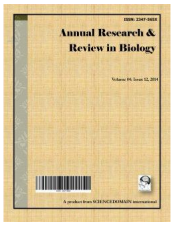

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Amino Acid Composition of Sour-milk Drink with Encapsulated Probiotics

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AK and ZK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GM, AB and ST managed the analyses of the study. Authors MJ, GZ and ZY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

At present time, there has been a growing interest in the consumption of probiotic food products. This study presents the results of the determination of amino acids in sour-milk drink with encapsulated *Lactobacillus casei* probiotics. Probiotics were encapsulated in pectin capsules by extrusion method. Totally, the sour milk drink contains 40.61 g/100 g of essential amino acids, which is higher than the FAO/WHO requirement (36.0 g/100 g). However, limiting amino acids such as, valin (4.86 g/100 g) and methionine+cystein (2.77 g/100 g) are lower than the FAO/WHO data for valine (5.0 g/100 g) and methionine+cystein (3.5 g/100 g). Developed sour milk drink contains 18 essential and non-essential amino acids with large amount of glutamic acid (440.59 mg/100 g), leucine (244.96 mg/100 g), proline (240.64 mg/100 g) and lysine (225.92 mg/100 g). The microbiology analyses of sour milk drink with encapsulated probiotics revealed the absence of pathogenic bacterium, like *Salmonella* and *Staphylococcus St. aureus.*

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1. INTRODUCTION

At present time, there has been a growing interest in the consumption of probiotic food products. These types of products contain live microorganisms of beneficial microflora of gastro-intestinal tract [1]. Functional milk products especially sour milk drinks with live-cell probiotics have a special role in human functional nutrition. The definition of the word "probiotics", approved by FAO/WHO in 2001, is live microorganisms which using in adequate quantities exerts beneficial influence on human health [2].

From the latest research, effect of probiotics is not only limited to correction of the microflora, but also their clinical effectiveness is based on immunomodulatory functions and participation in a metabolism. The revealed effects of probiotics allow to expand indications to their appointment and to design medicines with the given properties [3,4].

Widely used probiotics are *bifidobacteria, lactobacilli, streptococci*, etc. In particular *Lactobacillus casei* is capable to change structure and metabolic activity of intestinal microflora due to the expansion in the number of bifidobacteria and decrease of activity of beta glucuronidase in intestines, and also inhibit growth of *Helicobacter pylori* [5,6].

However, due to the low survivability of these bacteria in the product the preservation of probiotics in a viable state is a difficult issue. The considerable proportion of probiotic cells loses its activity affected by bacterial destruction during the storage of fermented milk products, and passing through the digestive tract. The main reasons are the low values of pH in fermented milk products, influence of the hydrochloric acid and pepsine of the gastric juice, influence of the antimicrobial components, etc. The survivability of probiotic cultures depends on various factors including storage conditions, fermentation, etc. and is the individual characteristic of each separate strain [7].

The most perspective method of saving the live-cell is the encapsulation. Probiotics encapsulation is becoming increasingly interested in food biotechnology since increasing the survival rate of probiotics, it protects cells from the bacteriophage; survivability enhancement of cells during drying and freezing; and increasing storage life of milk products [8].

For solution of such problem the most promising direction is to use of the process of immobilization of bacterial cell – encapsulation. The encapsulation process in addition to increasing the survival ability of probiotic cultures in milk products and in the conditions of the digestive tract, also promotes protection of cells against bacteriophages, increase their survivability during the drying and freezing process, stability of quality indexes and to improve the shelf life of the products. Besides, the encapsulated cultures provide larger stability of cells and high metabolite producing [9].

Viability of encapsulated probiotics depends on the physicochemical properties of capsules. These capsules must be water-insoluble and not dissolved in food and intestinal digestive tract. The materials used in encapsulation are alginate, gellan gum, chitosan, pectin and etc. [10].

The encapsulation process of microorganism is a process of forming polymer coatings in the forms of capsules around the immobilized microbial cells. The most important aspect of encapsulation is a type of encapsulation, including spray drying, spray freezing, and emulsification and extrusion method [11].

The extrusion method is widely used in encapsulation. Extrusion is a physical method of forming capsules from hydrocolloids gels by extruding it through the syringe needle [12].

Thus, the development of probiotic cells protection methods against adverse conditions of digestive tract and preservation of the maximal number of viable cells in milk products is urgent.

The goal of this study was to develop the technology of sour milk drink with encapsulated probiotics and investigate the chemical and amino acid composition.

