Fermented drink based on secondary raw milk materials

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Keywords:

Milk
Fermentation
Milk whey
Laurel cherry
Biological value

Article history:

Received 01.03.2024
Received in revised form 16.09.2024
Accepted 30.12.2024

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DOI: 10.24263/2304-974X-2024-13-4-6

Abstract

Introduction. The aim of the research was production of fermented milk drink with increased biological value for preventive nutrition based on secondary milk processing raw materials.

Materials and methods. Skim milk, demineralized milk whey, retentate, the bacterial starter cultures, laurel cherry fruits, fermented acid drink were used in the research. Titrated acidity, the content of proteins, fat, ash, and pectins were determined by ISO standards, the content of sugars was estimated by Bertrand's method, the content of total phenols by Folin Chocalteau method, and the number of viable bacterial cells by plate counting method.

Results and discussion. The fermentation process of demineralized whey, skim milk or retentate under the influence of direct addition of matsoni, kefir, yogurt or propionic bacterial starters was investigated. The best results were observed with the use of matsoni starter. After a 12-hour fermentation of demineralized milk whey, the number of viable cells of matsoni bacterial starter was 118±38x10^6 colony formed units (CFU)/mL, in skim milk it was 135±62x10^6 CFU/mL, and in retentate it was 127±52 CFU/mL. After the bacterial starter culture of matsoni, the best results were shown by the bacterial starter cultures of kefir, yogurt and finally propionic. In the fermentation process, the desired result of titrated and active acidities (800T, pH 3.62) was achieved by using the bacterial starter culture of matsoni.

To achieve the final optimal acidity of a prophylactic fermented milk drink (80–1000T, pH 4.2–4.5), out of different compositions made by using bacterial starter cultures of matsoni, kefir, yogurt, and propionic, two compositions were considered the best, in which the ratio of matsoni, yogurt, and propionic was 1.0:0.5:1.0 and 1.0:0.5:0.5, respectively. During this time, the optimal duration of the fermentation process was 5-6.5 hours. In the composition of the fermented milk drink, laurel cherry fruits were used as fillers. Based on the study of their chemical composition, it was determined that laurel cherry fruits are characterized by a high content of main components – sugars (16.20±0.42%), nitrogenous substances (1.92±0.09%), vitamin C (16.10±0.22%), pectin substances (0.78±0.21%), and total phenolic compounds (420±0.35 mg/100 g). Based on the sensory evaluation, the optimal amount of laurel cherry puree (3–5%) was determined for the fermented milk product. Prophylactic fermented milk drink technology was proposed.

Conclusions. Fermented milk drink was proposed based on secondary raw materials of milk and vegetable raw materials (laurel cherry fruits).

Introduction

Due to the shortage of food resources in the world, the maximum utilization of valuable secondary products of processing raw materials is of particular importance (Ivanov et al., 2021; Jolanta et al., 2016; Stabnikova et al., 2021, 2024a). Secondary raw materials for milk processing deserve special attention, especially milk whey, whose utilization factor is currently unacceptably low. Only 35–40% of milk whey is recycled, and it is mostly poured into the sewer system, causing environmental pollution. Especially serious problems are caused by curd whey due to its high acidity (70–75 °T). The full utilization of milk whey and other secondary milk raw materials for use in the production of high biological value, low-calorie, and low-glycemic functional fermented milk drink is still a worldwide problem (Ayed et al., 2023; Kochubei-Lytvynenko et al., 2022).

The dairy industry in Georgia loses thousands of tons of that valuable raw material every year, of which milk whey deserves special attention (Rukhadze et al., 2021). It is a low-calorie product (18-20 kcal/100 g) and has a low glycemic index (24), which is why it is considered a universal bio-eco-friendly raw material of natural origin for the production of dietary and functional food products (Ahmed et al., 2023; Jolanta et al., 2016).

The production of liquid fermented beverages, which does not require large expenditures, can be considered as one of the areas of cost-effective use of whey (Jeličić et al., 2008). The development of food products based on the beneficial substances of milk passed into the milk whey, especially functional fermented milk drinks, remains very relevant today (Reis et al., 2021).

