USEGE OF MILK PRODUCTION WASTE FOR OBTAINING FUNCTIONAL FOOD PRODUCTS FOR BABY AND DIETETIC NUTRITION

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An integrated dairy production waste management technology for obtaining functional food prod-ucts for baby and dietetic foods was developed. This technology has been obtained through the use of physicochemical methods, organoleptic estimation and microbiological parameters. As a result, experimental sam-ples of functional food products, standard process flow diagram for production of functional foods from the dairy industry waste have been developed.

Keywords: the Lowry method, enzymatic hydrolyzate, physical and chemical criteria, biological criteria, microbiological criteria, organoleptic criteria

When using a traditional technology for milk separation, smetana, butter, cheese, quark and milk protein production there are some by-products (skim milk, buttermilk, whey) obtained. Presently according to the GOST R 51917–2002 «Dairy and Milk Containing Products. Terms and Definitions» these by-products have an umbrella term – «secondary raw dairy material».

Skim milk, buttermilk and whey, or in other words secondary raw dairy material of AIC sudcomplex, should be used fully and effectively. When combined with whole milk and cream secondary raw dairy materials form a complex that can be called by the term «raw dairy material». In the production of 1 ton of butter up to 20 tonnes of skimmed milk and 1,5 tons buttermilk are produced, in the production of 1 ton of cheese or quark – up to 9 tons of whey are produced. Skim milk is also obtained while milk standardizing [3, 4, 5].

The composition of skim milk, whey and buttermilk indicates that it is a high-grade raw material, and according to its biological value it is practically not inferior to whole milk. However, the energy value of skim milk and buttermilk almost 2 times and whey almost 3,5 times less than the energy value of whole milk (Table 1). This determines the applicability of using skim milk, buttermilk and whey in the production of dietary food products [1].

Table 1

The extent of major milk nutrients transition into secondary raw materials

Milk components (100%)	Extant of transition, %		
	Skim milk	Buttermilk	Whey
Milk fat	1,4	14,0	5,5
Protein, total, including:	99,6	99,4	24,3
Casein	99,5	99,5	22,5
Whey proteins	99,8	99,6	95,0
Lactose	99,5	99,4	99,5
Mineral salts	99,8	99,6	98,0
Solids	70,4	72,8	52,0

The use of whey, skim milk and buttermilk as a basis for the creation of functional food products is contingent the fact that at the minimum energy value and low content of substances (like fat, etc.) they contain very important complexes of biologically active substances (Table 2). The widespread use of secondary raw dairy material in the diet can have therapeutic-preventive effect in the prevention of obesity and cardiovascular pathology.

The research objective is to develop a comprehensive technology of waste processing for obtaining functional dairy products for baby and dietetic nutrition.

As the part of the research physicochemical, biological, organoleptic and microbiological criteria of quality and safety of functional foods derived from dairy waste products were studied.

Materials and methods of research

Common, standard and original methods were used while performing this research work.

Theoretical and experimental studies were carried out using the current methodology of the study of complex phenomena using conventional, standard and original methods of biochemical, physicochemical, structural and mechanical analysis using the latest advances in science and technology.

Table 2

Physicochemical parameters of the secondary dairy raw materials

Dairy raw materials	Physicochemical parameters					
	Weight fraction, %			Acidity		Density, g/cm^3
	Protein	Fat	Lactose	Titrated, ºT	Active, pH	
Skim milk	3,00 ± 0,18	0,05 ± 0,01	4,25 ± 0,25	15–18	6,70	1,0325
Buttermilk	0,60 ± 0,05	0,20 ± 0,01	4,80 ± 0,29	13–75	6,5–4,5	1,0270
Whey	3,20 ± 0,19	0,50 ± 0,03	4,70 ± 0,28	15–50	6,6–4,9	1,0300

Sampling and preparation for analysis was performed in accordance with GOST 26809 «Dairy and Milk Containing Products. Acceptance Procedures, Methods of Selection and Preparation of Samples for Analysis», GOST 9225 «Dairy and Milk Containing Products. Methods of Microbiological Analysis», GOST 26929 «Raw Materials and Food Products. Preparation of Samples. Mineralization for Toxic Elements Determination».

