years in the beer industry is the buying up of large and average regional manufacturers by transnational corporations (Hartwall PLC, Carlsberg Breweries AS, Scottish&Newcastle, Heineken, Efes Beverage Group, SABMiller) and the replacement of those who is independent yet. The specified transnational corporations own 85% of the Russian beer [3] market.

The report of the Educational and Methodical Center of Agricultural Consultation and Retraining of Personnel of Agro-Industrial Complex "Participation of the Foreign Capital in the Russian Food Industry" (2014) [4] provides the data that the share of foreign capital in the Russian food industry is 60%. In most subindustries of the Russian market of food and drinks the greatest share of the market belonged to foreign corporations: nearly 60% of the market of conversion of milk; more than 70% of the market of juice products; about 80% of the market of refrigerated vegetables and fruit; more than 90% of the market of fruit and vegetable preservation. More than 70% of the Russian market of juice products belonged to two western corporations – PepsiCo and Coca-Cola. Due to the imposing of countersanctions from the Russian government, the situation begins to change in favor of the omestic producers.

The domestic companies hold a leadership position in the markets of meat processing and bakery. It should be noted that there is also a tendency of taking over smaller companies by large domestic and foreign corporations in these segments.

There is a decrease the last two years in the number of foreign competitors entering again in the Russian markets. In case of the food industry, such dynamics is caused by the peculiarities of business activity in Russia due to the imposition of sanctions from the USA, the European Union and a number of other countries, and also of food embargo from Russia. At the same time, it should be noted that the significant amount of segments of the Russian food market is already occupied with foreign companies, which are co-owners of domestic enterprises.

Factors influencing the intensity of oligopolization

The enlargement of the companies functioning in food markets acts as a universal tendency. The factors influencing the growth of rate and intensity of

oligopolization are revealed and the peculiarities of these processes in food markets are designated in the course of the research.

1. Change of scope and structure of the consumer demand. It is reasonable to differentiate the processes of oligopolization under the conditions of increasing, stable and decreasing demand.

The level of competition between oligopolists under the conditions of increasing demand (of the growing industry, of the increasing market) is rather low, there are possibilities of development without the violation of interests of competitors. The rates ofoligopolization are usually high.

The so-called procedure of "pushing out" from the market (price wars, the establishment of oligopolistic collusions and of model of partial oligopoly are characteristic of this stage) begins under the conditions of stable demand. The increase in market shares of oligopolists is only possible due to the narrowing of market shares of competitors under the conditions of stable demand, the rates of oligopolization slow down. The periods of relatively stable demand are characteristic of the food industry and Russian food markets now.

The model of shrinking demand is characteristic of either a stagnating industry or the period of economic crisis. While companies develop rather independently in the period of increasing demand, the strengthening of concentration by merging and taking over companies for the purpose of increase in production efficiency is characteristic of the period of shrinking demand. The shrinking demand forces oligopolists to broaden the spheres of their activities, increasing the chains of added value due to the provision of servicing and repair of the issued goods. "Commitment to the client" becomes an important competitive advantage under the conditions of decreasing demand. In this regard the control over distribution channels is of particular importance.

It is also necessary to take into account the change of consumer preferences concerning foodstuffs, especially in the developed countries. The demand shift from simple natural products to deep-processed products with a higher added value is noted. Today special requirements are imposed to food in the economically developed countries the main of which are the creation of high consumer value which is expressed in time saving, the convenience of acquisition, an advantage to health and the satisfaction of various taste preferences; in the compliance of quality of foodstuffs to the national and international standards [5, p. 39]. At the same time, the issue of providing the population with food for the purpose of hunger elimination is particularly acute in the poorly developed countries. The consumption in the countries with a low income level is about 2400 kcal per capita a day (while the consumption in the economically developed countries is 3500 kcal per capita a day) and consists generally of grain, root crops and tuber crops, though proteins and fats contained in such foodstuffs as meat, dairy products and vegetable oils are quite important [6]. An acute problem for the underdeveloped countries is providing the population with at least the necessary mix of nutrients [7]. In this case a demand for cheap and the most available food is created.

2. Discrepancy of the investment policy of oligopolists. The capabilities of intra-branch expansion of capital are constantly decreasing in the course of oligopolization. The increase in production capacities requires the corresponding increase in its share in industry sales which becomes more and more difficult to reach. The interbranch capital inflow in the traditional form of starting one's open business in other branch encounters essential obstacles, as well. At the same time, the sustained profits made thanks to group monopoly reinforces the process of capital accumulation. The contradiction is resolved by means of new forms of interbranch movement of capital, first of all, of the diversification and conglomeration performed mainly by means of taking over and merging.

Companies penetrate other branches with the purpose to strengthen their positions in the primary market. New types of productions more and more often emerge across various branches. The lines that earlier separated and isolated branches become more and more conventional. The new equipment and technological processes unite the earlier separated branches. The communication between branches as mutual suppliers and consumers becomes more and more close.

In addition to it, the aspiration of oligopolists to invest in innovative industries because of the enhancing interwining of various areas of new knowledge and the need of further development of scientific research should be noted. The companies realize the importance of creation and introduction of innovative products into the market. According to M. Hristofi and E. Leonidi, innovative products enhance the role of the organization and increase its financial performance due to the creation of stable benefit, which increases the economic aspects of stability [8].

Referring to global companies which are the leaders in the field of innovations it should be noted that the companies functioning in the food industry are not among the most innovative companies of the world determined by the rating agency Forbes and the Boston Consulting Group (BCG). Apple (the USA), Google (the USA), Tesla Motors (the USA), Microsot Corp. (the USA), Samsung Corp. (South Korea), Toyota (Japan), BMW (Germany), Glead Scienses (the USA), Amazon (the USA) and Dimler (Germany) [9] are on top of the rating of BCG.

According to Rosstat, [10] the percentage of the organizations of the food industry (including the production of drinks and tobacco) in Russia that perform technological innovations was 9.3% in 2012, 9.0% in 2013 and 10.3% in 2014 which is a little more than the average Russian indicators. The leaders in the field of technological innovations were the following branches: the production of coke and oil products; the production of electric equipment, electronic and optical equipment; the production of vehicles and equipment; the production of machines and equipment. The share of costs of the food industry, including the production of drinks and tobacco, in total of costs for technological innovations was 3.4% (25.9 billion

rubles) in 2014. The insufficient innovative activity of Russian enterprises is related to the deficiency of financial resources, a high risk level, the availability of bureaucratic barriers, the persistence of thinking, the deficiency of qualified personnel.

The processes of oligopolization are in many respects determined by the specifics of investment policy of oligopolists. P. Suizi draws attention to the fact that, on the one hand, there is an increase in the flow of profit, on the other hand, there is a decrease in demand for additional investments in the markets which are more and more managed by few [11]. The author expresses a concern for a possible negative effect of oligopolists on the processes of capital accumulation. The situation when there is more and more profit and fewer and fewer profitable investment opportunities precedes the delay of capital accumulation and the decrease in the rates of economic growth.

The interbranch capital flow becomes difficult in the process of further oligopolization of competitive sector and as fast as the possibilities of formation of new branches which do not compete with the already existing ones decrease. In the course of oligopolization the interbranch capital flow is performed within oligopolistic structures themselves. The interbranch communication consists in the creation of joint industrial complexes, research centers, the advertizing and sales machinery, general funding channels, and also is shown in communications with authorities. This method of redistribution of resources has a number of benefits. First, on being introduced in a new field of activity, the company is provided with the necessary financial, human and scientific and technical resources, secondly, the management system of the company is sufficient, thirdly, considerable savings on advertizing expenses is possible, fourthly, the company has an opportunity to sell new products in the geographical markets which are already developed by it.

A number of leading food companies are characterized not only by intrabranch, but also interbranch investment. Under the influence of needs of the countries with an emerging economy, which have a large population, for food import, and also of lack of land and water resources the amount of investment into their agricultural production increases.

The Report of UNCTAD on world investments of 2009 [12] is devoted to the issues of penetration of transnational corporations into agricultural production. It is noted in the Report that the transnational corporations using agricultural contracts and other non-equity forms of participation in the agricultural industry operated worldwide in more than 110 countries of Africa and Latin America. For example, in 2008 the Nestle corporation (Switzerland) had contract agreements with at least 600000 farmers in more than 80 developing country and countries with economy in transition as dropshippers of different types of agricultural products [9]. The agrarian sphere is the object of interest of a lot of global food companies.

The attention of transnational corporations is also drawn by other spheres. Thus, Nestle performed portfolio investments into the enterprises of the perfumery and cosmetics and pharmaceutical industry. Archer Daniels Midland Company, in addition to the agricultural industry, is coming into the sphere of production of fuel ethanol and biodiesel of rape. There are quite a lot of similar examples. At the same time, the global food companies that have kept their profile for decades continue to function. Danone, Coca Cola, PepciCo and other companies are among them.

Active foreign investment is characteristic of the global food companies from the developed countries. The developing countries and the countries with emergent markets often become the object of their interest. A lot of transnational corporations came to the Russian markets with the schemes of penetration worked out in Central and Eastern Europe.

Short-term profitability is not always the determining factor for making decisions about penetration. The share of investments into the Russian project is often an insignificant share of total of investments for the largest foreign companies, therefore they are usually interested in taking over and retention of the maximum market share counting on the receipt of considerable income from the market growth in the long-term.

