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Research Article

Nutritive and Biological Value of Mare's Milk Ice Cream

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Abstract

Background: Among milk products, ice cream is growing in popularity. Mare's milk instead of cow's milk can be used to make ice cream due to its low fat content and balanced amino acid, vitamin and mineral composition. **Objective:** The purpose of this study was to develop new methods of making ice cream and study the nutritive and biological value of mare's milk-based ice cream. **Materials and Methods:** Amino acid and vitamin composition levels were quantified using the Shimadzu LC-20 Prominence liquid chromatography system equipped with fluorometric and spectrophotometric detectors. Mineral composition was detected by Inductively-Coupled Mass-Spectrometry (ICP-MS). Ice cream samples were produced in the following proportions: Sample 1, 100% mare's milk (MM-100); sample 2, 40% MM and 60% cow's milk (MM-40); sample 3, 30% MM and 70% cow's milk (MM-30). **Results:** The fat contents of MM-100, MM-40 and MM-30 were 2.1, 7.3 and 8.5%, respectively. The carbohydrate content decreased from 26.0 g in MM-100 to 22.7 g in MM-40 and to 21.2 g in MM-30. Protein content of MM-100 was slightly lower than those of the MM-40 and MM-30 ice creams; however, no differences were found among the three types of ice creams in ash content. Using both mare's and cow's milk in ice cream mixtures resulted in increased mineral content in MM-40 and MM-30 in comparison with MM-100. Thus, combination of mare's and cow's milk enriched the ice cream with methionine, glycine, histidine, proline, threonine and valine. **Conclusion:** These findings suggest a beneficial role of mare's milk in making ice cream with low-fat content.

Key words: Mare's milk, ice cream, technology, amino acids, vitamins, minerals

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nutrition is an important factor in a person's overall health and well-being and can help to enhance the human body's resistance to the negative impact of the environment¹. Milk and milk products are the most nutritive and biological valuable foods. Presently, there are hundreds of types of functional milk products, including yogurt, curd, cheesecake, pudding and ice cream. These functional products are not just sweet treats but have a therapeutic effect on the human body².

In Kazakhstan, goat, mare and camel milk are widely available. These types of milk are known for their dietary and therapeutic properties but, hubat and koumiss (milk products from camel and mare's milk, respectively) are less often used in manufacturing^{3,4}.

Mare's milk has a neutral acidity and sweet taste, is fresh, and ranges from bluish-white to slightly brown in coloration. Important components of this type of milk include lactose, protein, fat, vitamins, enzymes and minerals^{4,5}.

Mare's milk also has high nutritive value. Its protein and fat are well digested and its fat has a low melting temperature (21-30°C) in comparison with cow's milk. Mare's milk has 6.5% lactose, higher than cow's milk⁶ and mare's milk lactose is a high active bifidogenic factor that is irreplaceable in infant and health nutrition products⁷. Infants with severe food allergies often tolerate mare's milk since mare's milk is closer in composition to human milk than that of any other mammal^{8,9}. A variety of milk desserts are widely manufactured in countries with a milk industry. These milk desserts are produced from milk and different supplements, fillings, taste and flavor additives. In Kazakhstan, the assortment of milk dessert products is expanding through the development of new recipes and processing of milk products, including low-fat milk products¹⁰. The purpose of developing dietary milk products involves correcting for protein, fat, mineral and vitamin composition and enrichment with biologically active compounds to improve flavor, nutritive and biological value. Among the milk products, ice cream is growing in popularity. Ice cream is characterized as a frozen milk dessert product comprised of milk, sugar, stabilizer, emulsifier and flavoring⁹. It has a complex food colloidal system including air bubbles, ice crystals and partially destabilized fat globules dispersed in a continuous aqueous phase^{11,12}.

Rivalry within the ice cream market requires product extension and production of quality food at a reliable price and calls for development of new methods to produce this product.

The purpose of this study was to develop new technology and study the nutritive and biological value of a new healthy food-mare's milk-based ice cream.

