Researching of fatty acids and amino acid structure of yogurts with use of mare's milk

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Abstract. Mare's milk is special dietary raw materials with treatment-and-prophylactic properties. The only product released by the industry from mare's milk in Russia is koumiss. However, koumiss is an alcohol-containing product. There is a problem of processing of mare's milk on functional fermented milk products. The most popular fermented milk product, among the population, is yogurt. We developed compoundings of two types of yogurt with use of mare's milk, the patent application is submitted No. 2017117840 RU for one of them of 22.05.2017 "Fermented milk product". The purpose of work is a research of fatty acids and amino acid structure of new yogurts with use of mare's milk. For studying two samples of yogurts were made. As raw materials mare's milk of the Bashkir breed was used. Production and researches of prototypes of yogurt were conducted on the basis of "Bashkir State Agrarian University"(Ufa), the accredited laboratory center FGBUN "FITs food and biotechnologies" (Moscow). The analysis of fatty acids composition of samples of yogurts showed that they have high content olein, γ-linolenic, α-linolenic and arakhidonovy acids. Data on the content of amino acids in yogurts with use of mare's milk show that they are characterized by the high content of the irreplaceable amino acids proving the high biological value of ready-made products. The research is executed with financial support FBSI "Fund of Assistance to Development of Small Forms of the Enterprises in the Scientific and Technical Sphere" within state contract No. 1202GS1/21741 of 05.05.2016.

1. Introduction

Scientists and practicians of various countries of the world work on a problem of development of the balanced food allowance by decrease in a mass fraction of high-calorific fats and increase in a share of proteins. In a food allowance of the numerous people the deficiency of protein which lack, especially biologically full, affects a physical condition of the person, decrease in his working capacity is noted, causes irreversible changes in an organism and contributes to the development of serious diseases [1–5]. One of solutions of this problem is development and production of food with the set structure and properties. Recently the question of correction of fatty acids structure of products is relevant.

Fats differ with the chemical composition and physiological properties [1, 2, 4–6]. The origin of raw materials from which receive fats is the basis for one of classifications of fats. They are divided into two main groups – vegetable and animal. Each of groups is subdivided into solid and liquid fats depending on their consistence which is defined by the chemical nature of triglycerides. Palmitic or oleic acid are necessary for protection of heart and vessels, prevent strokes and heart attacks. Stearin acid provides protective functions of an integument to influence of temperature conditions. Linoleic
acid belongs to the irreplaceable fatty acids necessary for normal activity; this acid comes to a human body with food. She supports immune, cardiovascular and reproductive systems, provides normal development and adaptation of a human body to adverse factors of the environment [7].

Follows from the analysis of the current state of nutrition, biological value of milk of different types of farm animals (cow's milk, sheep milk, buffalo milk, camel milk, milk of a horse, asinine milk, goat milk, etc.) that milk of a horse is comparable on composition of protein to female milk, has hypoallergenic properties and has rich amino-acid structure [7–9]. Mare's milk, mix of mare's and cow's milk is an important raw reserve for production of foodstuff. Mare milk in proteinaceous structure is similar to milk of a she-ass. From the world literature it is known that mare's milk has treatment-and-prophylactic properties and can be used proceeding from the amino-acid and fat and acid structure when developing the new functional food enriched with protein with the lowered maintenance of a mass fraction of fat [7, 10]. In the last several years research works on creation of products of functional purpose with application of mare's milk were sped up [11]. However, the problem of processing of mare's milk remains relevant and till present that interferes with development as dairy horse breeding, and to creation of new food with application of mare's milk. The only product released by the industry from mare's milk in Russia is koumiss. Koumiss - an alcohol-containing product that limits a circle of his consumers. The most popular fermented milk product, among the population, is yogurt. The average European annually uses about 16 kg of yogurt, in the Russian Federation this indicator doesn't exceed 5 kg on the person. Influence of consumption of yogurt as dietary product at all stages of human life plays an important role in model of healthy adequate food [12–14]. The work purpose is development and a research of fatty acids and amino-acid structure of prototypes of yogurt with use of mare's milk.

2. Experimental research
Pilot and theoretical studies have been conducted in 2016-2017 on the basis of Bashkir State Agrarian University (Ufa) in partnership with LLC «National products and services» (Ufa).

Researches were carried out in 2 steps. The purpose of the first stage – preliminary development, production and a research of prototypes of yogurt on the basis of mare's milk with the analysis of organoleptic properties and nutrition value of a product.