Physicochemical parameters were determined by standard methods: mass fraction of moisture in accordance with GOST 30305.1 «Canned Condensed Milk. Methods for Moisture Mass Fraction Measuring».

Enzymatic hydrolysis was carried out using the static method in a thermostat with stirring at a temperature and pH, optimum for the enzyme used, and according to the recommendations the manufacturer. Hydrolysis was carried out for 4–24 hours, the ratio of the enzyme protein concentration to the substrate protein concentration was 1:25, 1:50, 1:100.

Determination of total nitrogen was performed using a protein analyzer RAPID N ELEMENTAR in accordance with European standards. The principle of the method consists in determining nitrogen by burning a known mass of analyte at high temperature (about 900 ° C) in the chamber in the presence of oxygen, which leads to the release of carbon dioxide, water and nitrogen mass fraction detected by the device.

The total protein content was calculated by multiplying the total nitrogen conversion factor for milk proteins, constituting 6,38.

Determination of amino nitrogen was performed using a spectrophotometric method with 2,4,6-trinitrobenzenesulphonic acid (TNBS). The method is based on the spectrophotometric determination of chromophores that are formed by the reaction of primary amines with TNBS. Amount of amino nitrogen in the test hydrolysates were determined from the calibration graph, constructed for the standard dilutions of a known substance.

The degree of hydrolysis was determined as the ratio of amino nitrogen to total nitrogen.

Titratable acidity was determined in accordance with GOST 3624 «Milk. Titrimetric Methods for the Acidity Determination», active acidity was measured by potentiometric analyzer in accordance with GOST 26781 «Milk. Method of the pH Measuring».

Determination of amino acids was carried out with an automatic amino acid analyzer Aracus PMA GmbH, approved by guidelines 98/64/EU and 2000/45/EU. The principle of the method is a cation exchange separation of amino acids with a step gradient of pH and post-column ninhydrin derivatization. This sample was pre-subjected to an acid (6n. hydrochloric acid, 110° C for 24–72 h) or enzymatic hydrolysis.

The molecular weight distribution of proteins and peptides in derived hydrolysates was assessed using the Laemmli method of protein electrophoresis. Denaturing polyacrylamide gel (12 % – separating and 4 % – focusing) with 0,1 % SDS-Na was used for protein separation. Electrophoresis was performed on a single electrode buffer supplemented with 0,1 % SDS-Na at 15 mA. The gel was stained with 0,2 % Coomassie R250 (prepared with glacial acetic acid) at elevated temperature for 7–10 min, then it was washed three times with distilled water.

Viewing and photographing of the gels was carried out on UV transilluminator TCP-20M («Vilber Lourmat», USA) at the wavelength of 312 nm. Preservation and processing of data was performed using gel-documenting system Vitran-Photo.

The amino acid sequence of the formed peptides was determined by gas chromatography-mass-spectrometer system Agilent 5975 C by MALDI-TOF method, which consists in the separation of ions by the ratio of the mass/charge.

The rheological characteristics of the samples were determined on a rotational viscometer VT550. Embedded microprocessor based on a rotating speed value n (c^{-1}), torque M_{torque} ($N \times m$) and the geometry of the measuring system, counted towards a shape coefficient f, calculates major structural and mechanical characteristics:

$$\eta = \frac{f M_{torque}}{Mn}; \gamma = M \cdot n; \tau = f M_{torque};$$

where η – effective viscosity, Pa·c; γ – sliding velocity, c^{-1}; τ – shearing strength, Pa.

