

Research Article

The Effect of Bovine Colostrum on the Lactic Flora of Yogurt and Kefir

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Abstract

The objective of this study was to determine the effect of colostrum on microbial populations of yogurt and kefir. For this purpose, raw bovine colostrum is freeze-dried and added to yogurt and kefir on 8% and 16% (w/w; colostrum/product) dilutions. The results showed that, effect of colostrum on total mesophilic aerobic bacteria counts of yogurt and kefir are negligible. *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* counts were 0.26-0.29 log CFU/g and 0.38-0.67 log CFU/g higher in colostrum added yogurt samples, respectively. In kefir, lactic streptococci and lactobacilli counts were higher than that of yogurt. However, they weren't affected from colostrum addition, statistically. This study showed that, colostrum, which contains antimicrobial substances (immunoglobulins, lactoferrin, lactoperoxidase, lysozyme and cytokines), don't have an adverse effect on specific microbial floras of fermented dairy products such as yogurt and kefir. As a result, colostrum can be added to yogurt and kefir to increase their functional properties.

Keywords

- Colostrum
- Yogurt
- Kefir
- Lactic acid bacteria

ABBREVIATIONS

LAB: Lactic Acid Bacteria; CFU: Colony-Forming Unit

INTRODUCTION

Colostrum is the first natural food for the newborn calf. It is secreted by the mammary gland in the initial 24 to 96 h of the postpartum period. It is a rich source of basic nutrients; protein, fat, lactose, vitamins and minerals, and in addition, it plays a fundamental protection role with its antimicrobial substance content which contains immunoglobulins, lactoferrin, lactoperoxidase, lysozyme and cytokines. In addition, raw colostrum contains valuable microflora, including *Lactobacillus* and *Bifidobacterium*, which are known as probiotics [1]. The comparison of general composition of colostrum and whole milk is shown in Table (1).

The most important bioactive components in colostrum include growth factors and antimicrobial factors. Growth factors promote the growth and development of the newborn calf while antimicrobial factors provide passive immunity and protect against infections during the first weeks of life. The antimicrobial activity of colostrum is due mostly to immunoglobulins, although colostrum also contains other antimicrobial factors; lactoferrin, lysozyme and lactoperoxidase [2]. In bovine milk and colostrum, lactoferrin and lactoperoxidase are the most dominant and best studied non-specific antimicrobial components and many *in vitro* experiments have proven their activity against all kinds of

microorganisms. Lysozyme is a potent antimicrobial enzyme but, in contrast to human milk, the concentration in bovine milk and colostrum is probably too low to significantly contribute to the overall bacteriostatic and bactericidal activity [3].

The direct bacteriostatic effect of lactoferrin depends on underlying iron existence. In addition to this bacteriostatic effect, lactoferrin also exhibits an iron-independent bactericidal activity

Table 1: The composition of milk and colostrum serum.

Composition	Milk serum	Colostrum Serum
Dry matter, g / kg	122 g	153-245 g
Crude protein	34 g	41-140 g
Lactose	46 g	27-46 g
Fat	37 g	39-44 g
Ash	7 g	5-20 g
Lysozyme	0.07-0.6 mg/l	0.14-0.7 mg/l
Lactoperoxidase	20mg/l	30 mg/l
Lysine	4.9	43.4 mg/ml
IgG	2	496 mg/ml
Lactoferrin	<0.1	10.6 mg/ml
IGF-I	33 ng/g	2500 ng/g
IGF-II	12 ng/g	25 ng/g
Insulin	<1 ng/g	<1 ng/g

[4]. The bactericidal activity is related to the direct binding of lactoferrin to the microbial membrane, which alters the membrane permeability through dispersion of lipopolysaccharides and leads to death of the organism [5]. *In vitro* studies have shown that lactoferrin inhibits the growth of *E.coli* and *Staphylococcus* strains [6-8].

Interest in understanding the composition of colostrum is initiated by the desire of infant formula manufacturers to adjust cow's milk-based infant formulas so as to mimic as closely as possible, human breast milk. In human milk glycoproteins, glycolipids and lactose-derived oligosaccharides are now considered to be soluble receptors for pathogenic microorganisms, viruses or endotoxins, and hence may exert anti-infective properties. Some of these components were proved to be present in cow's milk and colostrum [9]. Shahani et al.[10], found that, yogurt cultured colostrum had significantly inhibited tumor cell counts of male Swiss mice, while non-cultured fresh colostrum had any effects on these tumor cells.

In spite of its numerous immunological benefits, colostrum is still an under-utilized food in the dairy industry. The use of colostrum or any of its ingredients in functional dairy foods is still very limited owing to consumer resistance and its high perishability. Relevant efforts have been developed for the processing of colostrum in order to obtain a stable and readily available product. Scammell [11] has used centrifugation and thermal treatments to diminish the microbial load in colostrum, thus providing a product which may be added to a variety of dairy food products or beverages to improve their functionality and potential to stimulate the immune system. Elfstrand et al.[12], investigated the reduction effects of freeze-drying and pasteurization in immunoglobulins, growth factors and growth hormones of bovine colostrum.