The main methods of increasing the biological value of fermented milk drinks and ensuring their pre- and probiotic properties are the use of highly effective multi-component bacterial starters (Amiranashvili et al., 2015; Pereira et all., 2015; Pescuma et al. 2010) with the addition of fruit juices (Chatterjee, 2015; Idan, 2021; Thakkar et al., 2018), fruit's pulp (Elkot et al., 2023a), extracts of phyto-raw materials (M'hir et al., 2023; Seyhan et al., 2016), germinated grains of various cereals and legumes (Criste et al., 2023; Emkani et al., 2022), soybean oil as a source of polyunsaturated fatty acids (Vlaseva et al., 2012), and algae (Elkot et al., 2023b).

The popularity of using plant extracts in fermented milk products is due to the presence of a wide range of biologically active substances such as vitamins, flavonoids, antioxidants, tannins, macro- and microelements. The combination of lactic acid microflora and biologically active substances of fruits and berries allows to significantly expanding the range of functional products (Farmani et al., 2024; Szafrańska et al., 2024).

Based on the above, it can be concluded that increasing the dietary and health properties of fermented milk products by enriching them with probiotic bacteria and prebiotics are a relevant area in the creation of functional food products.

The research aims to develop a high biological value fermented milk drink based on the secondary raw materials of milk processing to be used for preventive nutrition.

Materials and methods

Chemicals

All reagents used were of USP purity or higher. All solvents, including water, were used with an LC/MS label.

Materials

Secondary raw materials from milk processing, namely, skim milk, demineralized milk whey, and milk retentate; the bacterial starters, in particular, matsoni, kefir, yogurt, and propionic starters; a plant-based supplement (laurel cherry fruits); structure stabilizer (puree of fruits) were used as materials in the research. Skim milk, demineralized milk whey, and milk retentate were obtained from the milk processing enterprise LLC "Atinati".

Skim milk is a by-product in the production of cream, sour cream, and butter. The most valuable components in it are protein and carbohydrates. Skim milk contains high biological value protein (up to 4%), water- and fat-soluble vitamins.

Demineralized milk whey was obtained from LLC Atinati with a level of demineralization of 85–90% and a level of acidity neutralization of pH 6.0–6.5 (Rukhadze et al., 2021). Demineralized milk whey is a source of important food nutrients (Kochubei-Lytvynenko et al., 2023). It contains more than two hundred biologically active substances, almost all water-soluble and finely dispersed components of milk.

Milk retentate, or a milk ultraconcentrate, is a concentrated milk protein, which is obtained because of ultrafiltration of milk, semi-skim, or skim milk.

Matsoni, kefir, yogurt, and propionic starter cultures were purchased from LLC "SAMAIA".

Matsoni starter culture contains *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, and *Lactobacillus delbrueckii* subsp. *lactis* (Amiranashvili et al., 2014; 2015).

Kefir starter culture contains *Lactococcus lactis* subsp. *lactis, L. lactis* subsp. *cremoris, Leuconostoc mesenteroides* subsp. *cremoris, L. lactis* subsp. *lactis biovar diacetylactis, Lactobacillus delbrueckii* subsp. *bulgaricus,* and *Saccharomyces cerevisiae*.

Yogurt starter culture consists of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus lactis*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*.

Propionic starter culture was a propionic acid bacteria *Propionibacterium shermanii* – KM 186 strain.

A plant-based supplement laurel cherry fruits, grown in the Adjara region taken from the harvest of 2023 at perfect ripeness. Samples were stored in refrigerated chambers prior to analysis (Figure 1).

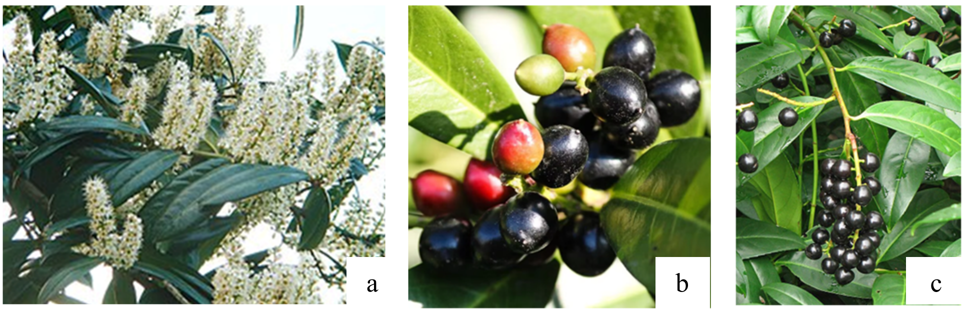

Figure 1. Laurel cherry during flowering (a); ripening fruits (b); ripe fruits of the wild-growing laurel cherry (c)

The laurel cherry is widespread in the subtropical zone of Georgia, especially in Adjara. The fruits of the plant have an individual, distinctive, unforgettable, velvety taste and aroma. The laurel cherry fruits contain polyphenols, among which anthocyanins are predominant, glycosides, tannins, and possess high antioxidant activity and can be used for the production of functional food (Minadze, 2006; Minadze et al., 2006; Stabnikova et al., 2024b).

