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Technology of a new fermented milk beverage enriched with lactic acid and propionic acid bacteria

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Abstract. Probiotics used in the food industry mainly include lactic acid bacteria (LAB), bifidobacteria, propionic acid bacteria (PAB) and yeasts. Today, creation a functional food - fermented milk product based on endemic starter culture with probiotic and biochemical activity is actual. The aim of our work was to develop a probiotic fermented milk product for drink using propionic acid bacteria and autochthonous lactic acid bacteria. As a result of our researches, based on organoleptic properties, the multicomponent combination of LAB and PAB was selected; The technological parameters for obtaining the fermented milk product were determined: tithable acidity of milk - 17±2˚T;

fermentation time in a thermostat - 2 hours, fermentation temperature - 43±1 ˚C, storage temperature - 4±2 ˚C; shelf life - 14 days; The selected consortium and the established technological process flow chart can be used to obtain probiotic fermented milk beverage enriched with LAB and PAB.

Keywords: Fermentation time; Fermented milk product; Probiotics; Sensory analysis.

Introduction

Researches made in the current century have shown a relationship between the consumption of fermented milk products and improved human health. Moreover, fermented milk products are associated with the prevention of obesity, chronic diarrhea, hypersensitivity, irritable bowel syndrome, treatment of gastroenteritis or relief of symptoms closely related to an unhealthy lifestyle. Lactic acid bacteria (LAB, propionic acid bacteria (PAB), bifidobacteria and yeast are widely used in the production of dairy products (Bintsis, 2018). During fermentation, they form organic acids, ethanol and aromatic substances, which improve the development of organoleptic properties of food products, prevent their spoilage and are therefore very useful for the food and dairy industries. Probiotics (bacteria and yeasts), in live form in properly amounts, improve the health of the host and can be used for controlling, prevention, or treatment of certain diseases (WHO/FAO, 2006; Hati, Mandal, & Prajapat, 2013). They enrich the intestinal microflora and protect the intestine from colonization by pathogenic microorganisms. They are also characterized by: anti-mutagenic and anti-carcinogenic action, stimulation of the immune system, reduction of cholesterol levels in the blood serum, etc. (Perez, Zendo, & Sonomoto, 2014). Fermented milk products containing probiotics prevents the impact of pathogens on normal microbiota of the microbiota of gastrointestinal tract, improve human health due to the growth of beneficial bacteria in the intestine and the production of metabolites by them (Bintsis, 2018).

The goal of our work was to elaborate a technology for the production of new fermented milk beverage using propionic acid bacteria and autochthonous lactic acid bacteria isolated and selected by us.

Main Part

To obtain the fermented milk product, we used the commercial propionic acid bacterium *Propionibacterium freudenreichii* subsp. *shermanii* (Lactoferm PP4, Biochem, Italy), recommended by the manufacturer for the production of various types of cheese (Grouvier, Emmental, Eldamer), as well as LAB isolated by us from milk and dairy products, collected from small family farms in different regions of Georgia and selected by probiotic, biotechnological and organoleptic properties (Gagelidze, et al., 2004).

Skimmed milk powder (Covbar Food Industry) was used for making of the product. Milk was reconstituted according to GOST 3624-92 (GOST 3624-92, 2009); Fat, protein, lactose and ex. water were determined by Lactoscan (Bulgaria). For homogenization and pasteurization of reconstituted milk, a Thermomix (France) was used, which allows processing reconstituted milk in the laboratory as in a factory: heating at the temperature of 91°C±1°C for at least 30 seconds and subsequent rapid cooling to a suitable temperature. The effectiveness of pasteurization was tested by measuring of peroxidase activity in pasteurized milk (GOST 3623-2015, 2019). The titrable acidity (˚T) of milk and fermented milk products were determined according to GOST 3624-92 (GOST 3624-92, 2009).

For obtaining of fermented milk product, propionic acid bacteria (0.02 g/l) and various combinations of selected LAB (in liquid form) in amount of 5% of the total volume (n × 10^{-6} CFU/ml) were added to the reconstituted, pasteurised and homogenised milk adjusted to the required temperature (44 °C). The amount of microorganisms were determined on MRS agar by the method of limiting dilution (Harley, 2005). Fermentation was conducted in a thermostat at 43°C temperature. For thickening/coagulation of milk by each combination required different time; The titrable acidity and pH was measured after incubation. The obtained product was transferred to the refrigerator at a temperature of 4±2°C and after 10±2 hours, it's acidity(°T), pH and organoleptic indices were studied (Table 1).

For sensory analyses of fermented milk products hedonic test was used; Analysis was conducted in the sensory laboratory of the Technical University of Georgia in a separate booth. 10 employees of the Georgian Technical University and Agricultural University of Georgia took part in the tasting. 5-point scale were used by volunteers for rating of each fermented milk product according to products liking: from 1 point (very bad) to 5 points (very good).