The reinforcement of positions of foreign oligopolists in Russia can be developed in various forms: the neutralization of local competitors by taking them over; the introduction of dumping and discrimination prices; carrying out "restrictive business practice" (for example, the restriction of sale of technology; imposing of use of a certain trademark; the fixation of prices of the products produced according to patents and licenses; the obligatory purchases of semifinished products and equipment from the specific suppliers and so forth). Oligopolists can buy enterprises and subsequently reduce or stop the release of goods to restrict the volume of national production.

Foreign food companies actively penetrate Russian markets. 23705 organizations with the participation of foreign capital (without small enterprises) had operated in the Russian Federation by October 1, 2015, 493 enterprises (2.1% of total of the enterprises with the participation of foreign capital) of them operated in the sphere of production of foodstuffs, including drinks and tobacco [13]. About 5% of the work power employed at the enterprises with the participation of foreign capital is employed at these enterprises. Fifty largest foreign companies operating in Russia are 10% of their total number, at the same time their revenue is about a quarter of the total revenue of the companies with the participation of foreign capital [calculated by 13 and 14].

Table 1 provides the information on the total revenues of the largest companies with the foreign participation operating in Russia in the food industry. These companies are included into the rating of Forbes "50 Largest Foreign Companies Operating in Russia" [14].

Table 1. The largest foreign companies operating in Russia in the food industry in 2014 included into the rating of Forbes "50 Largest Foreign Companies Operating in Russia"

Position in the rating	Name of the Russian company / name of the foreign investing company	Revenue for 2014, billion rubles (change of revenue)	Share of the Russian revenue in the global economy, %	Country	Year of entry into Russia
8	Pepsiko Kholdings / PepsiCo	171 (+9%)	7	the USA	1974
17	Mars / Mars	102 (+42%)	8	the USA	1991
19	Nestle Rossiya / Nestle	97 (+13%)	3	Switzerland	1995
21	Danon Rossiya / Danone	96 (-3%)	9	France	1992
23	Baltika / Carlsberg Group	83 (-5%)	20	Denmark	1993
25	KKEBSE / Coca-Cola Hellenic Bottling	68 (+7%)	21	Switzerland	2001
26	Makdonalds / McDonald's	66 (+4%)	6	the USA	1990
28	Mon Delis Rus' / Mondelez International	63 (+13%)	5	the USA	2012
48	San Inbev / Anheuser-Busch InBev	36 (-5%)	2	Belgium	1999

Source: 50 krupneyshikh inostrannykh kompaniy v Rossii [50 Largest Foreign Companies Operating in Russia]. – URL: http://www.forbes.ru/rating/50-krupneishikh-inostrannykh-kompanii-v-rossii/2015?full=1&table=1 (accessed 03/19/2016).

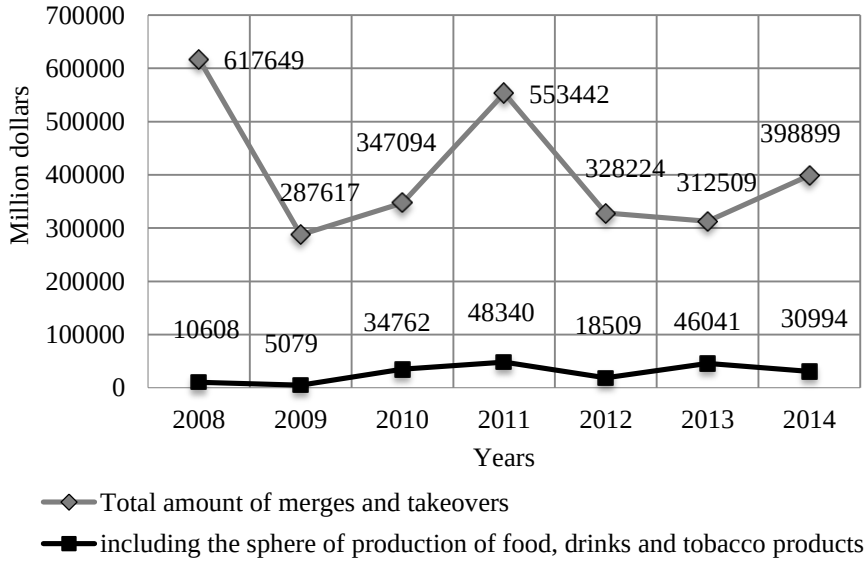
According to the rating, of 50 largest foreign companies operating in Russia nine companies operate in the food industry. Their revenue is nearly 16% of the total revenue of fifty companies. Six of nine companies had positive revenue dynamics in 2014. The share of Russian revenue in the global economy varies from 2% (Anheuser-BuschInBev) to 21% (Coca-ColaHellenic Bottling). Thus, the Russian food market and, consequently, the Russian food industry become more and more significant for foreign investors with the course of time. At the same time, it should be noted that the peak of merging and taking over from foreign investors is already behind. Now the task of the foreign companies functioning in Russian markets is to hold the positions.

The largest foreign companies operating in the food industry take rather high positions in the rating of RAEX Ekspert-RA "Ranking of 600 Largest Russian Companies following 2014" [15]. Thus, among the first ten food companies included into the rating there are PepsiCo Holdings (the 56th place in the total rating), Nestle Rossiya (the 108th place), Baltika (the 124th place), Mars (the 128th place), KKEBSE (the 140th place), Mon Delis Rus' (the 159th place) and McDonald's (the 189th place). Thus, 7 companies with foreign capital are among the top ten leaders in sales volume in the food industry. Cherkizovo Group (the 148th place), EFKO group of companies (the 163rd place), United Confectioners (the 210th place) are among the domestic companies included into the top ten. Such an arrangement of places in the rating tells us about the strong positions of the companies with foreign capital in the Russian economy.

3. The advancing development of network of foreign subsidiaries, the acceleration of growth of transnational capital in the developed countries and the growth of activity of oligopolists from the developing countries. The power of oligopolists increases in modern conditions both due to the expansion of scales of production, and by means of redistribution of capital as a result of their centralization.

The growth of economic power and the expansion of the sales market of oligopolists during the last two decades occurs generally due to the buying up of already operating enterprises whereas in the 50-60-ies of the XX century 2/3 of foreign investments went to the construction and modernization of plants, and 1/3 went to the buying up of already operating enterprises. At the beginning of this century the direct foreign investments were less than 20% of total of foreign investments, and cross-border merges and takeovers – more than 80% of the volume of investments [16, pp. 743–744].

According to the data provided in the World Investment Report (2015) [17], the amount of merges and takeovers of companies was 2845.4 billion dollars from 2008 to 2014. The maximum amount of merges and takeovers was in 2008 (617 649 million dollars). The most active merges and takeovers were in 2014 in the financial sphere, pharmaceutics, metallurgy, communications and the media industry. The sphere of production of food, drinks and tobacco products was in the 6th place (7.8% of total of transactions) in the amount of merges and takeovers in 2014. Fig. 1 provides the dynamics of amounts of merges and takeovers in total in the world, including that in the sphere of production of food, drinks and tobacco products, from 2008 to 2014.



Note. Compiled from: World Investment Report 2015: Reforming International Investment Governance. – New York: UNCTAD, 2015. – URL: http://unctad.org/en/PublicationsLibrary/wir2015_en.pdf (accessed 02/20/2016).

Fig. 1. Dynamics of amounts of merges and takeovers in the world from 2008 to 2014.

The increase in the number of merges and takeovers is related to the aspiration to quick capitalization, the penetration into perspective markets, the desire to keep competitors under control and to keep stability during the deterioration in the condition of certain markets, the possession of considerable financial resources. The most considerable volume of transactions in 2014 accrued to service trades (53.4%) and the manufacturing sector (36.6%). In recent years there are rather smaller amounts of merges and takeovers in the primary sector.

As a result of merges and takeovers, and also of the concentration of capital, the scale of assets of oligopolists increases, and production volume increases, as well. In addition to it, there is the active formation of chains which integrate not only firms, but also national economies.

The balance of power between oligopolists of different groups of countries changes under the conditions of globalization. It is shown in the advancing growth rates of production volumes and investment of oligopolists from the developing countries and the countries with economy in transition.

According to UNCTAD [18], the investments into the states with economy in transition decreased by 51% in comparison with 2013 and made 45 billion dollars. The inflow of world direct foreign investments in 2014 decreased by 8% to 1.26 trillion dollars.

There is an essential decrease in the amounts of direct foreign investments to Russia: they have decreased by 70% to 19 billion dollars. Foreign investors, especially western ones, were restrained by geopolitical conflicts, anti-Russian sanctions and the negative outlooks of economic growth.

The aggregate inflow of investments was higher in the developing countries than that in the developed ones, having made 700 billion dollars (an increase by 4% in comparison with 2013). By the end of 2014 56% of all world investments had been directed to this group of countries. China in which 128 billion dollars were invested became the leader of 2014 in investment attraction. It was followed by Hong Kong with the attracted capital of 111 billion dollars. The USA are in the third place with the volume of investment inflow of 86 billion dollars: In fact, China has ousted the USA from the position of the leader the latter have held since 2003. The top five also included Singapore and Brazil.

The dynamism of transnational corporations of the developing countries and the countries with economy in transition contrasts with the delay of investment activity of transnational corporations from the developed countries. According to the conclusions of UNCTAD, the main factors of investment of developing countries are chasing consumers in the sphere of IT services (India), the competitive pressure from foreign oligopolists, the state policy of support of foreign investments (China), the growth of costs, especially that of work power (Malaysia); the crises or restraining factors in the home country, in particular, those which provide the growth of inflationary pressure (Turkey, Chile); the deficiency of major resources necessary for the expansion of economic activity; the threat of global competition in the domestic market [19].