The laurel cherry fruit puree was cooked in the following order: selected and washed fruits were peeled and blended. The obtained mass was rubbed through a sieve with a hole diameter of 0.5 mm, and then the mass prepared in this way was used in the preparation of a fermented milk drink.

Fermented milk drink was prepared from skim milk with a dry matter content of up to 9.5%, acidity of up to 20 °T; demineralized milk whey with a dry matter content of up to 7%, and milk retentate taken in a ratio of 1.0:2.0 :0.2 (Patent GEO No. 7334). All components were mixed, pasteurized at 72–75 °C, cooled to a temperature of 32–38 °C, and bacterial starter culture was introduced into it. Then, a sweetener and filler were added (laurel cherry fruit puree), which simultaneously played the role of a structure stabilizer and a flavor additive. Coagulation was carried out for 5.0–6.5 hours, at a temperature of 35–37 °C, until an acidity of 80–100 °T was reached. After that, it was pasteurized, homogenized, cooled, and bottled. The main components were used in the following ratio, wt. %: milky composition, 85–87; bacterial starter culture, 3–5; sweetener (palatinose), 1–3; laurel cherry fruit puree, 3-5.

Methods

Determination of dry matter content. The amount of dry matter in secondary milk raw materials and plant-based supplements was determined using a RADWAG MA 50r moisture meter (Finland), where drying was carried out under the influence of infrared rays. 5-10 g of samples were placed on the cup of this device, and drying was carried out at 105 ^{0}C. The screen recorded the moisture content in the analysis samples in %. The amount of dry matter was calculated as the difference: 100 minus the amount of moisture content.

Determination of acidity. Titrated acidity was determined by the titration method: for a plant-based supplement – according to ISO-750-1998 standard, for secondary milk raw materials – according to ISO/TS 22113:2012 standard, and active acidity was determined using PH-meter Metlter Toledo (USA).

Determination of total sugars. Total sugars were determined by the Bertrand's method (Surmanidze, 2023).

Determination of total phenolic content. Folin-Chokalteu method was used to determine the amount of phenolic substances in fruits (Bond et al., 2003; Tkesheliadze et al., 2022).

Determination of the total amount of proteins. The total amount of proteins was determined by the Kjeldahl method according to ISO 8968-1:2014.

Determination of the total amount of fats. The total amount of fats was determined by the Soxhlet method. Hydrolysis of the sample was carried out on the device HydrolEx H-506, and extraction was carried out on the extractor FatExtractor E-500. The resulting fat was dried to a constant weight and then weighed, and the fat content of the sample was calculated following ISO 14156:2001/IDF 172:2001.

Determination of total ash content. Total ash content was determined following ISO/CD 9877 IDF 258.

Determination of the amount of pectin substances. The amount of pectin substances was determined according to ISO 5773:2023(E).

Determination of vitamin C content. The quantitative determination of vitamin C in plant materials was carried out using a method based on reducing properties: a blue solution of 2,6-dichlorophenolindophenol is reduced to a colorless compound by plant extracts containing ascorbic acid (Surmanidze, 2023).