Table 1

Characteristics of the fermented milk product obtained using different combinations of lactic acid bacteria and propionic acid bacteria

Combination of strains	Incubation time, h	Titrable acidity, °T		pH	Point
		At the end of the fermentation	After storage (8 h) at +4 °C		
T-221 + G-26 + Pr	8	90	100	4.5	4
T-221 + T-190 + Pr	8	92	102	4.47	4
T-221 + T-365 + Pr	8:30	94	102	4.45	4
G-26 + T-190 + Pr	7	90	100	4.42	4
G26 + T-365 + Pr	8	94	104	4.5	4
T-190 + T-365 + Pr	7	92	100	4.52	4
T-221 + G-26 + T-190 + T-365 + Pr	6	90	102	4.51	5

Note: Pr - *P. freudenreichii* subsp. *shermanii*; T-221 - *L. delbrueckii* subsp. *lactis* T-221; G-26 - *S. Thermophilus* G-26; T-190 - *L. Delbrueckii* subsp. *Bulgaricus* T-190; T-365 - *S. thermophilus* T-365

11:00 a.m. was chosen for the tasting, as the best time by optimal sensitivity of the appraisers. Volunteers have taken into consideration the inadvisability of conducting tastings on an empty stomach or immediately after eating. Half an hour before the tasting, degustators are prohibited smoking, drinking, and eating (Lawless & Heymann, 2010).

Before the beginning of the procedure, to the volunteers were explained the goals, tasks and order of work of the tasting; They worked independently of each other and wrote down their opinion about each product.

In all combinations presented in table 1, the texture/consistency of the fermented milk product was good, the milk clot - glossy, the whey - little, the taste - slightly sour and pleasant, the aroma - pleasant, however - different in all combinations. The combination of LAB and PAB - T-221+G-26+T-190+T-365+Pr, was chosen according to the evaluations of volunteer tasters as the best, by more diverse taste.

The fermentation time was decreased to obtain the desired consistency, characteristic of a fermented milk beverage. Properties of milk used for the production of probiotic fermented milk beverage were: fat - 3.2, protein - 2.8, lactose - 4.7; acidity - 17±2˚T. To obtain the product, skimmed milk powder was placed in a Thermomix in water heated up to 35°C and mixed well; after dissolving of the milk powder, the butter was added and heated the mass up to 55 ˚C temperature (at this temperature the butter melts); after 10-15 minutes when the butter completely melted, the mass was pasteurized at 90-91 ˚C temperature (30 seconds), then the Thermomix was switched to homogenization mode. The homogenized mass was cooled to the coagulation temperature (43±1 ˚C). At this stage, was added the starter (selected combination) in the amount of 5% (the number of bacteria in the inoculum – 35×10^{-6} CFU/ml); After mixing, the obtained mass was poured into 300 ml plastic bottles.

For fermentation one variant of the samples was kept in the thermostat for 1.5 hours, the second one - for 2 hours, after which the bottles of both variants were transferred to the refrigerator at a temperature of +4±2 °C for 12 hours. The acidity (˚T) and the amount of microorganisms were checked both after the end of fermentation in the thermostat and after the storage in the refrigerator for 12 hours, and organoleptic characters and the amount of microorganisms - after storage in the refrigerator (Table 2).

Table 2.

Effect of duration of fermentation on properties of the milk fermented products

Duration of fermentation, h	Titrable acidity of product, ˚T		CFU/ml in cooled product
	After removing from the thermostat	After cooling (12 h) at +4°C	
1.5	48˚T	90	14×10^{-9}
2	52 ˚T	102 ˚T	68×10^{-9}

The taste of both products obtained by fermentation different duration time was mild and look like to fermented milk product received by appling of LAB, but the 2-hour fermentation variant was chosen based on the consistency and homogeneity of the product.

To determine the shelf-life of the product obtained by 2-hour fermentationt, we kept the product in the refrigerator for 18 days; The acidity (˚T) and the amount of living microorganisms (lg CFU/ml) of the fermented milk product were tested in dynamic with an interval of 3-4 days (Table 3).

Table 3.

Changes in the properties of fermented milk beverage during storage in cold conditions

Day	Quantity of microorganism, lg CFU/ml	Titrable Acidity, °T
At the beginning of fermentation	7.5	17
Before cooling	10.8	52
1st (after cooling)	11.7	102
4th	11.8	102
7th	11.9	105
11th	10.4	110
14th	10.1	112
18th	7.0	130

As it is seen from Table 3, the shelf-life of milk fermented product obtained by LAB and PAB can be considered 14 days, when the titrable acidity of the product corresponds to the established standards for the matsoni (Geographical indication: "Matsoni") and is pleasant for drink; In addition the number of probiotic microorganisms in the product corresponds to the requirements for probiotic foods (WHO/FAO, 2006).

Conclusion

Based on organoleptic properties, a consortium of lactic acid and propionic acid bacteria was selected - *P. freudenreichii* subsp. *shermanii* + *L. delbrueckii* subsp. *lactis* T-221 + *S. thermophilus* G-26 + *L. delbrueckii* subsp. *bulgaricus* T-190 + *S. thermophilus* T-365;

The technological parameters for obtaining the fermented milk product were determined: titrable acidity of milk - 17±2°T; fermentation time in a thermostat - 2 hours, fermentation temperature - 43±1 °C, storage temperature - 4±2 °C; shelf life - 14 days;

The selected combination of lactic acid and propionic acid bacteria and the established process flow chart can be used to obtain probiotic fermented milk beverage enriched with lactic acid and propionic acid bacteria.

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