Thus, the growth of rate and intensity of oligopolization is influenced not only by the expansion of activities of oligopolists from the developed countries, but also by the fixing of oligopolists from the developing countries and the countries with economies in transition in the global market which promptly gain steam and gradually reduce the gap with the leading oligopolists of the developed countries. It is remarkable that this tendency remained during the world economic crisis. However the strengthening of positions of the food companies from the developing countries and countries with economies in transition is not a clear tendency. The companies from the economically developed countries continue to take the leading positions.

Stages of oligopolization

Let us mark with the following main stages of oligopolization in the developed countries based on our research of tendencies of evolving of oligopolistic markets (Fig. 2). The maturity of processes of oligopolization is a criterion of periodization.

1. Horizontal oligopolization (the end of XIX – the beginning of the 20th century). During this period there was a transition from capitalism of free competition to monopolistic capitalism. Just then a lot of American, German, French, English and Russian oligopolistic companies were created by means of horizontal merges of large companies.

The first food trade transnational corporations were created in the 19th century. Subsequently they penetrated into the sphere of agricultural production, including that of low developed countries. During this period such companies were created as United Fruit Company, Nestle, Brooke Bond and others.

2. Vertical oligopolization (the 20-ies – the 60-ies of the XX century). In the 20-ies – the 70-ies of the XX century there was the formation of vertically integrated companies controlling several markets.

After World War II the transnational corporations operating in the food industry began to control not only the production, but also the conversion and sale of foodstuffs for the local population of the dependent countries. From 1960 to 1975 the subsidiary firms of such companies as General Foods, Coca-Cola, Standard Brand, Ralston Purina, General Mills, etc. were established in Central and South America. The subsidiary companies Unilever, Nestle, Del Monte, etc. took hold in Africa. In 1975 130 largest food companies had more than 800 branches in the developing countries [20, pp. 19–23]. The transnational corporations put the import of equipment and technologies and fertilizers under control, monopolized production and sale, had a considerable effect on the pricing of food and agricultural machinery by means of the developed branch network.

3. Increase in the external expansion of oligopolies (the 70-ies – 90-ies of the XX century). During this period there is the rise of global oligopolists and the stable division of a number of markets by oligopolistic companies.

The distinctive features of oligopolists of that period were the following: the global approach to production and sales; the good knowledge of competitors and methods of global competitive struggle; the considerable volume of funding of scientific research; the management and coordination of functioning of their branches on the basis of modern information technologies; the adaptability of structure to the constantly changing conditions; the conclusion of integration agreements with other oligopolists; the inclusion of transnational banks and financial institutions carrying out, on international scale, the operations in taking over and merging to other companies, leasing, crediting and investment into the structure of oligopolistic companies; the reinforcement of interaction with the medium and small business both in the home countries and in the countries of implementation of their activities.

At that time the transnational corporations subordinated such a considerable part of world production, conversion and sale of food that they had an opportunity to provide global flows of food. The concentration and centralization of capital of the largest companies increased. In the mid-seventies 50 largest food companies of 30 thousand companies functioning in this branch in the USA got nearly 90% of profit of this sector [20, pp. 19–23].

4. Global oligopolization (the 90-ies of the XX century – the present). At this stage there is not only the globalization of activities of oligopolists from the developed countries, but also the fixing of oligopolists from the developing countries and the countries with economies in transition in the global market. Practically all oligopolists perform their activities in world markets.

The largest oligopolistic companies have the following distinctive features: the combination of globality and locality of activities; the high level of innovative activity and advanced information technologies in all fields of activity; the high opportunities of providing stability; the concentration of highly skilled work power; the diversification, finansialization and virtualization of activities; the formation of the system of cooperation with the national and regional authorities to provide the loyalty of authorities of the home country and the host country; the participation in strategic alliances.

In the developed countries the dominant position is held by the companies competing with each other in the markets of the majority of countries. Generally they belong to the extractive, pharmacological, electronic, electrotechnical and automotive industries. The formation of global oligopolies is directly related to the globalization of activities of large companies of not only tdeveloped, but also developing countries.

Characterizing the present stage of oligopolization, we can draw a conclusion that the stable domination of oligopolies of developed countries is peculiar for the modern economy. It is shown in the huge, constantly extending scales of their activity, the high level of influence on other subjects and the submission of them to their interests.

At the beginning of the 21st century there is the further consolidation of power of the largest companies functioning in food markets. The global food companies become the initiators of formation of global chains of added value on the way "from field to fork", splitting up the process of production of goods into a lot of stages and fragmenting their spatial placement. The functioning of companies as the owners of global chains of added value can be shown from the point of view of the opportunity to create the considerable amounts of added value which will not be redistributed for benefit of other participants of the chain, and the opportunity to take it from other participants of the chain. The domination arises proceeding from the opportunities of coordination of all the production process and establishment of proportions of cost distribution. The stronger positions the control link holds, the higher the redistribution scales.

State regulation of food markets

Referring to the strengthening of positions of the dominating entities of the food industry in food markets, it is also necessary to consider the issues of state regulation of this sphere. Taking into account the strategic importance of food markets, the governments of developed countries use various methods of regulation of these markets and support of food companies. The package of measures aimed at the support of grain markets, the purchase of food surplus, the fixing of threshold prices for the imported goods and the establishment of subsidies for the exported products, the help in promotion of products in the foreign markets and other measures are accepted.

In Russia in recent years the authorities have redoubled attention to food markets in relation with imposing the food embargo. Speaking about the ban to import products to Russia from the European Union countries at the Meeting of the State Council of the Russian Federation, the President of Russia V.V. Putin declared that this measure is caused by the need to protect the interests of Russians. "The limitations introduced against our country are nothing but a violation by some of our partners of the basic principles of the WTO. The principle of equal access for all countries involved in economic activity to the markets of goods and services is being violated; the most favoured nation treatment in trade and the principle of fair and free competition is being ignored" [21].

The prices of food products are under the spotlight of authorities. Fig. 2 provides the dynamics of consumer prices of food products across the Russian Federation in 2001–2015. There is a heterogeneous price behavior in this period. The last price surge took place in 2014–2015. The maximum growth rate of the prices for the last thirty years was in 1992 and was 26.2%.

The Russian Competitiveness Report 2015 prepared by the Federal Antimonopoly Service of the Russian Federation (the FAS of Russia) states that the FAS of Russia according to the competence determined by the Law on Protection of Competition controls food markets on a regular basis [22]. This work is aimed to avoid an unreasonable increase in prices in the specified markets, to suppress both the agreements and coordinated actions of the participants of food markets, and the agreements or coordinated actions of public authorities of subjects of the Russian Federation, the local government bodies and economic entities which can provide or already provide the non-admission, restriction or elimination of competition, and also the facts of acceptance of the acts, limiting competition, by the executive bodies.

The main aspects of such work are the following:

(1) Performing the analysis of goods markets for the purpose of assessment of competitive situation.

(2) The monitoring of markets of basic socially important goods for the purpose of control of monopolistic activities of economic entities regarding the establishment of economically unreasonable prices of food products by them.

(3) The implementation of control actions and checks in the markets of socially important goods.

(4) The control of actions of public authorities of subjects of the Russian Federation and municipal authorities aimed at competition restriction, including the establishment of restrictions of free movement of goods between the territories.

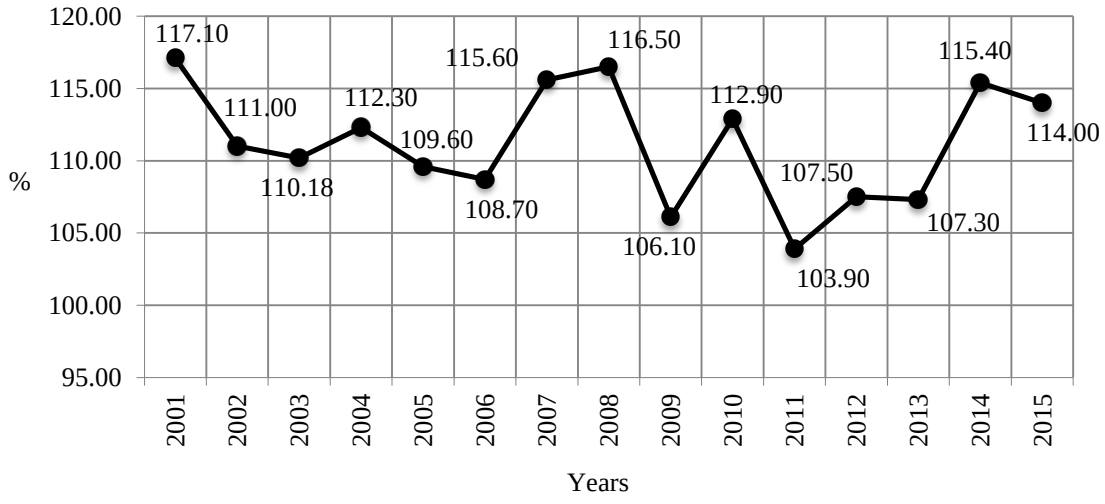
(5) The control of agreements of economic entities that provide the establishment and maintenance of prices in the market, the geographic division of the market according to the volume of sale or purchase of goods, and other violations.

(6) The control of observance of antimonopoly law when subsidizing agricultural producers.

(7) The control of providing with a non-discriminatory access to the infrastructure facilities of the grain market (elevators, warehouses, grain reception centres, grain terminals, rail and road transport).

(8) The decrease in administrative barriers.