Determination of the number of microorganisms. The number of microorganisms was determined by counting colonies. 10 g of product were taken and mixed with 90 ml of buffered peptone water or a 0.85%-solution of table salt. 10 test tubes were placed on the test bench, each of which contained 9 mL of buffered peptone water. After the initial dilution, 1 mL of the test product was added to the first test tube with a sterile pipette. Using a sterile pipette, 1 mL was transferred from the first test tube to the next test tube, and so on. As a result, dilutions of 1:10, 1:100, and 1:1000 were obtained. The time from dilution preparation to inoculation exceeded 45 min. Before sowing, the cup was assigned a number. From each dilution, 1-1 mL of the analyte compound was taken and applied to two Petri dishes (for parallel determination). Then thawed and cooled to 45 °C food agar was added in the amount of 12-15 mL. With a circular movement of the hand, the cup was moved on the horizontal surface of the table, so that the contents were distributed in the form of equal layers over the entire area of the bottom. The process continued until the agar solidified. The cup with the seed was placed in a thermostat upside down for incubation at 30 °C for 72±3 hours. After this time, the colonies grown both on the surface and in the depth of the agar were examined. Colonies were counted using a colony counter. The arithmetic mean was calculated for each dilution separately.

Sensory evaluation. The sensory evaluation of the fermented milk drink was carried out on a 10-point scale according to the following indicators: homogeneous, slightly viscous, with liquid whey, characteristic color of the product, pure lactic acid taste, moderately sweet, and moderate taste of filler. In advance, the characteristics of each score were introduced. The best indicator was valued at 10 points, and the unsatisfactory one at 1 point. Five tasters participated in the sensory evaluation. They were selected from the staff and students of the Department of Food Technology of Akaki Tsereteli State University. The final scores were calculated by the arithmetic mean of the points assessed by each taster (Karchava et al., 2022).

Calculation of caloric content. To determine the caloric content of the research samples, the amount of proteins, fats, and carbohydrates contained in them were taken into account, multiplied by their conversion coefficient (4, 9, and 4 respectively), and the results were summed up.

Statistical analysis

Mathematical processing of the experimental data was performed according to the results of 3-5 times repetitions of the tests with the methods of statistics and regression analysis using Windows IBM SPSS Statistics software program (version 20.0, IBM, Armonk, New York, USA).

These studies were conducted at the Department of Food Technologies of Akaki Tsereteli State University (Georgia, Kutaisi) and the Western Georgian Regional Center of Chromatography Chromatographic Center of Shota Rustaveli State University in Batumi.

Results and discussion

Characteristics of secondary raw milk materials used for fermented milk drink

Skim milk, demineralized milk whey, and milk retentate were chosen as base for fermented milk drink creation. Their proximate composition was studied and caloric content was calculated (Table 1).

Table 1

Proximate composition of secondary raw materials

Compounds	Milk whey	Skim milk	Retentate
Moisture, %	93.28±1.31	90.30±1.08	80.4±1.32
Protein, %	0.87±0.11	3.60±0.08	10.7±0.15
Fat,%	0.11±0.05	0.08±0.02	0.72±0.02
Carbohydrate, %	5.75±0.06	6.02±0.05	8.90±0.09
Ash, %	0.63±0.04	0.72±0.05	0.20±0.01
Calorie content, kcal/100 ml	24.9	36.32	77.60

The content of dry matter in the secondary raw milk materials was as follows: in demineralized milk whey: 6.72±0.11%, in skim milk 9.7±0.08%, and in retentate 19.6%±0.13. The highest contents of proteins and carbohydrates were observed in the retentate and they were 10.7±0.15% and 8.7±0.11%, respectively. Fat and ash contents were recorded in insignificant amounts and did not exceed 0.11±0.05% and 0.72±0.02%, respectively. As for caloric content, it was 24.91 kcal/100 ml for demineralized milk whey, 36.32 kcal/100 ml for skim milk, and 77.6 kcal/100 ml for retentate. All secondary raw materials of milk are low-calorie (Ahmed et al., 2023) and acceptable for the preparation of prophylactic fermented milk drinks (Reis et al., 2021).

Development of starter cultures in secondary raw milk materials

To produce a fermented milk product, cheese whey, skim milk, and retentate were proposed, so it was necessary to study the development of starter cultures in this environment. Considering the data of several studies that noted that during the production and storage of milk and fermented milk drinks based on whey, especially when using various supplements, liquid stratification (separation of whey) occurs (Chavan et al., 2018; Paula et al., 2021), therefore demineralized whey was used – purified from suspended protein fragments and mineral salts. The activity of the development of starter microflora within 12 hours during the fermentation process was estimated by titrated active acidity and the number of viable cells of starter microorganisms (Table 2). The experiments were conducted at a 5%-dosage of starter cultures.