2. MATERIALS AND METHODS

For production of sour milk drink the following ingredients were used:

- Milk (about 100 l) was obtained from the local milk farm;
- Probiotics *Lactobacillus casei* NCIMB 30185 (1XN37) were purchased from the

National Collection of Industrial, Food and Marine Bacteria (NCIFMB, United Kingdom);
- The cultures of L. acidophilus and "Bifilakt - B" were provided by Siberian Cheese Research Institute (Barnaul, Russia);
- Pectin (amidated, low methoxyl and high methoxyl) (HERBSTREITH & Fox KG, Germany);
- Inulin ("Ryazanskie Prostory", Moscow, Russia).

Chemical and physicochemical properties of sour milk product were determined by the methods:

- ISO 2446:2008. Milk. Determination of fat content.
- GOST 23327-98. Milk and milk products. Determination of mass part of total nitrogen by Kjeldahl method and determination of mass part of protein.
- GOST 3624-92. Milk and milk products. Titrimetric methods of acidity determination.
- GOST 3625-84. Milk and milk products. Methods for determination of density.
- GOST 9225-84. Milk and milk products. Methods of microbiological analysis.
- GOST 28283-89. Milk. Sensory analysis. Determination of odour and taste.
- ISO 5534:2004 Cheese and processed cheese. Determination of the total solids content.

2.1 Encapsulated Probiotics Preparation

Lactobacillus casei NCIMB 30185 (1XN37) was placed in Petri dish with MRS Agar and was incubated for 48 h at 37°C. Then, the bacteria colony was inoculated to 10 ml of MRS Broth and incubated for 24 h a 37°C. After bacterial growth, the broth was centrifuged for 10 min at 4°C (rotation speed was 3200 rpm). The bacteria sediment washed with a saline and mixed with a 3% pectin solution.

Pectin capsules were obtained by extrusion method, whereas 1 ml of 3% pectin solution by extruding it through the 0.8 mm diameter needle to 50 ml 0.15M calcium chloride solution. The distance between the needle and solution was 10 cm. The capsules were immediately formed in the solution and stored for 30 min. After hardening the solution was filtrated and the pectin capsules were collected. Then these capsules were placed onto 10 ml of 0.4% chitosan solution and mixed for 10 min. After mixing the capsules were removed from the solution by filtration.

2.2 Sour Milk Drink Production

For preparation of 100 kg of sour milk drink 87 kg of the normalized milk with fat content of 2,5% was taken, 2.5 kg of sugar beet was added. Then the mixture was carefully mixed and homogenized at the temperature of 50-55°C and pressure of 10-12 MPa. The homogenized mixture was pasteurized at the temperature (78±2)°C for 30-40 s, and cooled up to the temperature of 40°C. Then to the cooled mixture 0.5 kg of calcium alginate and 5.0 kg of the ferment consisting of acidophilic bacteria of *L. acidophilus* and "Bifilakt - B" bacterial preparation in the ratio 1:1 were added and mixed. Given mixture ripened at the temperature of 38.0°C for 5.0±0.5 h. After ripening 4.0 kg of encapsulated probiotics added and mixed for 20 min. Obtained sour milk drink filled into the containers and stored at 4-6°C.

Lactobacillus casei encapsulated probiotics application allows to receive a product with the increased pro-biotic properties. The invention provides activity preservation (10^9 CFU/ml) and viability of pro-biotic cultures in the conditions of the digestive tract (pH 2,0).

2.3 Statistics

Differences between samples were evaluated using the t-test which were considered to be statistically significant at $p<0.05$. Statistical analyses were performed using the free software R 3.02 developed by R Core Team.

3. RESULTS AND DISCUSSION

During the production of new milk products special focus is placed on the sensory, physical-chemical and microbiology properties. The obtained milk product has a homogeneous consistency with 2-3 mm capsules, which does not influence to the overall sensory characteristics (Table 1).