MATERIALS AND METHODS

The experiments were conducted in 2015-2016 at S. Seifullin Kazakh Agrotechnical University and with Limited Liability Partnership (LLP) "Gormolzavod" Milk Company in Kokshetau city in the Republic of Kazakhstan.

The Ethics Commission of S. Seifullin Kazakh Agrotechnical University approved the methods of this study under No. PEPPS SMK 11010/46-2013.

Ice cream preparation: For ice cream preparation, mare's and cow's milk was used in different proportions as follows: 100% of mare's milk (MM-100); 40% mare's milk and 60% of cow's milk (MM-40); 30% mare's milk and 70% cow's milk (MM-30). For a control sample, ice cream produced by national standard GOST 31457-2012 was used.

The flow process of ice cream production is shown in Fig. 1.

For ice cream preparation, mare's and cow's milk with good organoleptic quality and Therner degree of no more 21°T, which conforms to requirements of regulatory documents, were used. Milk was poured into an agitation vat and mixed with granulated sugar.

Before pasteurization, the composition was filtered by separating the mechanical and insoluble parts of the composition.

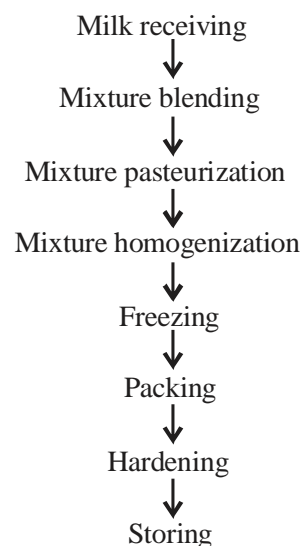


Fig. 1: Ice cream preparation flow process

The ice cream mixture was pasteurized at 85°C for 20 sec. Pasteurization kills spore and spoilage microorganisms and helps hydrate some of the components (proteins and stabilizers)^{13,14}.

After pasteurization, the ice cream composition was homogenized under a pressure of 12.5-15.0 MPa. The homogenization process breaks down or reduces in size the fat globules found in milk or cream to less than 1 µm. After homogenization, the ice cream composition becomes soft and homogenous with milk fat-pronounced flavor.

From the homogenizer, the ice cream composition was cooled to 2-6°C in the pasteurizing-cooling installation and mix was aged for at least 4 h. This allows time for the fat to cool and crystallize and for the proteins and polysaccharides to fully hydrate. Aging the mix cools it down before freezing, allowing the milk fat to partially crystallize and gives the proteins stabilizers time to hydrate, improving the whipping properties of the mix.

During the next stage, the mixture was frozen and pumped into continuous freezers. The temperature inside the freezers was kept at -40°C using liquid ammonia as a freezing agent. While the ice cream was in the freezer, air was injected into it. When the mixture leaves the freezer, it has the consistency of soft-serve ice cream.

Packaging and storing of ice cream: After the particulates have been added, the ice cream was packaged in 1 kg containers and was placed into a blast freezer at -30 to -40°C where most of the remainder of the water was frozen. Below -25°C, ice cream is stable for indefinite periods without the danger of ice crystal growth; however, above this temperature, ice crystal growth is possible and the rate of crystal growth is dependent upon the temperature of storage, limiting the shelf life of the ice cream¹³.

Sensory evaluation: Sensory evaluation was performed by a panel of six skilled persons using a 100-point system as follows: The maximum score for taste and flavor was 60 points; for structure and consistency was 30; for color was 5 and for packaging was 5. In case of defects in flavor and aroma (inadequate pronounced flavor, weedy flavor and slightly acid flavor), consistency and structure (powdery, faulty ice cream overrun and ice crystals presence), color and packaging, the score mark was reduced for each defect according to the special sensory evaluation scale.