Table 2

Comparative characteristics of starter cultures in skim milk, whey, and retentate

Indicators	**Starter culture**			
	Propionic	**Matsoni**	**Kefir**	**Yogurt**
Demineralized cheese whey				
The number of vital cells, CFU/mL	90±51	118±38	110±42	102±62
Titrated acidity, °T	47±2	80±1	72±2	68±3
Active acidity, pH	4.75±0.01	3.62±0,02	4.02±0,01	4.35±0,01
Skim milk				
The number of vital cells, CFU/mL	125±68	135±62	127±52	119±82
Titrated acidity, °T	71±2	87±2	82±2	69±2
Active acidity, pH	4.06±0.01	3.92±0.01	4.21±0.02	4.42±0.01
Retentate				
The number of vital cells, CFU/mL	118±68	127±52	123±65	116±58
Titrated acidity, °T	68±2	79±2	71±1	62±3
Active acidity, pH	4.3±0.01	4.13±0.01	4.26±0.02	4.37±0.01

Based on the comparative analysis of the data presented in Table 1, it was revealed that the activity of bacterial starter cultures in demineralized milk whey, skim milk, and retentate did not differ significantly. Considering all indicators, the best results were achieved using the bacterial starter culture of matsoni. After a 12-hour fermentation of demineralized milk whey, the number of viable cells of matsoni bacterial starter was 118±38x10^6 CFU/mL, in skim milk 135±62x10^6 CFU/mL, and in retentate 127±52 x10^6 CFU/mL. After the bacterial starter culture of matsoni, the best results were shown by the bacterial starter cultures of kefir, yogurt, and finally propionic (Gungor et al., 2024). As for the titrated and active acidities (80 °T, pH 3.62), the bacterial starter culture of matsoni was considered dominant in this case as well.

Changes in titrated and active acidities during process of fermentation (coagulation) of secondary milk raw materials using different bacterial starter cultures.

The fermentation process in secondary raw milk lasted for 12 hours. Determination of titrated and active acidities was done every 3 hours (Figures 2 and 3).

As shown in Figures 3 and 4, the best results were achieved by using the matsoni starter culture in different secondary milk raw materials for 12 hours (80 °T, pH – 3.62). The lowest activity was observed with propionic. Considering that the properties of starter cultures of matsoni and kefir, as well as their fermentation activities are close to each other, the bacterial starter culture of kefir was excluded from the composition of bacterial starter cultures for further experiments.

Determining the ratio of bacterial starter cultures for a milky composition

Further, with different ratios of the starters used in the bacterial composition, the time required for curd formation and the final acidity of the fermented milk base were established. The following ratios of starter microflora were compiled:

1. M :Y : P= 1.0 : 1.0 : 1.0 ; 2. M : Y : P = 1.0 : 0.5 : 1.0 ;
3. M : Y: P = 1.0 : 0.5 : 0.5 ; 4. M : Y: P = 2.0 : 1.0 : 1.0 ;
5. M :Y: P = 1.0 : 0.5 : 1.0 .

(M – marsoni starter: K – kefir starter: Y – yogurt starter: P – propionic starter)

