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Quality of Cow's and Goat's Fermented Bifido Milk during Storage

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Summary

The fermented bifido milk, with and without supplements (skim milk powder and whey protein concentrate powder) from commercially available goat's and cow's long-life milk was produced. In goat's milk samples pH decreased faster and at the end of fermentation was lower (from 4.64 to 4.83) than in cow's milk samples (from 4.96 to 5.24). In both milk types the growth of *Bifidobacterium bifidum* Bb-12 during fermentation was better in supplemented samples. At the end of fermentation, the lowest viable count (log(CFU/mL) =8.05) was in control cow's sample, and the highest (log(CFU/mL)=8.56) in goat's milk samples supplemented with whey protein concentrate. On the ninth day of storage the viable count in fermented cow's milk samples had an average value of $N=1.1 \times 10^8$ CFU/mL, and in fermented goat's milk it was $N=2.3 \times 10^8$ CFU/mL. Supplements had no influence on bifidobacteria survival. Sensory properties of supplemented goat's samples were significantly better than those of coresponding cow's samples, especially with whey protein concentrate powder.

Key words: goat's milk, bifidobacteria, fermentation, storage, quality

Introduction

Fermented milk products have been consumed in Europe for about 4000 years (*1*). Recently, in most European, Asia-Pacific, and American countries the consumption of probiotic products has increased dramatically, and more than 90 % of this products contain *Lactobacillus acidophilus* and/or *Bifidobacterium* spp. (*2*). Live cultures of these probiotic bacteria in the diet are claimed to provide several therapeutic benefits such as the maintenance of intestinal microflora balance, improvement in lactose utilisation in lactose malapsorbtion, reduction in the level of serum cholesterol, and prevention of cancer (*3–5*). According to Fukushima and co-workers (*6*) consummation of the products with live *Bifidobacterium bifidum* Bb-12 notably increases the number of this bacterium in faeces. Bifodobacteria and lactobacilli are the most common anaerobes in microflora of human colon. This has a beneficial influence on the host, especially at soothing and preventing digestion disorders (*7*). Furthermore, feeding of a formula containing *Bifidobacterium bifidum* Bb-12 enhances the IgA production in the intestine of healthy children, which has an important role to the immune system, especially in infants (*8*).

Depending on the milk type, different characteristics of fermented milk beverage products are obtained. It is well-known that goat's milk have better digestibility in comparison with cow's milk because of smaller size of the fat globules (*9*), more easily hydrolysed triacylglycerols containing short-chain fatty acids and more essential amino-acids (*10,11*), higher proportion of

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soluble minerals (*12*) and smaller size of casein micelles (*13–15*). Although goat's milk is used for therapeutic purposes, especially because of its antiallergenic effect (*16*), it was still not thoroughly investigated.

The presence of different supplements in milk used for fermentation can also improve sensory and nutritive characteristics of the products. Besides the milk powder, which is commonly used as a supplement in the production of fermented milk beverages, the whey protein concentrates, either in liquid or powder (*17–19*) form, can be used as well. Whey proteins have supreme biological value due to favourable composition of the essential amino acids (*20*). Apart from increased nutritive value of fermented milk beverages, the whey proteins stimulate the growth of several strains of lactic acid bacteria, as well as bacterial fermentation products, including primary metabolites such as lactic acid, acetic acid, carbon dioxide, diacetil and acetaldehyde. This is extremely important for intestinal lactic acid bacteria, which grow slowly in milk and are also poor producents of mentioned metabolites (*5,21*). The *Bifidobacterium* ssp. strains, that grow slowly in milk and produce insufficient taste and aroma in products, are often combined with other lactic acid bacteria to facilitate acidification (*2,22*), although the milk beverages fermented with monoculture of bifidobacteria can be found on the market as well.

In this paper the influence of whey protein concentrate and skim milk powder addition on cow's and goat's milk fermentation with probiotic bacterium *Bifidobacterium bifidum* Bb-12 was investigated. Also, microbiological and sensory quality of produced beverages were determined during nine days of storage at refrigerator temperature.

Materials and Methods

Materials

The commercial goat's and cow's long-life milk typified on 3.2 % fat was used. Skim milk powder (mp) contained 0.05 % milk fat, 4.0 % moisture, and pH=6.7, and the whey proteins concentrate powder (wpc) contained 60.0–60.5 % proteins, 3.1 % moisture, 4.7 % ash, and pH=6.5, obtained by kidness of LURA d.d. Dairy Company, Zagreb. Direct Vat Set (DVS) culture *Bifidobacterium bifidum* Bb-12 (identified as *Bifidobacterium lactis* (*8*)) was obtained from Christian Hansen's A/S, Denmark.