The addition of encapsulated probiotics to the sour milk drink recipe has not significantly change the sensory properties. The sour milk drink has pleasant sweet flavor and contains 2.5% of fat, 3.4% of protein. Our findings were in accordance with the results obtained by Luneva N.M. (2007), who reported that the amount of fat

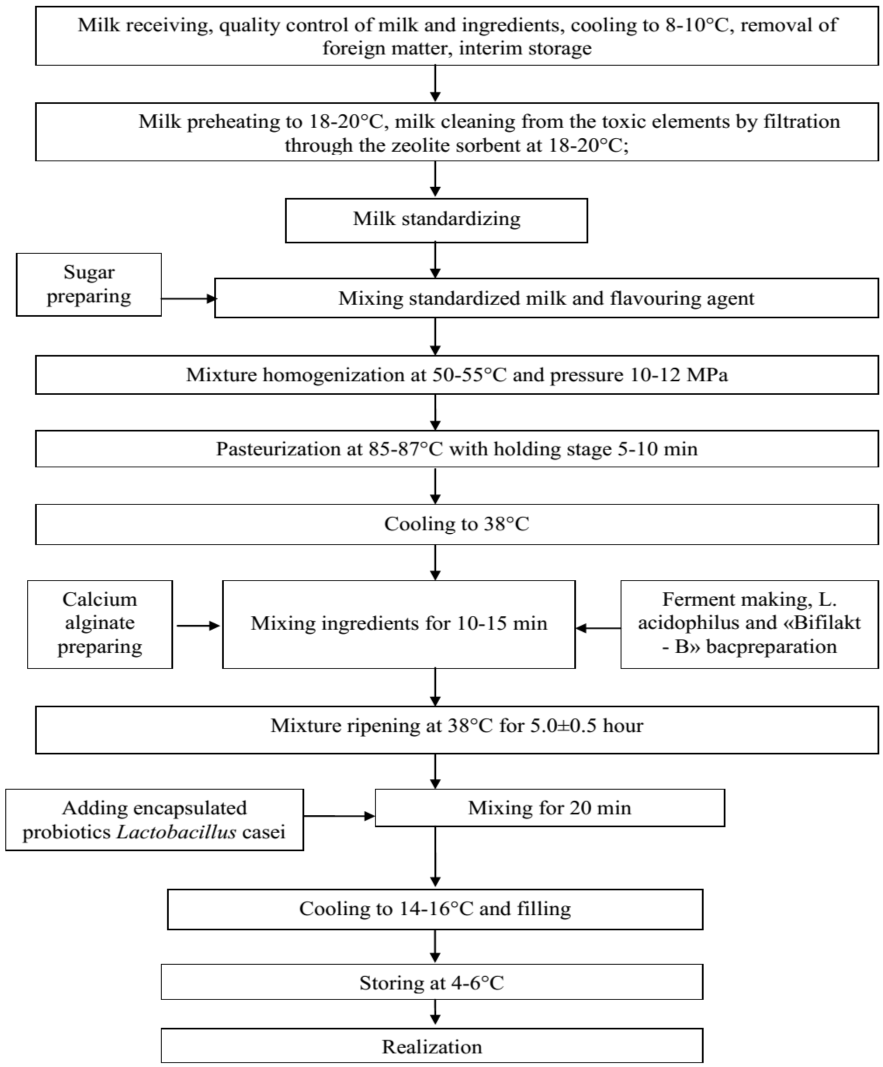

Fig. 1. Sour milk drink manufacturing flow chart

Table 1. Organolept and chemical composition of sour-milk drink

Indicator name	Characteristic
Appearance and consistency	Homogenous, liquid, viscous with presence of 2-3 mm capsules
Flavor and aroma	Pleasant, sour-milk, sweet
Colour	White
Fat, %	2.5±0.2
Protein, %	3.4±0.2
Dry solids weight ratio, %	At least 13.7
Acidity: active (pH)	4.5-5.0
titratable acidity (°T)	80-85
Outlet temperature, °C not exceeding	6.0

and protein in sour milk drink with *Streptococcus thermophilus* and bifidus bacteria were 2.83% and 3.5%, respectively [13]. Simonenko et.al. (2010) determined the high protein (6.5-6.7%) and low fat (1.2-1.7) contents in sterilized milk product for young athletes and sportsmen [14]. Ostretsova N.G. and Chekaleva A.V. developed fermented milk products containing 0.8% of fat and 4.8% of protein. The culture used were *Lactobacillus bulgaricus, Streptococcus salivarius subsp. thermophilus* and *Bifidobacterium bifidum* in ratio 1:4:2 [15].

The most important characteristics of milk products are its safety and microbiological susceptibility. The microbiology analyses of sour milk drink with encapsulated probiotics revealed the absence of pathogenic bacterium, like *Salmonella* and *Staphylococcus St. aureus.* The presence of fungus and yeasts are under the allowable level (Table 2).