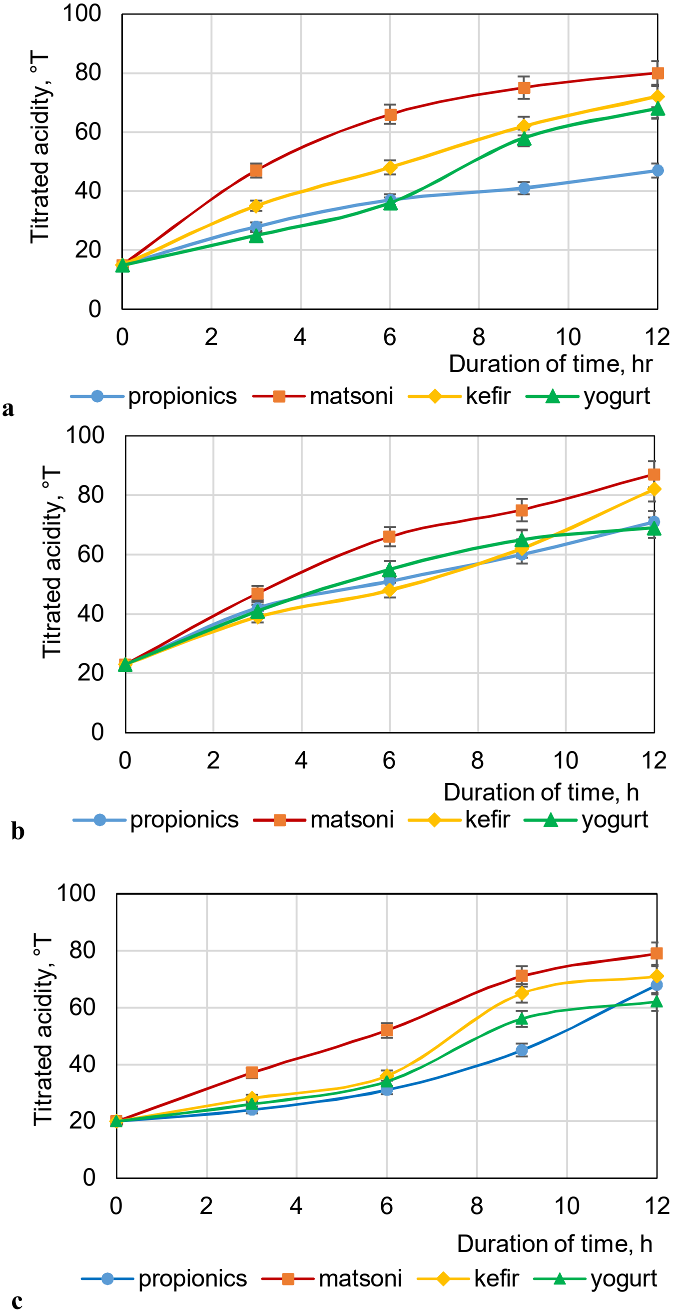

Figure 2. Change in titrated acidity during fermentation process using different bacterial starter cultures in: milk whey (a); skim milk (b); in retentate (c)