Determination of mineral elements: One to two grams of the sample was placed in a high-pressure Teflon container. Each sample was combusted at 400°C for 4 h and then to

600°C for 2 h using a muffle furnace. Approximately 1 g sample (dry weight) of ashes was digested with 3 cm³ HNO₃ and 2 cm³ of HF and was placed in a microwave at 200°C for 20 min (Berghof Speed Wave microwave system, Germany). After microwave digestion, the samples were diluted with 1% HNO₃ in a 10 cm³ vessel.

The content of elements in the samples was determined with an Inductively-Coupled Plasma-Mass Spectrometric Method (ICP-MS, Varian-820 MS, Varian Company, Australia). The method was validated with certified reference materials. Calibration standards Var-TS-MS, IV-ICPMS-71A were used to calibrate the mass-spectrometer (Inorganic Ventures Company, USA). The sensitivity of the mass-spectrometer was tuned using a diluted calibration solution Var-TS-MS with concentrations of Ba, Be, Ce, Co, B, Pb, Mg, Tl and Th of 10 µg L⁻¹. Three calibration solutions were used for the detector calibration as follows: IV-ICPMS-71A of Cd, Pb, Cu and Zn elements diluted to 10, 50 and 100 µg L⁻¹. Discrepancies between certified values and concentrations quantified were below 10%. The operating parameters of the Inductively-Coupled Plasma Mass Spectrometer Varian (ICP 820-MS) were as follows: Plasma flow, 17.5 L min⁻¹; auxiliary flow, 1.7 L min⁻¹; sheath gas, 0.2 L min⁻¹; nebulizer flow, 1.0 L min⁻¹; sampling depth, 6.5 mm; RF power, 1.4 kW; pump rate, 5.0 rpm and stabilization delay, 10.0 sec.

All analysis were performed in triplicates and results given in mg kg⁻¹ wet weight, were expressed as Mean ± SE.

Determination of amino acids and vitamins: Liquid chromatography was used to quantify amino acids and vitamins. The instrument used was a Shimadzu LC-20 Prominence liquid chromatography system (Shimadzu, Japan) equipped with fluorometric and spectrophotometric detectors. The chromatographic column used was SUPELCO C18 5 µm (Sigma-Aldrich, USA) with a surface area of 200 g⁻¹ m². The chromatographic analysis was performed under the linear gradient with an eluent flow rate of 1.2 mL min⁻¹ and the temperature of the column heated in an oven was 400°C. Amino acids were detected by fluorometric and spectrophotometric detectors at wavelengths of 246 and 260 nm following acidic hydrolysis and treatment with a phenylisothiocyanate solution in isopropyl alcohol to give phenylthiohydantoin.

Statistical analysis: 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.

RESULTS AND DISCUSSION

There were significant differences ($p < 0.05$) in fat levels across the three different types of ice creams. The fat contents of MM-100, MM-40 and MM-30 were 2.1, 7.3 and 8.5%, respectively. The carbohydrate contents decreased from 26.0 g in MM-100 to 22.7 g in MM-40 and to 21.2 g in MM-30; however, no differences were found among the three types of ice creams in ash content. The protein contents of MM-100 were slightly lower than those of MM-40 and MM-30 ice creams (Table 1).

Mineral elements, among other food components are responsible for the high biological value of milk. According to previous studies, the mineral composition of mare's milk is similar to human milk¹⁵. The mineral element composition is presented in Table 2.

Adding mare's and cow's milk to ice cream increased mineral content in MM-40 and MM-30 in comparison with MM-100. The calcium concentration increased from 89.97 mg/100 g in sample 1 to 136.7 mg/100 g in MM-40 and to 130.3 mg/100 g in sample 3. Potassium levels increased as well, in MM-40 and MM-30 to 123.22 mg/100 g and 128.4 mg/100 g, respectively, compared to 64.72 mg/100 g in MM-100.

Table 3 shows the amino acid composition of ice cream samples with different proportions of mare's milk.

Ice cream types differed in amino acid composition. Ice cream with only mare's milk had the lowest content of amino acid compared with MM-40 and MM-30. Thus, a combination of mare's and cow's milk enriched the ice cream with methionine, glycine, histidine, proline, threonine and valine.