The device allows you to set the change test conditions program: constant rotor speed, gradually increasing speed, step increasing speed, combine different speed conditions. With help of the systems like SV-DIN (f = 369,4, M = 1,29) and SV-DIN (f = 61,4, M = 1,29) it is possible to determine the viscosity in the range of 10 mPa × c at maximum speeds, with the MV-DIN up to 100 mPa·c and with the SV-DIN at minimum speed. Reliable results are obtained with the values of torque more than 0,15 N·cm

Microbiological parameters were determined in accordance with the current regulatory framework: SanRaN 2.3.4.551 «Milk and Dairy Products Production»; SanRaN 2.3.2.1078 «Hygienic Safety and Nutritional Value of Food Products».

Determination of the toxic elements, pesticides, antibiotics, and radionuclides content:

– lead – GOST R 51301 «Food Products and Raw Materials. Inversioned-voltammetric methods for the determination of toxic elements (cadmium, lead, copper and zinc)», GOST 26932 «Raw Materials and Food Products. The lead determination methods», GOST 30178 «Raw Materials and Food Products. Atomic absorption method for the determination of toxic elements», GOST 30538 «Food Products. Analysis of toxic elements by atomic-

emission method» and MG 4.1.986 «Method for measuring the mass fraction of lead and cadmium in the food products and food raw materials by electrothermal atomic absorption spectrometry. Methodical instructions»;

– arsenic – GOST R 51766 «Raw Materials and Food Products. Atomic absorption method for the arsenic determination», GOST 26930 «Raw Materials and Food Products. Method for the arsenic determination» and GOST 30538 «Food Products. Analysis of toxic elements by atomic-emission method»;

– cadmium – GOST R 51301 «Food Products and Raw Materials. Inversioned-voltammetric methods for the determination of toxic elements (cadmium, lead, copper and zinc)», GOST 26933 «Raw Materials and Food Products. Methods for cadmium determination», GOST 30178 «Raw Materials and Food Products. Atomic absorption method for the determination of toxic elements», GOST 30538 «Food Products. Analysis of toxic elements by atomic-emission method», MG 4.1.986 «Method for measuring the mass fraction of lead and cadmium in the food products and food raw materials by electrothermal atomic absorption spectrometry. Methodical instructions»;

– mercury – GOST 26927 «Raw Materials and Food Products. Methods for mercury determination» and MG 5178 «Methodical guidelines for mercury determination in food products»;

– strontium-90 and cesium-137 radionucleotides – MG 2.6.1.1194 «Radiation Monitoring. Strontium-90 and Cesium-137. Food Products. Sampling, analysis and hygienic assessment».

The experimental data was processed by the method of mathematical statistics on the computer. For further processing computer software WinStat or Statistica 5.0 was used.

Results of research and their discussion

Biological indicator. This indicator is presented in determination of the residual antigenicity of enzymatic protein hydrolysates. **Residual antigenicity** is a characteristic that is crucial for the possibilities of their usage in hypoallergenic milk-based products. Especially stringent requirements are shown to the reduction of antigenicity of the protein component of therapeutic use hypoallergenic products.

Until now, such a reduction of AG (10^{-6} and less of the original protein AG) could be achieved only for mixtures based on casein, put into deep proteolysis (eg, a mixture of «Nutramigen», «Pregestimil» and «Peptamen»).

Requirements for residual AG hydrolysates used in functional foods, are less stringent, but it is preferable to reduce AG at least to the level 10^{-5} of the original protein in this case also. At higher antigenic structures you can not exclude the presence of the immunogenic and sensitizing properties in the product, which can cause allergic reactions in genetically susceptible individuals [6, 7, 8].

The residual antigenicity (AG) of cow's full cream milk and its hydrolyzate peptide was determined by the Lowry method. This method shows that the trypsin hydrolyzate filtrate is set to AG $7{,}5\cdot10^{-6}$ 10 kDa through the membrane.; the chymotryptic hydrolyzate filtrate is set to AG $3{,}7\cdot10^{-6}$ 10 kDa through the membrane; the thermolysis hydrolyzate filtrate is set to AG $5{,}8\cdot10^{-6}$ 10 kDa through the membrane [2].