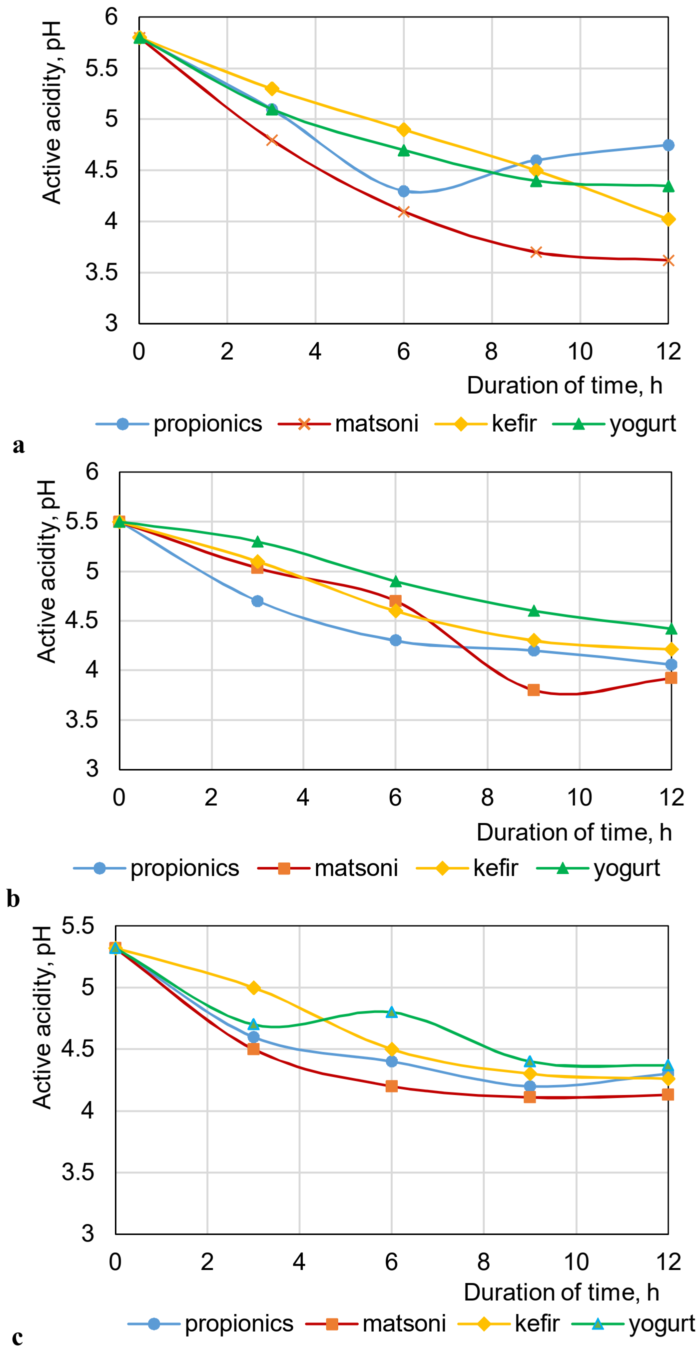

Figure 3. Change in active acidity during fermentation process using different bacterial starter cultures in: milk whey (a); in skim milk (b); in retentate (c)

Table 3 presents the activity of development of probiotic microorganisms in milk-whey medium with different initial ratios of starter microflora.

Table 3

Curd formation and changes in acidity during fermentation of milk-whey fermented drinks

Proportion of starters in the bacterial composition (dosage – 5%)	Time required for curd formation, h	Acidity	
		°T	pH
M : Y : P = 1.0 : 1.0 : 1.0	8.5	74±2	4.76±0.01
M : Y : P = 1.0 : 0.5 : 1.0	6.5	88±1	4.58±0.02
M : Y : P = 1.0 : 0.5 : 0.5	5.0	92±1	4.51±0.01
M : Y : P = 2.0 : 1.0 : 1.0	10.5	75±3	4.76±0.01
M : Y : P = 2.0 : 0.5 : 1.0	14.0	79±2	4.72±0.02

To achieve the predetermined final optimal acidity of a prophylactic fermented milk drink (80-100 ^{0}T, pH 4.2-4.5), the best result was shown by the composition of bacterial starter cultures in the following ratios: M : Y : P = 1.0 : 0.5 : 1.0; M : Y: P = 1.0 : 0.5 : 0.5.

Plant-based supplement for a fermented milk drink

The fruits of laurel cherry were used in the form of a plant-based supplement, as a structure stabilizer in the process of fermentation and delaying the milky composition.

Chemical composition of the laurel cherry fruits

The overall chemical composition of these fruits and the fractional composition of their pectin substances were determined (Table 4, Figure 4).

Table 4

Chemical composition of laurel cherry fruits

Characteristics	Samples of laurel cherry	
	Black-fruited, large	Wild-growing
Mass fraction of dry matter, %	21.80±0.21	17.10±0.32
Total sugars, %	16.20±0.42	11.71±0.35
Acidity, % (in terms of malic acid)	0.26±0.11	0.24±0.12
Pectin substances, % (in terms of the raw mass)	0.78±0.21	0.48±0.33
Total phenols, mg/100 g	420±0.35	480±0.15
Nitrogenous substances, %	1.92±0.09	1.87±0.10
Vitamin C, mg%	16.10±0.22	13.7±0.16

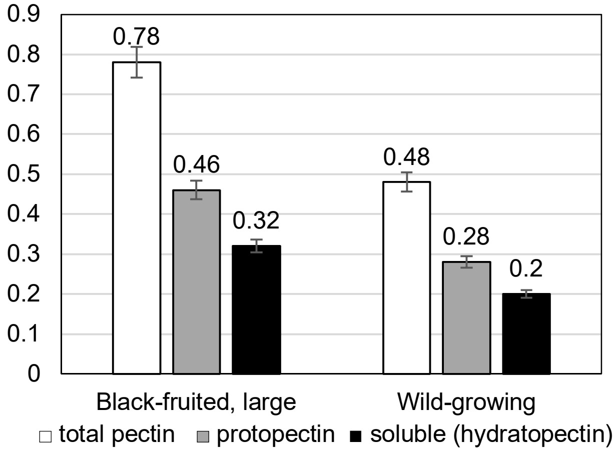

Figure 4. Fractional composition of pectin substances of the laurel cherry fruit

It was determined that the studied raw materials are valuable not only for their taste properties, but also due to their composition, high content of pectin substances, and phenolic compounds. These results are close to the results of research conducted by different researchers at different times. In particular, the chemical composition of the fruits of laurel cherry common in the subtropical zone of western Georgia and Adjara has been determined for use in the technology of food coloring (Surmanidze, 2023), confectionery (Minadze, 2006) and alcoholic beverages (Japaridze et al., 2005). Based on the analysis of the chemical composition of the fruits of laurel cherry (black-fruited large and wild growing), the black-fruited large fruits were used for further research.