(9) The development of relations between the retail chains and suppliers of food products, the control of observance of the law "'On the Fundamentals of State Regulation of Trade Activities in the Russian Federation" regarding the anti-monopoly control.



Source: Consumer price indexes across the Russian Federation in 1991-2016 – URL: http://www.gks.ru/free_doc/new_site/prices/potr/tab-potr1.htm (accessed 22 March 2016).

Fig. 2. Dynamics of consumer prices of food products across the Russian Federation in 2001–2015.

The FAS of Russia also performs a weekly monitoring of wholesale selling prices of specific types of food products. The list of products subject to monitoring includes: beef (except boneless meat), pork (except boneless meat), hen (except chicken quarters), whole frozen fish, butter, drinking milk, potatoes, fresh white cabbage, common onion, carrots, apples and buckwheat.

The subjects of monitoring are the companies and entrepreneurs included in the Register of Economic Entities (except for financial organizations) having the share in the market of certain goods in the amount of more than thirty five percent or holding the dominant position in the market of certain goods if the cases of recognition of provisions of economic entities dominating, and also the economic entities occupying an essential share in the corresponding goods markets, carrying out the production and/or wholesale of the goods included in the list, are established in relation to this market by other federal laws for the purpose of their application. The retail chains that often hold up the prices of food products got under the spotlight of regulatory authorities, as well. In addition to it, retail chains began to penetrate directly the sphere of food production, controlling all the process "from field to fork".

However, the situation in the food market can not be regarded stable yet. The development of new productions, the well-reasoned price policy, the expansion of measures of support of domestic agricultural enterprises and food productions is required. It will allow to increase the level of food security of the country and the competitiveness of Russian food companies.

CONCLUSION

The functioning of global food companies has a significant effect on the subjects of the economic relations, the food security of the country, the level and quality of life of the population. In modern conditions there is the strengthening of economic power of the largest companies by the redistribution of capital, property and imperious relations for the purpose of profit maximization. This process provides the formation of oligopolistic structure of the market or the formation of a tendency for oligopolization.

The peculiarities of functioning of global companies functioning in food markets will be the following:

– The considerable sales volumes, the inclusion into the leading world ratings;

– The expansion of range of produced goods to satisfy the requirements for food both of inhabitants of the developed countries (the demand shift from simple natural products to deep-processed products with a higher share of added value;

– The creation of high consumer value which is expressed in time saving, the convenience of acquisition, health benefit and the satisfaction of various taste preferences; in the compliance of quality of food products with the national and international standards), and of the low developed countries in which the demand for cheap and most available food is created;

– The availability of both diversified and profile companies functioning in food markets;

– The formation of tendency of entry of trading companies into the sphere of food production;

– The average level of innovative activity of food enterprises;

– The domination of companies of developed countries in the implementation of foreign investments while the companies from the developing countries and the countries with emergent markets are active foreign investors in the bank sphere, extractive industry, electronics and other branches. The developing countries and the countries with emergent markets, including Russia, are often the objects of investment of the companies from the developed countries;

– The preservation of considerable amounts of merges and takeovers in the sphere of production of food, drinks and tobacco products in the world (the maximum is in 2011);

– The domination of the companies with foreign capital in the markets of developing countries and the countries with emergent markets. This tendency is also characteristic of a number of Russian food markets.

Taking into account the strategic importance of food markets, the governments of developed countries use various methods of regulation of these markets and support of food companies. In Russia in recent years the authorities have redoubled attention to food markets in relation with imposing the food embargo. In the developed social and economic conditions the development of new productions, the well-reasoned price policy, the expansion of measures of support of domestic agricultural enterprises and food productions is required. It will allow to minimize the negative consequences of enlargement of dominating entities in the food industry, to increase the level of food security of the country and the competitiveness of Russian food companies.

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PROVIDING FOOD SECURITY IN THE EXISTING TENDENCIES OF POPULATION GROWTH AND POLITICAL AND ECONOMIC INSTABILITY IN THE WORLD

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Abstract: Food security, namely, the stability in availability of food for the population is important for the wellbeing and health of all mankind. In the modern world the destabilization of food the basic reasons of which are population growth, poverty, lack of investments into the agricultural industry, climate and weather, wars and resettlement, etc., is noted. The existing forecasts of the dynamics of population growth till 2050 are limited to 8–11 billion. The most part of this growth will accrue to the developing countries of Africa and Asia while the population of the developed countries, on average, will remain unchanged (except the United States of America due to international migration). The geographical distribution of undernourished people looks similarly, less than 5% of the population accrue to the developed countries, more than 12% – to the developing ones, on average in the world – more than 10%, with the prediction of further decrease. In spite of the fact that the percentage of mankind of the general biomass of our planet is insignificant, its activity is comprehended and anthropogenous as it became one of the most important forces changing processes in the biosphere. The interrelation of population growth and the necessary dynamics of food using the example of the protein, carbohydrate and fatty components in the world and in the former Soviet Union is considered in the work. The average forecast of population is used for the analysis. In case of the realization of the corresponding biotechnologies, the opportunity, if not of providing food security, then that of reduction of the number of undernourished people in the world, of food and energy resource conservation and of negative effect on the environment is quite achievable.

Keywords: Food security, growth of the number of mankind, hunger, Customs Union, Eurasian Economic Community

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INTRODUCTION

The mankind, in a varying degree, has faced hunger from the moment of its emergence. It is common practice to designate the decrease in agricultural production, industrialization and urbanization, armed and ethnic conflicts, the deterioration in the ecological situation as the reasons of hunger, however the uncontrollable population growth in the developing countries is considered to be the main one [1–2]. The global food deficiency is, as a rule, during world wars, natural cataclysms, epidemics, but it was until the 20th century when attempts to provide food security were made [3–6]. In 1945 the Food and Agriculture Organization (FAO) the main objective of which consisted in the assistance to agriculture for the reduction of the problem of hunger and poverty was created on the basis of the United Nations. In 1962 the World Food Programme of the UN was organized to confront world hunger. Ten thousand of people who annually help on average about 100 million undernourished people in 75 countries of the world (*www.ru.wfp.org*) work in the organization. In 1996 the member states of the UN adopted the Millennium Declaration (MPG) to improve the welfare of mankind by elimination of hunger, poverty and diseases (*http://www.un.org/millenniumgoals*) which resulted in the decrease in hunger by half during 1990–2015. In 2009 FAO organized the world summit on food security. Collateral events: the expert forum "How to Feed the World in 2050", the Committee on World Food Security and the World Food Day on which the declaration (WPS) with the statement for the decrease in hunger by half by 2015 (*ru.wikipedia.org*) was adopted. According to FAO (Fig. 1–3), (*http://www.fao.org/hunger*) the number of undernourished people in the world in 2015 was 793 million people (malnutrition and hunger, directly or indirectly, are the reasons of more than half of child deaths [7–10], the main reasons for death and invalidization of the person, owing to the reduction of body's immunity and the decrease in its immunodefences because of lack of nutrients [11–15]).

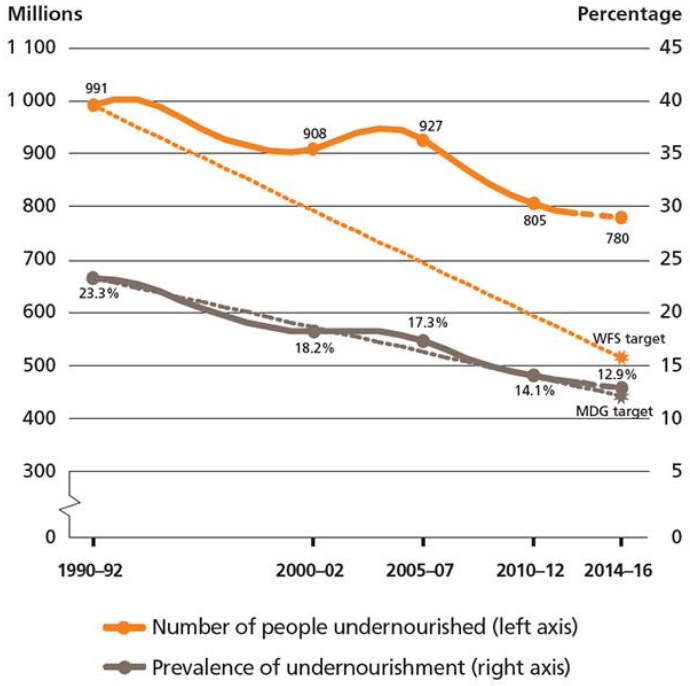
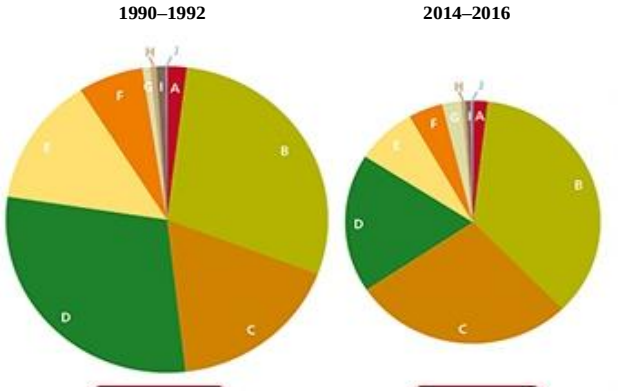


Fig. 1. Distribution of the number of hungry people in the developing regions: the real and expected result (Data for 2014–2016 are preliminary estimates, MPG is the Millennium Development Goals, WPS is the the World Food Summit).