In MM-40 and MM-30, alanine content decreased by 20%. In MM-40, alanine content was 114.8 mg/100 g, in MM-30 content was 110.6 mg/100 g against an MM-100 content of 140.0 mg/100 g. There was no significant change in arginine content.

Unlike other vital food nutrients (essential amino acids and polyunsaturated fatty acids), vitamins are not used as an energy source. Vitamins are involved in different chemical reactions and regulate metabolism, thus ensuring biochemical and physiological processes in the body.

Vitamins A, C and B₅ were measured in the control sample (ice cream was made from cow's milk) and developed ice creams. As shown in Table 4, these vitamins were increased in the mare's milk-based ice cream. Vitamin C content in sample 1 was 0.71 mg/100 g, while in control sample it was 0.41 mg/100 g. Vitamin C strengthens the immune system and increases protective functions of the human body against viruses and infection.

Sensory analysis of the ice creams is presented in Table 5. Skilled panelists gave high scores to MM-40 ice cream. Mare's milk contributed significantly to reduce the evaluation score because the ice cream made from 100% mare's milk had a slightly acid taste with non-dense flakes and sensible ice crystals.

In a study conducted by Burmagina *et al.*¹⁶, a positive effect was found in ice cream by adding 40% malt extract instead of granulated sugar; the fat content level ranged around 10%.

Kazakova¹⁷ emphasized on the importance of low-calorie food and developed a recipe for low-calorie ice cream using food fibers, polyols, maltodextrins and sweeteners that reduced the energy value from 30-80%; fat content varied between 0.2-2.0%.

Table 1: Chemical composition of ice cream samples

Index (g)	Ice cream samples			
	Control	MM-100	MM-40	MM-30
Protein	3.4	2.5±0.4	3.15±0.3	3.16±0.2
Fat	8.3	2.1±0.5	7.30±0.5*	8.50±0.3*
Carbohydrate	19.5	26.0±0.4	22.70±0.3	21.20±0.4
Ash	0.5	0.6±0.1	0.70±0.8	0.70±0.1

*Values are statistically significant at $p < 0.05$

Table 2: Mineral composition of ice cream samples

Elements (mg/100 g)	Ice cream samples			
	Control	MM-100	MM-40	MM-30
Macroelements				
K	156	64.720±0.98	123.22±0.87	128.40±1.34
Ca	148	89.970±0.88	136.71±2.72	130.30±2.41
Mg	22	9.050±0.08	12.07±0.17	18.14±0.20
Na	50	30.880±0.47	31.48±0.40	44.75±0.72
P	107	54.480±0.66	69.95±1.34	91.17±1.46
Microelements				
I	0.100	0.0700±0.001	0.0800±0.001	0.0910±0.001
Mn	0.014	0.0030±0.0001	0.0070±0.0001	0.0107±0.001
Cu	0.015	0.0220±0.001	0.0120±0.001	0.0171±0.001

K: Potassium, Ca: Calcium, Mg: Magnesium, Na: Sodium, P: Phosphorus, I: Iodine, Mn: Manganese, Cu: Copper

Table 3: Amino acid composition of ice cream samples

Amino acids (mg/100 g)	Ice cream samples		
	MM-100	MM-40	MM-30
Essential			
Valine	110.33±0.75	158.73±0.92	166.80±1.53
Isoleucine	117.00±1.47	160.20±2.05	167.40±2.57
Leucine	187.73±2.78	244.89±4.70	254.42±4.96
Lysine	209.11±2.78	240.20±2.12	245.40±3.04
Methionine	23.80±0.21	59.32±0.57	65.24±1.14
Threonine	76.30±0.58	122.32±1.98	129.99±1.54
Tryptophane	31.00±0.44	42.40±0.42	44.30±0.79
Phenylalanine	150.40±1.82	165.98±1.91	167.66±2.45
Non-essential			
Alanine	140.60±1.15	114.82±0.98	110.63±1.35
Arginine	121.03±1.35	121.61±1.83	121.71±0.78
Aspartic acid	171.57±2.80	200.03±2.33	204.77±2.88
Histidine	37.60±0.37	69.04±0.81	74.28±1.37
Glycine	18.70±0.31	35.68±0.64	38.51±0.47
Glutamine acid	377.37±4.95	456.35±5.81	469.51±7.07
Proline	127.62±2.15	217.60±2.52	232.70±3.19
Tyrosine	114.00±1.57	156.61±3.07	163.54±2.03