Optimal amount of plant-based supplement for fermented milk drink

At the next stage of the research, the fermented milk product was mixed with the puree of the laurel cherry fruits in different percentages: 1, 3, 5 and 7%. To determine the optimal amount, an organoleptic evaluation was carried out. A fermented milk product with the addition of 3-5% of laurel cherry puree was considered the best. The results are illustrated in Figure 5.

Based on repeated experiments, the amount of filler and the technological parameters of the drink preparation were specified, which was reflected in the developed and proposed technology to produce a fermented milk drink, which is described in the section: “Materials and methods”.

The resulting fermented milk drink has a uniform, gentle consistency, natural color, and original aroma of the vegetable component – laurel cherry fruits. The ratio of secondary products of milk processing, bacterial starter culture, and fillers was selected not only based on their biological compatibility, but also based on the taste properties, to achieve a uniform organoleptic original range, which will additionally have a preventive effect on the human body.

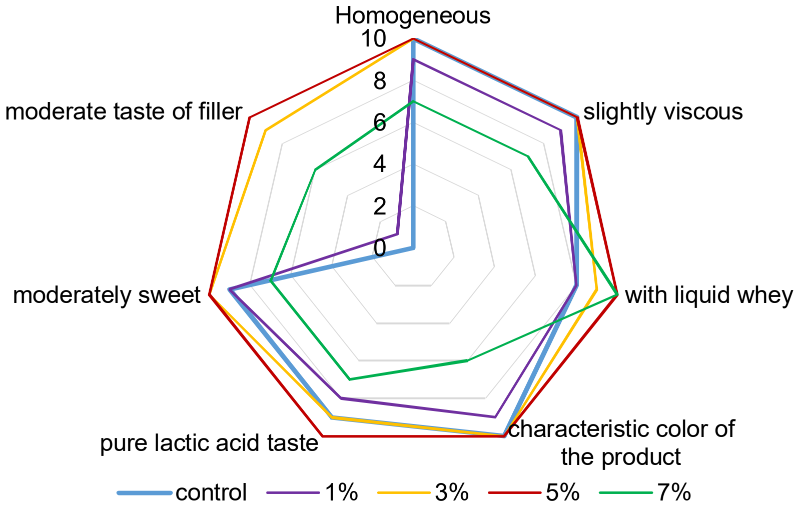

Figure 5. Influence of doses of laurel cherry puree on sensory parameters of fermented milk product

Conclusions

1. In the fermentation process of demineralized milk whey, skim milk, and retentate, with the action of direct application of the bacterial starter cultures – matsoni, kefir, yogurt, and propionic, the best results were observed with the use of matsoni bacterial starter culture. After a 12-hour fermentation of demineralized milk whey, the number of viable cells of matsoni bacterial starter was 118±38x10^6 CFU/mL, in skim milk 135±62x10^6 CFU/mL, and in retentate 127±52x10^6 CFU/mL. After the bacterial starter culture of matsoni, the best results were shown by the bacterial starter cultures of kefir, yogurt and finally propionic.
2. In the fermentation process, the desired result of titrated and active acidities (800T, pH 3.62) was achieved by using the bacterial starter culture of matsoni. The lowest activity was observed with propionic.
3. Various compositions of bacterial starter cultures were prepared. To achieve the final optimal acidity of a fermented milk drink (80–100 °T, pH 4.2–4.5), the composition of bacterial starter cultures was considered the best in the following ratios: M : Y : P = 1.0 :0.5 :1.0; M : Y: P = 1.0 : 0.5 : 0.5. At this time, the optimal duration of the fermentation process was 5-6.5 hours.
4. There was determined the feasibility of using a plant-based supplement (laurel cherry fruits) as a filler in a fermented milk drink due to its taste properties, chemical composition, sugars (16.20±0.42%), nitrogenous substances (1.92±0.09%), vitamin C (16.10±0.22%), considering the high content of pectin substances (0.78±0.21%) and total phenolic compounds (420±0.35 mg/100 g).
5. Based on the sensory evaluation, the optimal amount of laurel cherry puree for a fermented milk drink was determined as 3–5%.

Acknowledgements. This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) (FR-23-3721 Development of innovative technology for production of whey-based functional lactic acid drink).

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