		Population (mln)		Regional share (%)	
		1990–1992	2014–2016	1990–1992	2014–2016
A	The developed regions	20	15	2.0	1.8
B	South Asia	291	281	28.8	35.4
C	The countries of Africa to the South of the Sahara	176	220	17.4	27.7
D	South Asia	295	145	29.2	18.3
E	Southeast Asia	138	61	13.6	7.6
F	Latin America and the Caribbean region	66	34	6.5	4.3
G	West Asia	8	19	0.8	2.4
H	North Africa	6	4	0.6	0.5
I	The Caucasus and Central Asia	10	6	0.9	0.7
J	Oceania	1	1	0.1	0.2
	Total	**1011**	**795***	**100**	**100**

Note. * Including the data on Sudan which are not included in the indicators regarding the countries of Africa to the South of the Sahara, after the partition of the country in 2011 and the independence of South Sudan.

Fig. 2. Distribution of the number and percentage of undernourished people across the regions by 1990–1992 and 2014–2016. (The value of segments is proportional to the total number of undernourished people for every period. All the indicators are rounded. The data for 2014–2016 are preliminary estimates).

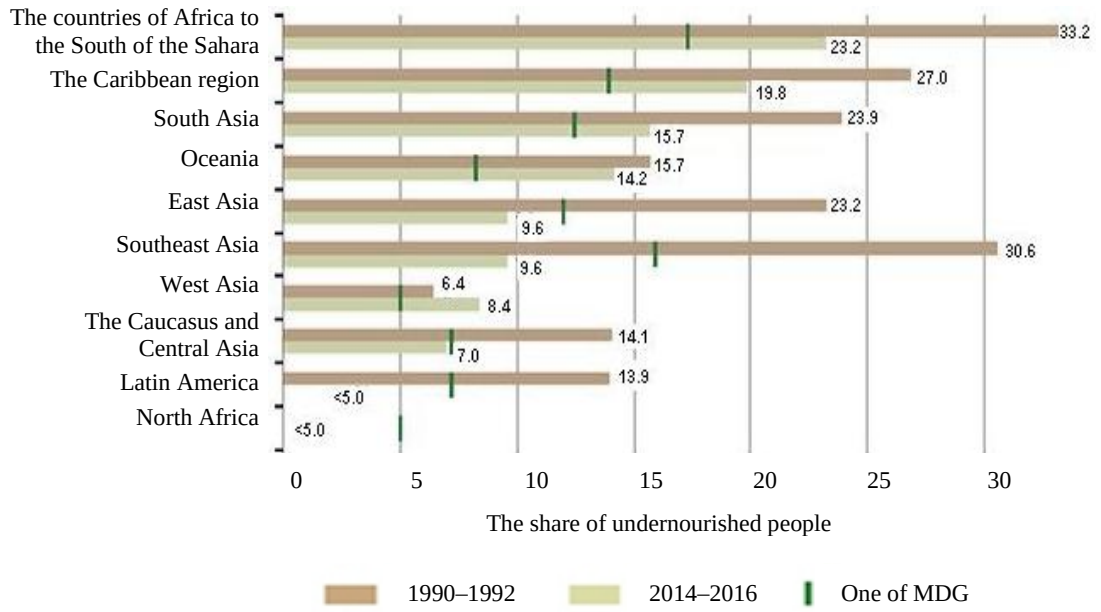


Fig. 3. Trends of decrease in malnutrition across regions.

In June, 2015 the head of FAO called for the global movement for full eradication of hunger, defining poverty as the reason for hunger. The last program of the UN "Zero Hunger" up to 2030 is based on a thesis about the possibility of eradication of the problem of hunger only after eliminating the interrelated reasons of poverty and, actually, hunger.

The actions and programs which performed by the UN act as the world platforms of discussion and development of the complex strategy of fight against hunger on the basis of scientific, ecological, technological, social and economic and political aspects [16–20].

The greatest intensity of addition of the population of the globe was in the 20th century, it is explained, first of all, by a significant industrial progress. According to different forecasts the population growth will continue [21–23], at least, to the middle of the 21st century and will have been from 8 to 11 billion people by 2050 (Fig. 4). For this reason, there is a need of providing all people with the available, good nutrition rich with nutrients [3, 6, 24–28]. The purpose of the overview is the analysis of opportunities of providing global food security and decrease in the number of undernourished people taking into account the population growth tendencies, including that of the developing countries (Belarus, Kazakhstan, Russia) of the Customs Union and the Euroasian Economic Community.

RESULTS AND DISCUSSION

The analysis and projection of population allow to regulate and, in case of need, to introduce amendments in the scenarios of distribution of the existing and the renewal of the missing resources. A long-term forecast allowing to distribute the efforts of mankind in the present circumstances of the civilization and distribution of natural resources is necessary for the the solution of the similar tasks.

The first attempt to estimate the population growth and the possibility of providing all people with food was made by the English economist Thomas Malthus [29, 30]. He considered that population increases exponentially if it is not restrained by any reasons, and it does not foretell anything good for the planet, a food and ecological crisis is ahead as the quantity of food resources grows only at an arithmetic rate.

There existed for a long time an opinion that the growth of the world population is subject to the hyperbolic law, however, the analysis of empirical data (past and the present) by modern science showed that the unrestrained growth is not possible under the conditions of the existing restrictions. And there was a delay of birth rate in the second half of the last century. The English biologist Julian Huxley gave the most exact forecast (1964) - the population of the planet will have reached 6 billion people by 2000, according to the UN, this date was October 12, 1999 [31, 32]. According to the UN, the increase in population is 74 million people now. The largest growth of the population occurs and will occur in the underdeveloped countries while the population of the developed countries will remain within the present limits. The USA can make an exception because of migration flows. According to FAO, it is supposed that the population of the planet will have exceeded 9 billion people by 2050.

Some of the most widely used forecasting models of population up to 2100 which, to some accuracy, have a single quality characteristic of dynamics are known, the difference is in quantitative values (Fig. 4).

These data include the forecasts of the UN, International Institute for Applied System Analysis (IIASA), the World Bank and the Russian science professor Sergey Petrovich Kapitsa who paid considerable attention to the study of problematics of population of mankind (Table 1).

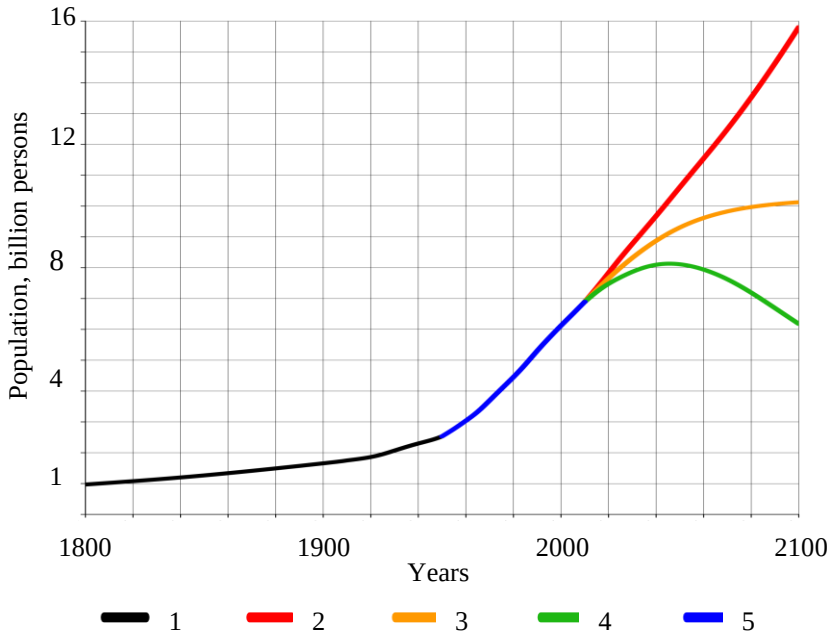


Fig. 4. Dynamics of growth of the world population with the results of the forecast till 2100 (*https://en.wikipedia.org/wiki/Projections_of_population_growth*): 1 – the calculated data (US Census Bureau historical estimates); 2 – the high case scenario of the UN; 3 – the medium case scenario of the UN; 4 – the low case scenario of the UN; 5 – the actual data.

Table 1. World Population Forecast by year (billion)

	2025	2100
UN	7.9–9.1	6.0–16.1
IIASA	8.1–9.91	9.2–16.0
WB	8.3	11.7
MK	8.1	11.0–12.0

The forecast of the UN is the total scenario of birth rate and mortality by nine regions of the world: the population of Earth will have reached the value of 11.600 billion by 2150 and further will be stabilized.

The forecasts of IIASA are based on the split of the world into six regions and the use of ten various scenarios of development, they will be in effect until 2100.

The World Bank is an international financial organization created for the purpose of providing the organization of financial and technical assistance to the developing countries, one of its activities is the strategic tasks of development of humanity and certain regions.

The mathematical model of Kapitsa S.P. (MK) defines the asymptotic transition to the limit of 12 billion in 2100, 11 billion are expected by 2050. Kapitsa's work showed that the growth of population of the Earth does not depend on additional variables during considerable time intervals, but only on the temporary variable and the population size. According to calculations, the stabilization of the world population is expected to be stabilized at the level of 14 billion persons by 2135 [33].

Fig. 4 provides the change of population of the Earth, taking into account the data of the UN (medium variant) and the existing forecasts. This situation will not go unnoticed in terms of providing the quality of life. There is already a certain territorial and food deficiency. Eleven children die of hunger every minute in the world. According to FAO, more than one billion people constantly suffer from hunger in the world. Most part of them lives in the Asian-Pacific Region and in Africa.