From the above it can be seen that the use of the enzyme chymotrypsin is significantly more effective in reducing the antigenic properties of the hydrolyzate. Even without using nanofiltration, AG in this case makes up $4\cdot10^{-6}$, which can be sufficient for the use of the hydrolyzate in the functional food products.

Thus, during the research of different processing options of hydrolyzed milk we concluded that, first of all, critical characteristic of the enzymatic hydrolyzate for use in enteral feeding is the kind of the molecular weight distribution which is corresponding to the predominance of medium peptides in the specimen. Such an enzymatic hydrolyzate has a combination of good digestibility properties with partially disturbed digestive function; low osmolarity and satisfying flavor properties. Secondly, these are hydrolysates, used in medical and health-care food, for which the critical feature is the residual antigenicity of cow's milk protein in the protein component of the product (guaranteed no more than $1{,}10^{-5}$ and $1{,}10^{-4}$ respectively).

Organoleptic and physicochemical indicators. In order to further research the properties of enzymatic hydrolyzate of milk proteins, organoleptic and physicochemical indicators were considered. Consistence of enzymatic hydrolyzate of milk proteins has the form of fine, dry powder with the presence of easy break up lumps. It has white with cream shade color. Smell and taste are clean and inherent for fresh milk mixture, with a specific taste. Mass fraction of dry solids has a value not less than 94–96 %. Mass fraction of the peptide has a value not less than 50 g per 100 g of protein.

Microbiological indicators. In terms of safety the most important thing is the control of microbiological indicators of hydrolysates. Standardized microbiological indicators (with the requirements set out in the «Hygienic requirements for safety and nutritional value of food products» SanPiN 2.3.2.1078-01) are presented in Table 3.

By the results of enzymatic hydrolyzate of milk proteins microbiological characteristics researches for specialized food products during storage at 4 ± 2 °C, the analysis of the results showed that by the content of sanitary-indicative and pathogenic microorganisms tested samples of hydrolysates have high reliability, because such microorganisms (as Escherichia coli, Staphylococcus aureus, Salmonella) in standardized mass product were not found.

Table 3

Standardized microbiological indicators of enzymatic hydrolyzate

Indicator	Permissible levels, mg/kg, not more
QMAFAnM, CFU/g, not more	$2 \cdot 10^3$
Escherichia coli in 0,01 g	not allowed
Pathogenic microorganisms, including Salmonella spp in 25 g	not allowed
S. aureus in 1,0 g	not allowed
Yeast, CFU/g, not more	10
Mould, CFU/g, not more	50

A different picture is set in the determination of molds and yeasts that were found in hydrolyzate, besides, on the two hundred and seventieth day content of the latter exceeded the upper limit (at 20%, accordingly). From this we can conclude that guaranteed storage life including two-term (regimented by the Federal Service) is 180 days .

By the end of storage we examined the safety record of the enzymatic protein hydrolyzate for functional food products that were packaged under nitrogen in bags made of the com-bined material

We found that there is no migration of toxic elements in the product, controlled potentially hazardous chemical substances are contained in the product in concentrations that do not exceed established specifications. Apart from the listed substances the content of cesium radionuclides-137 (standard is no more than 50 Bq/kg) and strontium-90 (standard is no more than 25 Bq/kg) is not found in the product.

We controlled the organoleptic characteristics of enzymatic hydrolyzate of milk proteins additionally at the end of the shelf life.

The research has shown that the enzymatic hydrolyzate has the form of fine, dry powder with the presence of easy break up lumps. In the reduced state it has a form of homogeneous liquid without sediment At the end of the shelf life enzymatic hydrolyzate has white color with cream shade. Smell and taste are clean that is inherent for fresh milk mixture, with a specific taste.

During the research, organoleptic indicators did not change that indicates the correctness of the chosen period and temperature storage regime

At the same stage of research osmolarity of the samples was determined by a standard cryoscopic method of the reduction the freezing temperature of the solution using the osmometer.