Optimisation of production

In preliminary experiments the optimal quantity of skim milk powder (mp) and whey protein concentrate powder (wpc) for fermentation were determined. Supplements were used in quantities of 1, 2 and 3 % (w/v). The amount of supplements was determined on the basis of sensory evaluation of fermented milk samples conducted after 24 h of storage at 5 °C.

Fermented bifido milk production

Goat's and cow's milk were divided in three portions. In the first two portions of milk, milk powder or whey protein concentrate were added in predetermined quantity. The third portion served as a control sample. In order to obtain whey protein denaturation and incorporation in coagulum structure, samples were heated to 90 °C and then cooled to fermentation temperature of 37 °C. All milk samples were inoculated with 2 % inoculum of DVS culture and 1.5 dL aliquots were distributed in sterile buttercups. Inoculum of DVS culture was prepared according to the producer's recommendation (2 g/10 L). Fermentation was conducted until pH=4.6 was reached. Fermented milk was cooled with water and stored in the refrigerator (5 °C) for nine days. The acidity, microbiological and sensory analyses were conducted periodically (3rd, 6th and 9th day).

Each experiment was repeated five times, and the results are presented as averages.

Analysis

Viable count was determined by the standard microbiological methods on MRS agar (Biolife, Italy). Anaerobe incubation was conducted in anaerobic jars at 37 °C for 48 h. The pH values were determined using a pH-meter (»Knick«, type 647-1). Titritable acidity (°SH) was determinated by the Soxhlet-Henkel method, proteins by the Kjeldahl method, total solids (drying at 105 °C until constant mass) and ash content (at 550 °C) were analysed according to the National Standard (*23*). Lactose was analysed by the Luff-Schoorl method (*24*). The sensory properties of fermented beverages were evaluated by the panel group of 5 sensory analysts, using 20 points scoring system. The points were obtained by multiplication of the scores for each property (1–5) with weighted factor (Fw) (*25*). The analysis of variance was used to examine the statistical significance between samples and between sensory scores of the same samples during storage.

Results and Discussion

Chemical composition and acidity of commercially available goat's and cow's long-life milk used for production of fermented bifido milk are shown in Table 1. Goat's milk had lower total solids (10.90 %) than cow's milk (11.39 %). Although the ash quantity from goat's milk was higher for 6.6 %, the proteins quantity was very low (2.25 %) in comparison with the cow's milk (3.03 %). The pH of both milks was almost identical (ΔpH=0.01), while titritable acidity of cow's milk was slightly lower (Δ°SH=0.40).

Table 1. Chemical composition and acidity of commercially available long-life cow's and goat's milk used for production of fermented bifido milk (n=5)

Composition and acidity		Cow's milk	Goat's milk
Total solids (%)		11.39	10.90
Ash (%)		0.71	0.76
Fat (%)		3.20	3.20
Lactose (%)		4.88	4.16
Proteins (%)		3.03	2.25
Acidity	pH	6.64	6.65
	°SH	7.60	8.00

Table 2. Sensory scores of fermented cow's (C) and goat's (G) bifido milk, without and with the addition of 1, 2 and 3 % milk powder (mp) or whey protein concentrate powder (wpc) (1 day after manufacture)

Sensory characteristics	Fermented samples						
	C	Cmp 1 %	Cmp 2 %	Cmp 3 %	Cwpc 1 %	Cwpc 2 %	Cwpc 3 %
Appearance	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Colour	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Odour	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Consistency	2.4	3.0	3.6	3.6	3.6	4.0	4.0
Flavour	6.0	10.8	10.8	10.8	9.6	9.6	9.6
Σ	12.0	17.8	18.0	18.0	17.2	17.6	17.6
	G	Gmp 1 %	Gmp 2 %	Gmp 3 %	Gwpc 1 %	Gwpc 2 %	Gwpc 3 %
Appearance	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Colour	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Odour	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Consistency	1.2	3.6	4.0	4.0	3.6	4.0	4.0
Flavour	9.6	10.8	10.8	10.8	11.4	12.0	12.0
Σ	14.6	18.4	18.8	18.8	19.0	20.0	20.0

The preliminary experiment was conducted in order to establish an optimal concentration of milk powder or whey protein concentrate powder added to milk. The results in Table 2 show the improvement of sensory scores by increased concentration of milk powder and whey protein concentrate from 1 to 2 %, without further improvement at higher concentration.