Table 2. Microbiology characteristics of sour milk drink with encapsulated probiotics

Parameter name	Allowable level
Pathogenic bacterium, including:	
Salmonella (in 25 cm^3)	Not detected
St. aureus 1.0 $см^3$	Not detected
Fungus, CFU/g, less than	10
Yeasts, CFU/g, less than	10

3.1 Amino Acid Composition

Developed sour milk drink contains 18 essential and non-essential amino acids with large amount of glutamic acid (440.59 mg/100 g), leucine (244.96 mg/100 g), proline (240.64 mg/100 g) and lysine (225.92 mg/100 g) (Table 3).

Thus, glycine participates in nitrogen metabolism and protein construction, play a vital role in brain function. Proline is an essential component and source of energy for muscles on exertion, where the deficiency of it lead to tiredness. Aspartic acid has a significant effect on nitrogen metabolism. Lysine is an essential aminoacid which assists with the formation of collagen and muscle tissue. Lack of lysine in food leads to the growth impairment of children; negative nitrogen balance ; functional abnormality of organism [16].

Overall, the essential amino acid content of sour-milk drink conformed to the FAO scale for ideal protein.

Table 3. Amino acid composition of sour milk drink with encapsulated probiotics

Amino acid	mg/100 g of drink	g/100 g of protein
Essential	*1380.63*	*40.61*
Isoleucine	163.60±3.45*	4.81
Leucine	244.96±6.74	7.20
Lysine	225.92±4.21	6.64
Tryptophan	43.28±1.23	1.27
Tyrosine	159.27±2.49	4.68
Cystine	22.51±0.52	0.66
Valine	165.33±4.45	4.86
Methionine	71.84±1.28	2.11
Phenylalanine	151.48±3.01	4.46
Threonine	132.44±2.68	3.90
Non-essential	*1340.81*	*39.44*
Aspartic	189.57±4.92	5.58
glutamic	440.59±10.99	12.96
Serine	161±4.30	4.74
Histidine	77.9±1.20	2.29
Glycine	40.68±0.87	1.20
Arginine	105.6±2.17	3.11
Alanine	84.83±1.43	2.50
Proline	240.64±5.44	7.08
Total	**2721.44**	**80.04**

** Results are mean±SD*

Essential amino acids cannot be synthesized by human body and must be consumed in sufficient quantity. Totally, the sour milk drink contains 40.61 g/100 g of essential amino acids, which is higher than the FAO/WHO requirement (36.0 g/100 g). However, limiting amino acids, such as valin (4.86 g/100 g) and methionine+cystein (2.77 g/100 g) are lower than the FAO/WHO data for valine (5.0 g/100 g) and methionine+cystein (3.5 g/100 g).

In comparison of other findings (Table 4), the total sum of essential amino acid is higher than in [17] and [18], but lower than in findings of [19] (total EAC 45.69 g/100 g) and [14] (49.22 g/100 g). Particularly, the content of phenylalanine+ tyrosine is 9.14 g/100 g which is higher than those data reported in [17] (6.81 g/100 g) and [14] (5.14 g/100 g). Similar results of phenylalanine+ tyrosine content is reported in [14] (9.49 g/100 g), but lower than in [19] (11.06 g/100 g).

Table 4. Calculated essential amino acid content in 100 g of protein

Amino acid	This study	[14]	[15]	[16]	[11]	FAO/WHO g/100 g
Essential	*40.61*	*38.32*	*34.28*	*45.69*	*49.22*	*36*
Isoleucine	4.81	4.18	6.57	10.12	5.43	4
Leucine	7.20	9.25	-	5.78	11.03	7
Lysine	6.64	6.56	7.43	7.05	7.52	5.5
Tryptophan	1.27	-	2.86	-	1.98	1
Phenylalanine + Tyrosine	9.14	6.81	5.14	11.06	9.49	6
Methionine + Cystine	2.77	7.42	2.57	2.96	3.8	3.5
Valine	4.86	-	5.14	4.6	5.09	5
Threonine	3.90	4.1	4.57	4.12	4.86	4

4. CONCLUSION

In this study, sour-milk drink manufactured using encapsulated *Lactobacillus casei* probiotics in recipe, which had good sensory properties. The pathogenic microorganisms were not observed. The most abundant amino acids in sour-milk drink with encapsulated probiotics were phenylalanine, tyrosine, glutamic acid, leucine, proline and lysine. However, valine, methionine and cystein were lower of FAO/WHO scale for ideal protein.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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