Fig. 5 provides the information about the percentage of undernourished people (*databank.worldbank.org*). Certain results of fight against hunger are obviously achieved. By 2012 the growth of the world population had been about 30% of the quantity in 1991 while the decrease in the number of undernourished people had been 25% against the background of the growth of total of the population. Considering the average prediction of population growth, the preservation of the existing tendencies of decrease in the number of undernourished people will have inherently provided the growth of their number by 2025. Neither programs of the UN nor the existing development of technologies including that in agriculture are effective to control this trend.

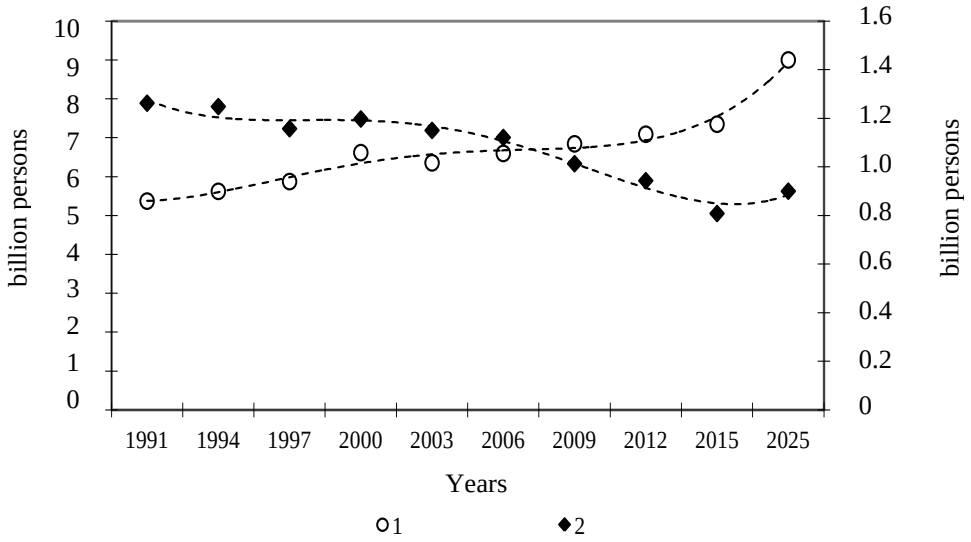


Fig. 5. Dynamics of growth of the world population and percentage of undernourished people in the world: 1 is population; 2 is the number of undernourished people.

There has been a sufficient increase in population growth with the absence of deficiency of livelihood [34] for centuries. During the last century the food production increased due to the development of technologies in the agro-industrial complex. However, the uncontrollable population growth results in lack of resources, poverty and hunger, on the one hand, and in problems of climate and more complex problems of mankind which in total will cause the shortage of food up to 25% of the necessary food (UNEP, 2009) in 2050.

On the one hand, the global problem of shortage of food is determined by the developed natural and ecological (lack of water, fertile lands, dense population) reasons, on the other hand, by political conditions (armed conflicts, economic crises) [35]. There is an opinion that any government, if desired, is capable to put an end to hunger [36]. Therefore providing food security is one of the primary needs of any state the effective solution of which is not possible without the integrated consideration of such problems as the dynamics of population growth, the availability of food resources, the amounts of food consumption, income level, etc.

There are more than 250 countries in the world, each of them has its own population and habits of food consumption. Meat, milk, cheese and eggs can be counted as the products used in the majority of the territories of the world, they can only differ [37] in type and grade. Anyway, however, not only providing food security of the country is desirable, but it is also necessary that this food should be both diversified and balanced. When planning rational nutrition, both age, sex, the level of physical activity and even climatic and national peculiarities are usually considered. The intensity of metabolic processes is determined by age and the its peculiarities of metabolic processes. According to (*total-rating.ru*) the data for July, 2013 the world population was 7.095 billion people, Table 2 gives the age distribution of them.

Table 2. Indicators of gender and age distribution of the world population

Age range, years	Percentage, %	Men, billions of persons	Women, billions of persons
0–14	26.0	0.956	0.894
15–24	16.8	0.614	0.578
25–54	40.6	1.479	1.447
55–64	8.4	0.298	0.312
> 64	8.2	0.265	0.331

Nore. For January 1, 2016 the ratio of men and women was 50.4% and 49.6%, respectively.

It is known that a balanced diet is necessary for the satisfactory functioning of the human body, the ratio of its main components is considered to be the following: proteins: fats: carbohydrates = 1 : 1 : 4. The protein intake is, on average, 1.5 g per 1 kg of body weight (not less than 30% of proteins must be of animal origin), the fats intake is 1 g per 1 kg of body weight (not less than 30–40% of them are vegetable fats), the carbohydrates intake is 6 g per 1 kg of body weight (only 10–15% of them are simple carbohydrates). Depending on physical activity, state and age there is variation of amount of protein from 30 to 150 g, carbohydrates from 350 to 500 g and fats from 40 to 170 g a day. During pregnancy (starting from 4th month) the protein intake increases to 2 g per 1 kg of body weight. The same amount of protein can be considered sufficient during the period of breastfeeding. During high physical activity (for athletes) the protein requirement increases up to 120–150 g [38–47]. To provide the growth and formation of the body and its systems children need both proteins and carbohydrates enough. With age, especially after 60, regardless of the intensity of work, there is a delay of rates of metabolic processes, the World Health Organization (WHO) recommends persons of this age to consider the reduction of energy

consumption approximately by 5%, which demands a decrease in the caloric value of food, first of all, on account of carbohydrates. In our country people adhere to the recommendations developed by the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences together with the Federal Service for the Oversight of Consumer Protection and Welfare taking into account the sex, age and the coefficient of physical activity (Table 3) [48].

Let us determine the necessary amount of consumed protein, fats and carbohydrates a day, considering the tendencies in the dynamics of population growth and the average body weight in relation to the necessary intakes. According to *expert.ru*, the average weight of the person in the world determined by the British scientists is 62 kg. Let us consider 93.0–124.0 g, 62.0–82.7 g and 372.0–496.0 g of protein, fats and carbohydrates a day, respectively, as the average indicator (Fig. 6).

Table 3. Physiological requirements of the human body for nutrients (g/day)

	proteins	fats	carbohydrates
Men	65–117	70–154	257–586
Women	58–87	60–102	257–586
Children*	36–87	40–97	170–420

Note. * older than 1 year old.

According to (FAO), 318 million tons of meat were produced during 2015: pork – 119 million tons, beef – 67.9 million tons, poultry – 111.8 million tons, the values of indicators for meat of turkey and rabbit are determined as approximately 5.0–6.0 million tons and 1.7 million tons, respectively. The production of meat is expected, in total, to be stable in 2016, and the production of poultry will increase. The world production of grain is predicted to increase by 2543 million (0.6%) in 2016 as compared to 2015. The production of wheat will decrease by 1.4%, barley – by 1.6%, the production of rice will increase only by 1%. The decrease is expected in Europe and is caused by the reduction of acreage, and also in Africa – because of dry weather, the most part of growth is planned in the countries of Asia, and also in Africa, North America and Europe. The production of corn in the United States of America, the world's largest producer of this crop, will increase. The world consumption of grain will be 2546 million tons in 2016/17 which is 0.9% higher than the corresponding indicator in 2015/16. The production of grain, from a global perspective, continues to improve though the expected world production and consumption of grain do not still correspond to each other. There are 37 countries needing the help from outside in providing with food, including 28 in Africa.

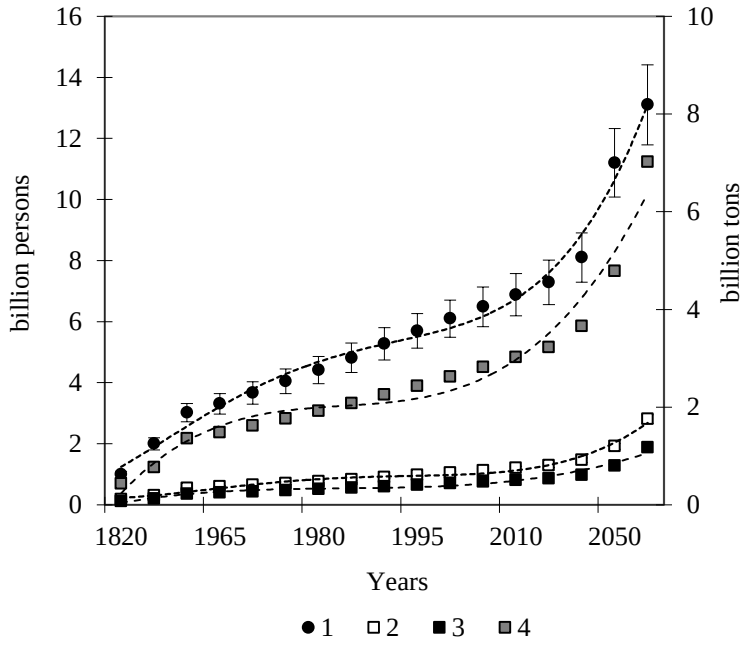


Fig. 6. Dependence of the world population and the protein intake by year: 1 – the world population, 2 – the necessary daily intake of protein, 3 – the necessary daily intake of fats; 4 – the necessary daily intake of carbohydrates.