Fermentations were conducted for 28 h with 2 % of inoculum at 37 °C. At these conditions all samples coagulated.

During fermentation, no expressive pH changes occurred between the samples (Fig. 1); the pH of goat's milk samples decreased faster and at the end of fermentation (the 28th hour) they were lower (pH=4.64 to 4.83) than in cow's milk samples (pH=4.96 to 5.24). It may be due to lower buffer capacity effect of goat's samples. At the end of fermentation, only the control goat's sample achieved the pH value of isoelectric point of casein (pH=4.64). Titritable acidity during fermentation (Fig. 1) was higher in supplemented samples, especially with whey protein concentrate addition, regardless of the type of milk. At the end of fermentation, titratable acidity was almost identical in all goat's samples (°SH=31.0–34.4) and higher than in cow's samples (°SH=24.8–30.2).

The viable count of bifidobacteria at the beginning of the fermentation was 3.3×10^6 CFU/mL. In both milk types the growth of bifidobacteria was better in supplemented samples (Fig. 1), while in goat's milk the whey protein concentrate had stronger influence on bifidobacteria growth. The growth of bifidobacteria was in all samples faster in the first seven hours of fermentation and the viable cell count increased at least for one logarithm in this period (in goat's milk sample supplemented with whey protein concentrate (Gwpc) even for 1.65 logarithm). At the end of fermentation the lowest viable cell count (log(CFU/mL)=8.05) was determined in control cow's sample (C), and the highest (log(CFU/mL) =8.56) in goat's sample supplemented with whey protein concentrate (Gwpc). In other samples the number of bifidobacteria was almost identical (log(CFU/mL) = 8.34–8.43).

Similarly, Rosenthal and Berstein (*26*) found log(CFU/mL)=7.63 of *Bifidobacterium bifidum* at pH=5.88 after 24 h of cow's milk fermentation at 37 °C.

Acidity was slightly enlarged during storage of fermented bifido samples (Fig. 1). In all goat's samples during nine days of storage the pH was lower than in cow's samples (ΔpH=about 0.5). In supplemented goat's samples the pH (Fig. 1) remained the same during storage. In control goat's sample (G) the pH slightly increased (ΔpH=0.23) during 6th day and remained unchanged until 9th day of storage (pH=4.72). In goat's samples no significant changes of titritable acidity were noticed after 3rd day of storage. In supplemented cow's samples (Cmp, Cwpc) the pH slightly increased during storage, while in the control sample (C) it did not change notably (Fig. 1).

The viable count during storage did not change considerably in any of the samples (Fig. 1). The most stable were both control samples, recpectively, while supplements had no great influence on bacteria survival. Generally, the viable count was higher in goat's samples having lower pH values. At ninth day of storage the average viable count in cow's samples was $N=1.1 \times 10^8$ CFU /mL, and in goat's samples $N=2.3 \times 10^8$ CFU /mL which classifies those products as probiotics ($N > 10^6$ CFU /mL) (*2*).

According to Klaver *et al*. (*27*) the bifidobacteria can hardly tolerate very acid media, but at pH=5.5 most bifido strains can survive well.

In this work the bifidobacteria survived well in most samples until the 9th day of storage and the pH slightly increased while titritable acidity decreased.

Sensory evaluation of produced fermented samples (Table 3) showed that the supplemented goat's bifido