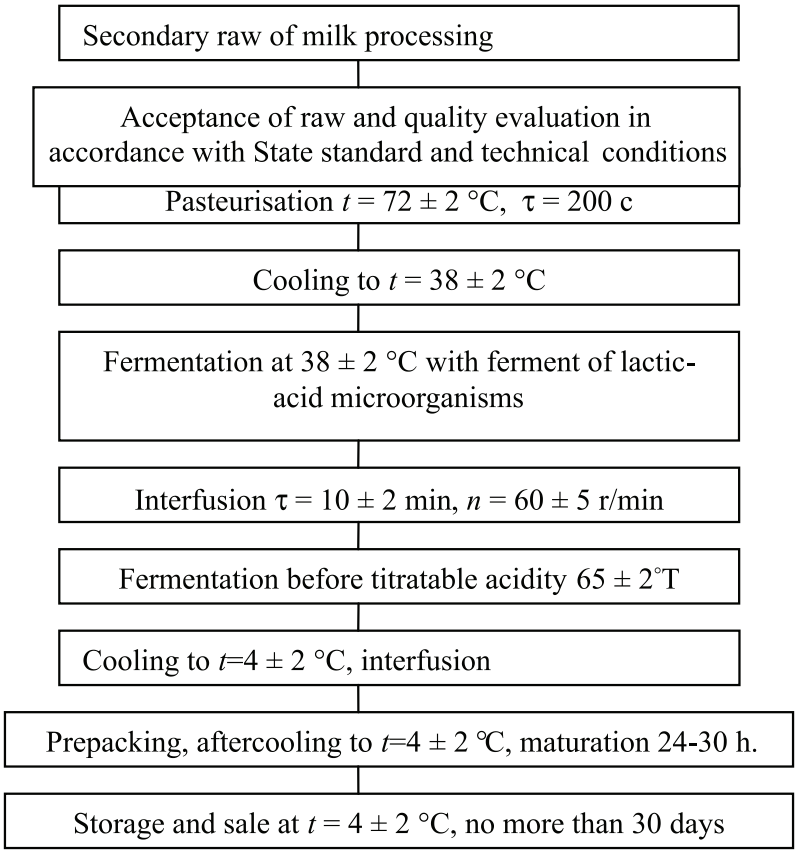
Mixtures based on unhydrolyzed protein have the lowest osmolarity and good organoleptic properties, however, it can be difficult for patients with deep dysfunction of digestive system, and, furthermore, with the injection of such mixtures to intracolonic probe, the absorption of the uncleaved protein significantly increases that can lead to food-allergic sensitization.

Based on the results of the research developed standard scheme of technological process of production of functional dairy products (Figure).

The process of reaching the functional product begins with the acceptance of raw materials and verifying its quality indicators in accordance with applicable requirements of technical documentation. Technological process of production functional dairy products consists of the following operations: reception and evaluation of the raw materials quality; cleaning; pasteurization, cooling, fermentation, fermentation of dairy raw materials; addition of flavoring and aromatic components (under recipe); packaging in market containers, maturation for several hours, storage and sale.

Conclusions

The research resulted in the examination of physicochemical and biological criteria of quality and safety of functional foods, produced from waste of dairy industry. We found that hydrolysates of milk can be used for children and dietary, as well as medical and preventive nutrition, for which the critical feature is the residual antigenicity of cow's milk protein in the protein component of the product (guaranteed no more than $1{,}10^{-5}$ and $1{,}10^{-4}$ respectively). It is shown that in the content of sanitary-indicative and pathogenic microorganisms, test samples of hydrolysates are highly reliable, because such microorganisms (as Escherichia coli, Staphylococcus aureus, Salmonella) in standardized mass product were not found. We found that there is no migration of toxic elements in the product, controlled potentially hazardous chemical substances are contained in the product in concentrations that do not exceed established specifications. Typical technological process of functional foods production based on secondary raw of milk processing was developed.



Typical technological process of functional foods production based on secondary raw of milk processing

Test samples of functional foods were developed.

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