FAO considers that it is necessary to increase the production of agricultural goods by 60% to provide the increasing population with food. According to the report on the agricultural markets for 2015/16, a possibility of achievement of the necessary increase by the investment of about $83 billion in the world sector of the agrarian and industrial complex is considered. It is obvious that to reach the necessary productivity (Fig. 5) of new territories and technologies, for example, modifications at the level of genomes, is not possible without investment attraction [49–52]. Critics claim that the development of agricultural technologies, including the creation of genetically modified organisms and products, provides an increase

in the income of the limited population group, but not globally at all to feed all the population. However this trend, sooner or later, will provide an environmental disaster. The remaining conflicts, degradation of agriculture and weather cataclysms are the main reasons for the destabilization of food security in 2016. Solving the arising problem of hunger of the planet needs to be begun already now. One of the obvious trends is the increase in sowing territories, the countries of the former Soviet Union have the largest potential in this perspective. At the time of the USSR, its territory occupied a sixth part of the land and approximately a tenth part of all the farmlands of the world. It is no wonder that a lot of developed countries the economic stability of which release them from hunger or malnutrition do not always show peaceful interest in these territories, especially in combination with the thoughts of open spaces of the undeveloped lands of the Russian Federation. It also explains both the imposed sanctions and the hostile rhetoric in a number of issues of the world culture. Providing with food security in the former Soviet Union was just one of the basic reasons for the creation both of the Customs Union and the Common Economic Space.

Table 4 provides the data (*databank.worldbank.org*, *faostat3.fao.org*, [54–60]) about the population and productivity by the main types of agricultural products. According to the physiological requirements of food, grain and grain crops, vegetable and animal oils and also meat and dairy products (have the property of interchangeability) can be referred to the group of main products. According to the offer of FAO, food independence is reached by the production of vital products at the level of not less than 80% of the requirement of the population.

The demographic recession in the former Soviet Union was determined by the political and economic processes accompanying the change of statehood. There is a positive change in the increase in the population both in the Russian Federation and in the Republic of Belarus last years, and Kazakhstan managed not only to restore the population to the Soviet maximum, but even to exceed it approximately by 7%. However, none of the considered models (Table 1) assumes a significant population growth in these territories, moreover, they insist on a considerable decrease in the population as well as in other developed countries of Europe and America.

Table 4. Dynamics of the population and production of foodstuffs (grain and meat products and fats) of the developing countries of the Euroasian Union: the Russian Federation, Kazakhstan and the Republic of Belarus

Years	1990	1994	1997	2000	2003	2006	2009	2013	2015
Russian Federation									
Population, millions of persons	148.292	148.336	147.915	146.597	144.648	143.0495	142.785	143.507	144.097
Cereal production, millions of tons	116.700	78.644	86.801	64.326	65.562	76.495	95.616	90.382	103.400
Meat production*, millions of tons	10.112	6.803	4.854	4.440	4.950	5.237	6.720	8.544	9.484
Fat production**, millions of tons	1.822	1.615	1.264	2.041	2.348	3.553	3.731	3.839	3.805
Kazakhstan									
Population, millions of persons	16.348	16.095	15.334	14.884	14.909	15.308	16.093	17.035	17.544
Cereal production, millions of tons	28.500	16.375	12.359	11.539	14.741	16.461	20.764	18.157	19.950
Meat production*, millions of tons	1.548	1.207	0.722	0.623	0.693	0.807	0.893	0.871	0.903
Fat production**, millions of tons	0.160	0.087	0.031	0.053	0.141	0.185	0.185	0.277	0.314
Republic of Belarus									
Population, millions of persons	10.189	10.227	10.117	10.005	9.797	9.604	9.507	9.466	9.513
Cereal production, millions of tons	7.000	5.938	5.924	4.565	5.117	5.685	8.154	7.233	9.104
Meat production*, millions of tons	1.181	0.743	0.632	0.598	0.605	0.768	0.921	1.172	1.150
Fat production**, millions of tons	0.087	0.094	0.098	0.0932	0.084	0.102	0.133	0.114	134.147

Note. * total of meat, offal and poultry; ** total of butter and ghee, sunflower oil and margarine.

Despite the increase in production of agricultural products, the food security of the developing member states of EEU is not reached. The best success in providing with food was achieved by Belarus, on some points – by Russia and Kazakhstan. Nevertheless, one should not postpone the solution of the problem of food security, as it is one of the reasons for creation of EEU. The productions of food by the states of the community must supplement harmoniously each other retaining the economic and political independence from other countries. The percentage of mutual export of the countries of Community, in its total amount, is at the level of 30%, import – of about 15%. The highest percentage of intraregional export accrues to Belarus (more than 85%), Kazakhstan and Russia – 10.7% and 14.9%, respectively. The highest percentage of intraregional import accrues to Kazakhstan (more than 40%), the lowest – in Russia (10–12%) [61].

CONCLUSION

An opinion has recently emerged [62–66] that the organic, i.e. non-synthetic, agriculture is the only that is capable to feed the world. The performed researches (FAO, 2014) led to a conclusion that small or family enterprises can provide food security and protect both certain regions and the world in general from an environmental disaster. The tendencies of increase in the productivity of grain, fruit and vegetable crops, the fruits and plants of which also act as the protein sources in the human diet, allow to consider the problem of the existing hunger as the one that can be positively solved [67–73]. And it is necessary to begin with providing with food for the population of each of the countries separately which will result in total in stability in the food security of the world. However, the increase in the productivity and quantity of food products on the existing farmlands is a necessary but not sufficient condition of achievement of the global food security. A third of all the produced foodstuffs spoils, but people die of hunger every day in the world. At the same time, in today's world (FAO, 2015), taking into account foodstuffs and vegetable raw materials of biofuel, enough food is produced for provide the day-to-day consumption of about 2850 calories for each person, at the same time it is not possible to put an end to hunger. Even if we assume that the production of food will remain at the present level, and the population will reach 9 billion, then the daily energy consumption will decrease to 2200 calories, this quantity is quite enough for the satisfactory functioning of the human body.

No more than 38% of total of acreage in the world have an agricultural purpose, more than 43% in Belarus, more than 80% in Kazakhstan and about 13% in the Russian Federation. There is obviously a certain potential of providing with agricultural products due to the increase in farmlands, especially for the Russian Federation. The development of agro-industrial complex of Russia will allow to provide with food not only for its citizens but for all the former Soviet Union. There was a certain opportunity to fulfil this stipulation by imposing economic sanctions and food embargo by a number of the European states in 2014 in relation to the events in Ukraine. To accomplish the purposes of agrofood policy of Russia the intensive import substitution of meat, milk, vegetables, seed potatoes and fruit and berry products was required. Unfortunately, the domestic manufacturer did not bring the expected support and instead of import substitution there was an "import exchange" of the producers of the EU by the producers of the countries of Latin America or the Middle East which affected without delay the prices of food [74]. As a result, we did not only come closer in providing food security, there was the aggravation of the existing problem, first of all, due to the power of consumption of Russians which had already been reduced by the considerable depreciation of internal currency at the end of 2014. And, though, according to FAO, there are no more than 5% of undernourished people in the countries of the Customs Union and Euroasian Economic Community, we should not forget about them, and all the efforts should be aimed at the increase in the availability of the received food, as well.

There is an opinion that poverty and the absence of political power are more paramount reasons of hunger and malnutrition than the capability of the region to produce foodstuffs. According to the estimates of FAO, there is a change of geography of poverty in the world markets, against the background of economy growth the income in a number of developing countries begins to be similar to the income of the developed countries. Before now, poor people generally lived in the countries with the low level of income, now 1 billion people who are below the poverty line live in the countries with the average level of income, for example, in India.

Therefore the global problems of hunger can not be solved by a simple increase in productivity, the integrated approach is needed including both the development of knowledge-intensive and breakthrough biotechnologies allowing to preserve the produced foodstuffs, at least, until its delivery to the consumer, the wide application of logistics and the reasonable development of agricultural productions locally. A lot of experts both in our country and abroad consider that a clear state policy in this area of the developed countries will help to address the problem of food security quite sufficiently.

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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.

Having been approved by the scientific consultant, the content submitted for publication undergoes reviewing. The reviewer is appointed by the editor-in-chief or the deputy editor.

The journal only publishes the manuscripts recommended by the reviewers. Both the members of the editorial board and highly qualified scientists and specialists from other organizations and enterprises can be invited to review the manuscripts. They are to have profound professional knowledge and experience in the specific field of science and are to be doctors of sciences and professors.

The reviewers are notified that the manuscripts are copyrighted materials and are not subject to public disclosure. The reviewers are not allowed to copy the articles for their private needs. Reviewing is performed confidentially. Violation of confidentiality is impossible unless the reviewer declares unreliability or counterfeiting of the article's materials. The reviews' originals shall be kept by the editorial board for three years since the publication.

If the reviewer says that improvement is necessary, the article shall be sent to the author for follow-on revision. In this case, the return date of the modified article shall be considered the date of the article submission.

If, on the recommendation of the reviewer, the article undergoes a considerable revision by the author, it is again sent to the reviewer who gave the critical remarks. The editorial board reserves the right to reject the articles in case of the author's inability or unwillingness to take into account the editor's recommendations.

In case of two negative manuscript reviews from different experts or one negative review on the article's modified variant, it is rejected without consideration by other members of the editorial board. After reviewing, the possibility of publication is decided upon by the editor-in-chief or, if necessary, by the editorial board as a whole.

The official responsible for publication sends a motivated refusal to the author of the rejected article. The reviewer's name may be reported to the author provided that the former gives consent to it.

The editorial board does not retain rejected articles. Accepted manuscripts are not returned. The manuscripts with negative reviews are neither published nor returned to the author.

As a rule, manuscripts are published in the order of their submission to the editorial board. In exceptional cases the latter has the right to change the order of priority for the articles.