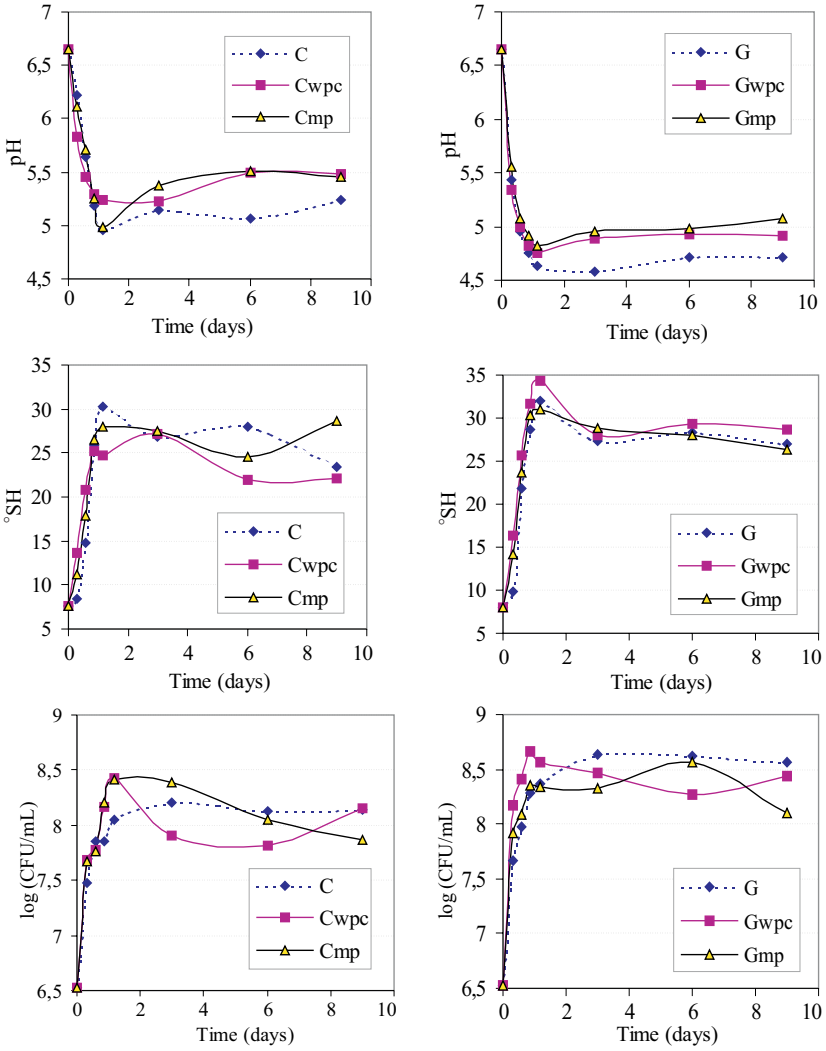

Fig. 1. Changes of pH values, titritable acidity (°SH) and viable cells of *Bifidobacterium bifidum* Bb-12 (log N/mL) during fermentation (28 hours) and storage (9 days) of fermented bifido milk from cow's (C) and goat's (G) milk with and without 2 % milk powder (mp) or 2 % whey protein concentrates (wpc) addition (n=5)

samples have much better characteristics than cow's samples in spite of notably lower proteins content (Table 1). The best scores were obtained for goat's samples supplemented with whey protein concentrate, and according to maximal scores (20.0) obtained during entire storage they were classified as extra class quality samples. The addition of whey protein concentrates improves consistency of fermented milk beverages without syneressis (whey banishment) (*18–20*).

The whey protein concentrate improved the consistency of fermented beverage from cow's milk (Cwpc) as well (Table 3). However, the flavour of these samples was slightly reminiscent of fresh cheese, and the products had less expressed flavour and acidity taste in comparison with other bifido milk samples. Control cow's sample (C) had poor taste and strange, unpleasant aroma. Cow's bifido sample supplemented with whey protein concentrate (Cwpc) had better consistency in comparison with the sample supplemented with milk powder (Cmp). In milk powder-supplemented cow's milk samples with unpleasant sweet taste dominated. During storage its sensory quality decreased constantly (Table 3). The scores dropped from 18.0 total points (the 3rd day of storage) to 16.4 total points (the 9th day of storage).

Control goat's sample (G) had a very poor, almost liquid consistency during entire storage, although the flavour of these samples, except at the beginning of storage (the 3rd day), got maximum scores (12.0). Bifido goat's samples supplemented with milk powder (Gmp) had a slightly sweet but pleasant taste and at the end of storage period this sample was also evaluated with maximum scores (Table 3).

Sensory evaluation of fermented bifido milk samples showed that differences between samples are statistically highly significant ($P<0.01$). Differences in sensory scores of the individual samples during the storage are not statistically significant ($P<0.01$) (Table 4).