Object of a research were the main components during the developing and production of yogurt: mare's milk and dairy mix with use of mare's milk, ferment for yogurt, powdered skim milk, cow's milk, the stabilizer (without stabilizer). The raw materials used for preparation of yogurt conformed to the national standards of the Russian Federation shown to raw materials for production of fermented milk products. Milk of mares of the Bashkir breed of State budgetary institution of the Republic of Bashkortostan the State factory stable was used the "Ufa" (Ufa).

At the first stage components of prototypes of yogurt have been picked up, prototypes of yogurts are developed and made. For compliance of prototypes of yogurt from mare's milk to definition "yogurt" in accordance with GOST 31981-2013 "Yoghurts. The general specifications" powdered skim milk in a certain quantity is added. Preparation of yogurts was carried out on the technology described in the patent for the invention of the Russian Federation No. 2 350088.

The main ingredients, technologies and trends on preparation of yogurts are stated in the public world literature [15–16].

Selection of amount of powdered skim milk varied in the range from 1% to 10% with a step of 1% of the lump of dairy mix: 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%. At selection of fermenting cultures for fermentation of mix of mare's milk with cow in the course of test seven types of ferments have been used. The list of ferments at preparation of prototypes of yogurt: ferment of direct introduction: YF-L811 (Chr. Hansen, Denmark); YF-L904 (Chr. Hansen, Denmark); YFHarmony 1.0 (Chr. Hansen, Denmark); YO-MIX 495 (Danisko, Denmark); AiBi Lbs series 22.11 (LLC «Green line», Russia); AiBiGoldenTime Lbs series 22.44 (LLC «Green line», Russia); KTS ferment-bakkoncentrat (Federal Public Budgetary Scientific Institution"All-Russian Research Institute of the
dairy industry", Moscow). As criterion for evaluation of process of fermentation served increase of acidity, and souring duration, density, conditional viscosity, organoleptic indicators.

For the purpose of optimization of structure and structure of a dairy basis from mare's milk and mix of mare's milk with cow 30 options have been tested. The optimum ratios of mix of mare's and cow's milk enriched with powdered skim milk are picked up. Types of ferments of direct introduction for fermentation which have in the best way affected physical and chemical and organoleptic indicators of a ready-made product are picked up, the qualitative and quantitative nature of change of characteristics (fat, protein, carbohydrates) of yogurt prototypes depending on a share of the brought components is established. When developing products for food special attention was paid to organoleptic indicators as the choice of a product is connected with her pleasant taste. Organoleptic assessment was carried out on 5 to a ball scale. The best organoleptic indicators at touch of assessment of prototypes of yogurt are received with ferment of direct introduction YF-L904 Thermophilic Yoghurt Culture - YoFlex ("Chr. Hansen", Denmark). The purpose of researches of the second stage – pilot study of fat and acid and amino-acid structure of prototypes of yogurt, assessment of safety of consumption. Researches are relevant in the light of the increased demand of the population to yogurt – dietary fermented milk product with a high mass fraction of solid and also gives the chance to development of dairy horse breeding, and promotes performance of strategy of import substitution of raw materials and dairy products with use of mare's milk.

3. Results and considerations

The power value of foodstuff is determined by the chemical composition. The main components bearing energy in mare's milk yogurt are fats, proteins, lactose. Therefore, first of all defined a mass fraction of fat, protein and carbohydrates. Nutrition value of a sample No. 1 (100 g of a product): fat-2.3 g; protein-4.37 g; lactose-5.85 g. Nutrition value of a sample No. 2 (100 g of a product): fat-2.36 g; protein-4.31 g; lactose-5.25 g. On the basis of results of tests calculation of power value of yogurt from mare's milk is made. The power value of prototypes of yogurt was: a prototype No. 1 – 61.4 kcal, a prototype No. 2 – 59.5 kcal.

Determination of fat and acid structure of 2 types of prototypes of yogurts has been made in the accredited FGBUN test laboratory center "FTTs of food and biotechnology". At the same time have determined content 15 saturated and 19 nonsaturated (from them 7 polyunsaturated) fatty acids.

The analysis of results of the conducted researches showed that from saturated fatty acids differ in high content in the presented yogurts myristic (9.61%-10.82%), palmitic (32.53%-34.61%) and stearin (6.76%-7.86%). Among monounsaturated fatty acids the high rate characterizes oleinic acid (20.06%-21.92%). From polyunsaturated fatty acids the greatest value was shown linoleic (4.36%-6.18%) and α-linolenic (1.84-2.82%) by acids.