If the editorial board does not share the author’s outlooks completely, it may give a footnote remark on this point. Manuscripts published for the purpose of discussion may be supplied with corresponding remarks.

The editorial board has the right to publish readers’ letters giving evaluations of the printed articles.

The authors assume responsibility for authenticity of the information presented in the article. It should be original and should not have been published before or submitted to other publishing organizations.

The editorial board is not responsible for falsity of the articles’ data. Any copyright violations are prosecuted by law. Reprinting of the journal’s materials is only allowed upon agreement with the editorial board. A written consent of the editorial board for reprinting and references to the journal *Foods and Raw Materials* for citing are compulsory.

The authors are not expected either to pay fees for publication or to be given a reward or free copies of the journal.

FORMATTING REQUIREMENTS FOR ARTICLES

The journal publishes original articles on problems of the food industry and related branches in the English and German languages.

Along with experimental works, the journal publishes descriptions of fundamentally new research techniques and surveys on selected topics, reviews, and news items.

Manuscripts submitted for publication should meet the following basic criteria: validity of data, clarity, conciseness, reproducibility of results, and

compliance with manuscript requirements. In discussing the results, it is mandatory to set forth a sound conclusion on the novelty of the content submitted for publication.

The articles are accepted in the text editor Microsoft Word using the font Times New Roman, font size 10. The manuscript should contain no less than 7–10 pages, typewritten with the single line spacing and having 2-cm-wide margins on all sides. The article size also includes an abstract, tables, figures, and references.

Each article sent to the journal should be structured as described below.

1. In the top left corner of the first page there is the UDC (Universal Decimal Classification) index.

2. Title. It is necessary to give a short, informative, and precise name of the work.

3. Name(s) and initials of the author(s).

4. The name(s) and affiliation to the institution(s) where the research was carried out, the country, city, zip code, e-mail address and phone (of the author).

5. Abstract. An abstract of 180–250 words should reflect fully both the main results and the novelty of the article.

6. Key words (no more than 9).

7*. Introduction. A brief review of the problem dealt with in the study and the validation of the approach taken are presented. References are given in square brackets and numbered (beginning with no. 1) in the order of their appearance in the article. With several references appearing in sequence, they should be placed in the chronological order. The aim of the study should be clearly formulated.

8*. Objects and methods of research.

– For describing experimental work, the section should contain a full description of the object of the study, consecutive steps of the experiment, equipment, and reagents. The original names of equipment and reagents should be specified, and the manufacturer's name (company, country) should be given in parentheses. If a method is not widely known or is considerably modified, please provide a brief description in addition to the reference;

– For presenting theoretical research, the section should contain the tasks, approximations and assumptions, conclusions, and solutions of basic equations. The section should not be overloaded with intermediate data and the description of well-known methods (such as numerical methods of solving equations) unless the authors have introduced some novelty into them.

9*. Results and discussion.

– The section should provide a concise description of experimental and/or theoretical data. Rather than repeating the data of tables and graphs, the text should seek to reveal the principles detected. The past indefinite tense in describing the results is recommended. The discussion should not reiterate the results. This section should be completed with a major conclusion that answers the question specified in the introductory part of the article.

* In case of surveys, these sections do not need to be entitled. The contents may present an analytical survey of the problem chosen and give the widest reflection of the existing points of view and data related to the theme. The article should necessarily contain the grounds for the problem's timeliness and the author's conclusion on the prospects of the approaches given for the solution of the problem analyzed.

Each table is to consist of no less than three columns and have a number and a title. The journal publishes black-and-white photographs and diagrams.

Recommendations for typewriting formulae: mathematical equations forming a separate line should be printed in the MathType formula editor as a whole.

It is not allowed to combine a table, a text, and an inserted frame within the same unit. For the equations printed in the MathType, it is necessary to keep to the standard style of symbols and indices, as well as their size and placing. Forced manual changing of individual symbols and formula elements is not allowable!

10. References.

They should be formatted according to the common standard. The list of papers is typed on a separate page in the order of their appearance in the text. All authors of each cited paper are indicated. If there is an English version of the paper, it is necessary to refer to it, indicating the DOI.

For cited journal articles, the names and initials of all authors, the name of the article, the name of the journal (for foreign journals, it is necessary to keep to the CASSI), year, volume, number, and page are indicated.

For cited books, the names and initials of the author(s), the name of the book, publisher, year, volume, number, part, chapter, and page are given.

For cited collections of articles, abstracts; conferences, symposiums, etc., the author(s), the name of the work, the name of the collections (conference, symposium), city (place of holding), publisher, year, volume, number, and the paper's first page are indicated.

References to web resources are to be given in the body of the text.

There should be no references to publications that are not readily available. These include institutional regulations, state standards, technical requirements, unpublished works, proceedings of conferences, extended abstracts of dissertation, and dissertations.

The absence of references to foreign authors and to the cited papers of 2–3 year novelty reduces the chances of a manuscript's publication. The references should reflect the actual impact of representatives of different countries on the investigation of a particular problem.

11. The following information should be sent in English: the title of the article, the authors' initials and surnames, an abstract, key words, the name of the institution (with the mailing address, telephone number, and e-mail).

Documents may be presented in the Russian, English, and German languages.

The following papers are sent to the editorial board.

1. A soft version of the article typewritten in MS Word. The file of the article should be entitled by the first author's surname, e.g., PetrovGP.doc. The data file must only contain a single document.

2. A printed copy of the article identical to its soft version. In case of discrepancies between them, the editor gives preferences to the electronic version of the manuscript.

3. Personal information: the full names of all authors, the place and the mailing address of their places of work, the subdivision and position, the academic degree and rank, the honorary degree, the telephone number, the personal mailing and electronic addresses, the date of birth, and a reference to the author's scientific profile.

The author to contact is indicated by an asterisk. The file should be entitled with the first author's name, e.g., PetrovGP_Anketa.doc.

4. A cover letter to the editor-in-chief from the responsible organization with the conclusion about the work urgency and recommendations for its publication, carrying the date, reference number, and the head's signature.

5. An external review on the article according to the sample, the reviewer's signature being authenticated by the corresponding HR subdivision.

6. A standard copyright agreement. The manuscript's electronic version may be e-mailed to the editorial office at fjournal@mail.ru.

RECOMMENDATIONS

For the article file use the format *.doc. Do not use Russian letters and spaces in the file's name.

The article file is to be identical to the printed original submitted to the editorial board.

The article's textual file is to include the title of the article, an abstract, a structured text, references, a separate sheet of captions for the diagrams, and tables (each on a separate sheet). Structural chemical formulae are placed in the body of text.

The articles are accepted in the text editor Microsoft Word using the font Times New Roman for texts, Symbol for Greek letters, and MathematicalPi2 for handwritten and gothic symbols. The standard font size is 14.

For tables, use Word (Table – Add Table) or MS Excel.

Recommendations for typewriting formulae

Mathematical equations forming a separate line should be typed in the MathType formula editor as a whole.

It is not allowed to combine a table, a text, and an inserted frame within the same unit. For the equations printed in the MathType, it is necessary to keep to the standard style of symbols and indices, their size and placing. Forced manual changing of individual symbols and formula elements is not allowable!

Dimensions

They are separated from the figure by a space (100 kPa, 77 K, 10.34(2) A), except for degrees, percent, and permille: 90°, 20°C, 50%, 10‰. Fraction dimensions: 58 J/mol, 50 m/c^2.

For complex dimensions, it is allowed to use both negative degrees and parentheses (J/ mol-1 K-1), {J/ (mol K) or J(mol K)-1}, if it makes reading easier. The main requirement is the unified manner of writing dimensions.

While enumerating and giving numerical spaces, the dimension is set for the last number (18–20 J/mol), with the exception of angular degrees.

For Celsius degrees: 5°C, not 5°. Angular degrees are not omitted: 5°–10°, not 5–10°.

Dimensions for variables are written with a comma (E, kJ/mol); for logarithms, in square brackets without a comma: ln t [min].

Spaces between words

References to diagrams and tables are typed with spaces (Fig. 1, tab. 2).

Inverted commas and brackets are not separated by spaces from the included words: (at 300 K), (a); not (at 300 K), (a).

There should be a space between the № or § sign: (№ 1; § 5.65), but no space in numbers with letters: (IVd; 1.3.14a; Fig. 1d).

In geographical names, there is a space after the period: p. Енисей, г. Новосибирск.

References to cited works

Initials are put after authors' and editors' names and are not separated by spaces: Иванов, А.А., Petrov, B.B.

The year, volume, number, etc. are separated from one another and from the figures by spaces: 1992, n. 29, № 2, С. 213. or 1992, vol. 29, no. 2, pp. 213.

To refer to the issue number of both Russian and foreign journal, use the № sign. In the titles, the word *journal* is shortened for Journ. / Журн.

Before the year of issue after the publisher's name or city (if no publisher), there is a comma.

Graphic material

The journal publishes black-and-white illustrative material.

While making graphic files, keep to the following recommendations:

Vector pattern, schemes, and diagrams are preferably to be presented in the format of the application in which they were carried out or in EPS. ***For other illustrations***, it is preferable to use TIFF and JPEG formats, optimum resolving capacity being 300 dpi.

Photographs should be submitted in 2 variants. The first one should correspond to the paper-based original, with marks and inscriptions; the second one should not contain any text, captions, etc. The advisable file formats are TIFF and JPEG, the optimum resolving capacity being 300 dpi. The gray color shade is allowed from 9 to 93%.

All illustrations are to be saved as separate files in the folder. Every file contains one picture.