Conclusion

Better sensory properties of bifido milk can be obtained by the addition of 2 % milk powder or whey pro-

Table 3. Sensory scores of fermented cow's (C) and goat's (G) bifido milk with and without addition of 2 % of milk powder (mp) or whey protein concentrate (wpc) during nine days of storage at 5 °C

Storage time /	Characteristics	C	Cmp	Cwpc	G	Gmp	Gwpc
3rd day	appearance	0.6	0.6	1.0	0.8	1.0	1.0
	colour	1.0	1.0	1.0	1.0	1.0	1.0
	odour	2.0	2.0	2.0	2.0	2.0	2.0
	consistency	2.4	3.6	4.0	1.2	4.0	4.0
	flavour	6.0	10.8	9.6	9.6	10.8	12.0
	Σ	12.0	18.0	17.6	14.6	18.8	20.0
6th day	appearance	0.4	0.9	1.0	0.6	0.9	1.0
	colour	1.0	1.0	1.0	1.0	1.0	1.0
	odour	2.0	2.0	2.0	2.0	2.0	2.0
	consistency	2.8	2.0	4.0	1.6	3.6	4.0
	flavour	4.8	12.0	9.6	12.0	12.0	12.0
	Σ	11.0	17.9	17.6	17.2	19.5	20.0
9th day	appearance	1.0	0.6	1.0	0.2	1.0	1.0
	colour	1.0	1.0	1.0	1.0	1.0	1.0
	odour	2.0	2.0	2.0	2.0	2.0	2.0
	consistency	3.2	3.2	4.0	1.6	4.0	4.0
	flavour	2.4	9.6	10.8	12.0	12.0	12.0
	Σ	9.6	16.4	18.8	16.8	20.0	20.0

Table 4. Analysis of variance using the results from Table 3

Source of variation	SS	df	MS	F	P-value	F crit
Rows	0.4311	2	0.2156	0.2225	0.8044	4.1028
Columns	163.0978	5	32.6196	33.6670	6.05×10^{-6}	3.3258
Error	9.6889	10	0.9689			
Total	173.2178	17				

tein concentrate in both goat's and cow's milk. During 28 hours of fermentation the pH in goat's milk samples decreased faster and at the end of fermentation these values were lower (from 4.64 to 4.83) than in cow's milk samples (from 4.96 to 5.24) of the same categories. In both milk types the growth of bifidobacteria was better in supplemented samples. At the end of fermentation the lowest number of viable cells was in control cow's sample (log(CFU/mL)=8.05), and the highest was in goat's sample supplemented with whey protein concentrate (log(CFU/mL)=8.56). In other samples the number of bifidobacteria was almost identical (log(CFU/mL) =8.34–8.43). Goat's samples showed lower but more stable pH values than cow's samples (ΔpH about 0.5) during nine days of storage. The presence of supplements had no notable influence on bifidobacteria survival. On the ninth day of storage an average viable count in cow's ($N=1.1 \times 10^8$ CFU /mL) and in goat's samples ($N=2.3 \times 10^8$ CFU /mL) was higher than 10^6 CFU /mL which classifies these products as probiotics. Sensory evaluations showed that goat's bifido samples have much better characteristics than cow's samples. Control goat's sample had a very poor, almost liquid consistency during the entire period of storage, but a much better flavour than control cow's sample. Sensory properties of both types of bifido milk were notably improved by supplementations. The best scores were obtained for goat's samples supplemented with whey protein concentrate.

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Kakvoća kravljeg i kozjeg fermentiranog bifido-mlijeka tijekom čuvanja

Sažetak

Od komercijalnog kozjeg i kravljeg trajnog mlijeka proizvedeno je fermentirano bifido--mlijeko, s dodatkom obranog mlijeka u prahu ili koncentrata proteina sirutke i bez njega. U uzorcima od kozjeg mlijeka pH-vrijednosti su opadale brže i na kraju fermentacije bile niže (od 4,64 do 4,83) nego u uzorcima od kravljeg mlijeka (od 4,96 do 5,24). U oba tipa mlijeka rast *Bifidobacterium bifidum* Bb-12 tijekom fermentacije bio je bolji u uzorcima s dodacima. Na kraju fermentacije najmanji je broj živih bakterija (log(CFU/mL)=8,05) bio u kontrolnom uzorku kravljeg mlijeka, a najveći (log(CFU/mL)=8,56) u uzorku kozjeg mlijeka s dodatkom koncentrata proteina sirutke. Devetog dana čuvanja broj živih bakterija u fermentiranim uzorcima od kravljeg mlijeka bio je prosječno $N=1,1 \times 10^8$ CFU /mL, a u fermentiranim uzorcima od kozjeg mlijeka $N=2,3 \times 10^8$ CFU /mL. Dodaci nisu utjecali na preživljavanje bifidobakterija. Senzorska svojstva uzoraka kozjeg mlijeka s dodacima bila su primjetno bolja od senzorskih svojstava istih uzoraka od kravljeg mlijeka, a osobito s koncentratom proteina sirutke.