Analyzing aliphatic and acid structure of exemplars of yogurts, it is revealed that the exemplar of yogurt from mix of mare's milk (68%) and cow's milk (28%) with addition of 4% of milk skim milk an exemplar No. 1 possesses high the maintenance of oleic – 21.92%, γ-linolenic – 0.03%, α-linolenic – 2.82% and also arachidonic – 0.24% of acids.

The exemplar of yogurt No. 2 of mix of mare's milk (48%) and cow's milk (48%) with addition of 4% of skimmed milk powder for which the content of acids is established is on the second place: oleic – 20.06%, γ-linolenic – 0.02%, α-linolenic – 1.84%, arachidonic – 0.21%.

Cooperative content of saturated fatty acids varies from 60.29 for an exemplar No. 1, up to 65.35% for an exemplar No. 2 respectively, and unsaturated fatty acids 39.36 and 34.28 of %. The relation of unsaturated fatty acids to saturated makes 0.65 and 0.52 respectively for an exemplar No. 1 and No. 2.

Thus, optimum aliphatic and acid structure from the presented products the exemplar of yogurt from mix of mare's milk (68%) and cow's milk (28%) with addition of 4% of skimmed milk powder (No. 1) has.

Compared the data on aliphatic acid structure on cow's milk yogurt of 3.2% of fat content provided in the reference book of chemical composition of the Russian food [4]. Having carried out the analysis of aliphatic and acid structure on reference data on cow's milk yogurt of 3.2% of fat content with
exemplars of yogurt from mix of mare's and cow's milk No. 1, 2 follows that the aliphatic and acid structure of a product changes and corrected in such a way that in yogurts from mix of mare's and cow's milk the content of C_{16}H_{23}O_2 palmic acid from 18% increases to 32.53%-34.61%, the content of C_{14}H_{28}O_2 tradecanoic acid decreases from 14% to 9.61%-10.82%, the content of stearic acid decreases from 9.6% to 6.76%-7.86%, and the content of linoleic polyunsaturated acid from 0.6% increases to 4.36-6.18%. The research of aliphatic and acid structure confirms the fact that at introduction to lactic mix, from cow's and mare's milk the aliphatic and acid structure of yogurts is corrected towards increase in content of polyunsaturated fatty acids. Increase in contents α-linolenic ω-3 acids as the human body does not reproduce ω-3 acid self-contained is especially important.

The biological value of foodstuff is characterized by the level of content of amino acids and especially – irreplaceable. The amino-acid structure was defined in the accredited FGBUN exploring laboratory center "FITs of a delivery and biotechnology" (Moscow).

Data on the content of amino acids in yogurts with use of mare's milk are presented in table 1.

**Table 1.** Amino-acid structure of yogurts.

| Amino acids | Structure of yogurt | Exemplar of yogurt from mix of mare's milk (68%), cow's milk (28%) and skimmed milk powder (4%) | Exemplar of yogurt from mix of mare's milk (48%), cow's milk (48%) and skimmed milk powder (4%) |
|-------------|----------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Protein     | 4.37 (±0.22) g / 100 mg | 4.31 (±0.22) g / 100 mg                                                             |                                                                                     |
| Amino acids | Interchangeable       |                                                                                     |                                                                                     |
| Glutamic acid | 4.0±0.5%            | 2.5±0.5%                                                                            |                                                                                     |
| Serine      | 1.0±0.5%             | 1.0±0.5%                                                                            |                                                                                     |
| Glutamine   | 11.0±0.5%            | 8.0±0.5%                                                                            |                                                                                     |
| Glycine     | 5.0±0.5%             | 5.0±0.5%                                                                            |                                                                                     |
| Arginine    | 0.5±0.5%             | 0.5±0.5%                                                                            |                                                                                     |
| Alanine     | 21.0±0.5%            | 20.0±0.5%                                                                           |                                                                                     |
| Thyrosinum  | 2.5±0.5%             | 2.0±0.5%                                                                            |                                                                                     |
| Cysteinum   | 9.5±0.5%             | 12.0±0.5%                                                                           |                                                                                     |
| In total    | 100.0±0.5%           | 100.0±0.5%                                                                          |                                                                                     |

Results of researches showed that yogurt contains 15 amino acids, including 7 irreplaceable (threonine, methionine, valine, phenyl alanine, isoleucine, Leucinum, a lysine).