Table 4: Vitamin content in ice cream samples

Vitamins (mg/100 g)	Ice cream samples			
	Control	MM-100	MM-40	MM-30
Vitamin A	0.048±0.001	0.055±0.001	0.051±0.001	0.050±0.001
Vitamin C	0.410±0.01	0.710±0.01	0.530±0.01	0.500±0.01
Vitamin B ₅	0.270±0.01	0.340±0.01	0.310±0.01	0.290±0.01

Litvinova and Bogdanova¹⁸ studied the effect of sea-buckthorn and sweet briar syrup on ice cream production. Starter bacteria (*Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium*) were used during acidification. In an analysis of the nutritive value, Litvinova and Bogdanova¹⁸ found the fat content to be 6.5%, protein content to be 3.17% and carbohydrate content to be 14.92%; calcium and phosphorous content were 290 mg/100 g and 129.96 mg/100 g, respectively.

Ivanovna and Vasil'evna¹⁹ made ice cream with probiotic, prophylaxis and biological properties. They reported that the ice cream consisted of milk, cream, sand sugar, stabilizer, vitamin complex and bacterial concentrate, with vitamin complexes including vitamins A, B and C groups and combined bacterial concentrate containing *Bifidobacterium lognum* B 379 M, *Lactobacillus acidophilus* 97 and *Propionibacterium shermanii* 12 AE. The bacterial concentrate was introduced in ratio of 1:1:1 in an amount of 1-5% based on mixture mass; the total fat content was 8%.

Miwa and Ohashi²⁰ described a low-fat ice cream or ice cream-like product, which has a fat content of 3-8% weight, based on the total weight of low-fat ice cream or ice cream-like product, which was produced by treating a milk material or a liquid ice cream mix with a protein deamidating enzyme to obtain deamidated milk protein.

Table 5: Sensory characteristics of ice creams

Sensory index Evaluation score	Ice cream samples		
	MM-100	MM-40	MM-30
Color	89	94	92
Texture and consistency	White with yellow shading uniformly distributed across the product	Yellowish white uniformly distributed across the product	Creamy white uniformly distributed across the product
Flavor	Non-dense with sensible ice crystals	Homogenous, soft and sufficiently dense without sensible ice crystals	Homogenous and sufficiently dense without sensible ice crystals
Taste	Sweet flavor without foreign smell	Sweet flavor without foreign smell	Sweet flavor without foreign smell
Appearance	Slightly acidic taste	Sweet taste	Sweet pronounced flavor
	Flat and smooth surface	Flat and smooth surface	Flat and smooth surface

Overall, these studies illustrate the flexibility of the ice cream process and how different additives impact the chemical and nutritive value of the food.

CONCLUSION

A current trend in milk dessert is the development and production of dietary low-fat ice cream enriched with different additives. A combination of cow's and mare's milk in an ice cream mixture decreased fat content and enhanced amino acid composition, including methionine, glycine, histidine, proline, threonine and valine. These findings suggest a beneficial role of mare's milk in ice cream production with low-fat content.

SIGNIFICANCE STATEMENT

This study found the nutritive value of mare's milk ice cream could be beneficial for low-fat nutrition. For ice cream production, mare's and cow's milk was used in different proportions. Based on the chemical, amino acid, mineral and vitamin composition findings, the ice cream with 40% of mare's and 60% of cow's milk was proposed for healthy nutrition.

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