The comparative analysis of amino-acid structure on reference data of yogurt from cow's milk of 3.2% of fat content with exemplars of yogurt from mix of mare's and cow's milk No. 1, 2 confirms
change of amount of irreplaceable and interchangeable amino acids as a part of a product [4]. Change of amount of irreplaceable amino acids: content of valine in exemplars of yogurt from mix of cow's and mare's milk increases from 6.5% to 8%-9% in comparison with cow's milk yogurt, the maintenance of Leucinum increases from 9% to 14%-15%, the content of phenyl alanine increases from 4.5% to 6.5%-9%. Change of amount of interchangeable amino acids: content of alanine in exemplars of yogurt from mix of cow's and mare's milk increases from 3% to 20%-21%, the maintenance of Cysteinum increases from 1% to 9.5%-12%.

Data on the content of amino acids in yogurts with use of mare's milk show that they are characterized by rather high content of irreplaceable amino acids.

For the characteristic of biological value of proteins of yogurt with use of mare's milk the value of amino-acid is calculated it is fast, showing the content of amino acids in this product, in comparison with contents them in the complete ideal protein taken for the standard according to a scale of FAO/WHO.

Earlier we received the mare's milk yogurt having an original compounding [17, 18]. Improvement of a compounding and structure of yogurt from mare's milk is carried out. Having compared amino-acid it is fast the new developed exemplars of yogurt No. 1, 2 with the previous compounding of yogurt follows that amino-acid considerably improved it is fast a squirrel on a scale of FAO/WHO: content of phenyl alanine increased from 71.7% to 108.3%-150%, the content of isoleucine increased from 125% to 150%-162.5%, the content of valine increased from 121% to 160%-180%.

Apparently from the data presented to table 1, content of irreplaceable amino acids (threonine, isoleucine, Leucinum, phenyl alanine) in an exemplar 2 exceed the content of these amino acids in an exemplar 1. Studying of amino-acid it is fast showed that the limiting amino acids in both cases are a methionine and a lysine (57.1 and 72.7% respectively), on other amino acids yogurt exceeds indicators of "ideal" complete protein.

For assessment of the major component of food adequacy of proteinaceous components of yogurt – their biological value the indexes and criteria offered by academicians of Russian Academy of Agrarian Sciences I.A. Rogov and N.N. Lipatov such as used: coefficients of distinctions of amino-acid it is fast (CDAAIIF) and biological value (BV).

The results of laboratory research which are carried out in the accredited FGBUN exploring laboratory center "FITs of a delivery and biotechnology" (Moscow) prove that the content of especially dangerous and toxiferous heavy metals (Hydrargyrum, lead, arsenic, cadmium) in exemplars of yogurts is not exceeded by admissible norms.

The main danger is constituted by aflatoxins. The results presented by the accredited FGBUN exploring laboratory center "FITs of a delivery and biotechnology" (Moscow) showed that their contents in exemplars of yogurts is not revealed.

Having analysed all data obtained during the researches of toxicological indexes in exemplars of yogurts give us the chance to draw a conclusion that the developed and made exemplars of yogurts from mix of mare's milk (48% and 68%), cow's milk (48% and 28%) and skimmed milk powder (4%), do not constitute danger to the consumer.

4. Conclusion

By results of the executed researches it is possible to draw the following conclusions:

- For the first time in Russia the compoundings on yogurt from mix of the mare's and cow's milk enriched with milk skim milk having the increased biological value are developed.
- The results of researches which are carried out in the accredited FGBUN exploring laboratory center "FITs of a delivery and biotechnology" (Moscow) prove the high biological value of the exemplars of yogurts developed by us with use of mare's milk.
- The possibility of use of at the same time combined lactic raw materials of different farm animals in production of yogurt was for the first time studied: milk of mare's whole, cow's
milk, milk skim milk, the optimum dose of an importation of skimmed milk powder (4%) which affects in the best way quality of a finished stock is picked up.

- In order that yogurt had good consistence, we used the skimmed milk powder (SMP) for increase in content of nonvolatile solids and protein in a finished stock and exceptions of use of stabilizers. We stopped on this component as it is available and is made practically everywhere. Compounding of two types of yogurt with use of mare's milk are developed, the patent application of the Russian Federation is submitted No. 2017117840 for one of them of 22.05.2017 "Fermented milk product". The conducted researches correspond to the modern directions on use of unique structure of mare's milk in new types of products. The problem of processing and use of mare's milk is most relevant, earlier were not engaged in it in the Russian Federation therefore careful study is required, starting with the analysis of a source of raw materials of the Republic of Bashkortostan, Russia, and finishing with development and researches of a line of new full-fledged functional food on the basis of mare's milk.

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