Probiotics are live microbial food supplements that beneficially affect by improving the microbial balance and they are used in fermented dairy products [13]. Kefir and yogurt are natural probiotics. Kefir grains contain a complex mixture of both bacteria (including various species of lactobacilli, lactococci, leuconostocs and acetobacteria) and yeasts (both lactose-fermenting and non-lactose-fermenting). Kefir has frequently been claimed to be effective against a variety of complaints and diseases [14-17]. Several studies have investigated the antitumor activity of kefir and of kefir grains and antimicrobial activity *in vitro* against a wide variety of gram-positive and gram-negative bacteria and against some fungi [18,19]. Yogurt is a coagulated milk product that results from the fermentation of lactic acid in milk, by lactic acid bacteria (LAB) *L. bulgaricus* and *Str. thermophilus* [20]. Many researches were focused on the role of probiotic characteristics of LAB in yogurt and effects of yogurt in lactose-intolerance people's gastrointestinal system [21]. LAB bacteria enables the inhibition of the pathogen infection through production of acetic acid, lactic acid and bacteriocins as well as stabilizing the intestinal associated to the intestinal tract microflora after long term antibiotics uses [22,23]. Fiorda et al.[24], examined for concentrations of some aromatic compounds and some other metabolites like glucose, lactose, lactic acid and ethanol in colostrum-based kefir. They found that, colostrum-based kefir beverage was very similar to

traditional milk kefir, with high lactose and lactic acid content and low ethanol concentration. Antioxidant capacities and exopolysaccharide contents of these two products were also similar according to that study.

The growth of undesirable pathogens and saprophyte microorganisms can be prevented by the dominance of starter cultures in yogurt and kefir. For this purpose, there are studies to improve the activity and the viability of starter cultures in fermented dairy products. In some studies, colostrum was inoculated with starter cultures, but this fermented colostrum was used only in calves nourishment. There was no observed effect of colostrum on the starter cultures viability. The main objective of this study is to investigate the effects of colostrum on the cultures of fermented products such as yogurt and kefir.

MATERIALS AND METHODS

Materials

Colostrum was milked from the first day milk of new offspring Simmental cow in a family farm, Sakarya. Thermophilic yogurt culture from "YO-MIX™ 499 LYO" (CHR-Hansen, Denmark) was used in yogurt production, which is a characteristic mixture of *Str. thermophilus* and *L. bulgaricus* in freeze-dried form. Cultures used in kefir production were from Yayla Maya (Maysa Gıda, İstanbul) that includes specific kefir bacteria and yeasts. UHT milk used in production of yogurt and kefir contains 11.5 % total solids, 3.0 % fat, 3.4% protein and obtained from BIM Ltd, Turkey.

Methods

Yogurt and Kefir Productions: Colostrum from the first day of lactation was immediately frozen after milking and then, freeze-dried in laboratory with Labconco, Freezone-6 (Kansas City). Before production, powdered colostrum was dissolved in the UHT milk with a blender (Braun, 400 Watt). Formulations of the products are given in Table (2). Yogurt culture was inoculated in 1/10.000 (g culture/g milk) dilutions and was in 1 / 3.000 (g culture / g milk) dilutions for kefir culture. Incubation conditions for yogurt and kefir were 43°C and 22°C, respectively. Incubation times were determined by pH measurements. Yogurts were incubated for 31/2 h, kefir were incubated for 23 h. After incubation, all samples were stored at 4°C until the analyses.

Analytical tests: All samples were analyzed in 3 days of storage. Dry matters of the samples were determined according to AOAC method [25]. The pH was measured at 4°C using pH/Ion meter (Mettler Toledo, Seven Compact S220). Total nitrogen was determined by Kjeldahl method [26], crude protein was calculated as 'total nitrogen x 6.38. Fat analyses were performed by Gerber method [27].

Viscosity and color analyses were performed at 4°C. Viscosities of yogurt and kefir samples were measured using a rotational viscometer (Fungilab, ALPHA H, and Spain) at the speed of 60 rpm with spindle R3 as Poise (P).

Colors of yogurt and kefir samples was analyzed by a tintometer (Lovibond RT3, England), in terms of L* (Brightness: 100: white, 0: black), a* (+: red, -: green) and b* (+: yellow, -: blue) parameters.

Table 2: Concentrations of colostrum and culture used in yogurt and kefir samples.

Group name	Group information	Colostrum content in powdered form	Culture content
YC	Control yogurt		1/10.000
Y1	8 % Colostrum milk containing group	1 % (w/w milk)	
Y2	16 % Colostrum milk containing group	2 % (w/w milk)	
KC	Control kefir		1/3.000
K1	8 % Colostrum milk containing group	1 % (w/w milk)	
K2	16 % Colostrum milk containing group	2 % (w/w milk)	

In the process of microbiologic analyses of yogurt and kefir samples, 10 g sample taken under aseptic conditions was homogenized using 90 ml of 0.1% peptone water. Preparing decimal dilutions, the appropriate medium was inoculated by standard analysis methods. For the count of total mesophilic aerobic bacteria in yogurt and kefir samples, Plate Count Agar was inoculated using pour plate method and colonies were counted after 48 h incubation at 37°C (PCA, Merck, Germany). The counts of *Str. thermophilus* in yogurt samples were enumerated on ST agar by incubating the plates aerobically at 37°C for 24+3 h. MRS agar (Oxoid) adjusted to pH 5.2 and anaerobic incubation at 43°C for 72 h were used for the enumeration of *L. bulgaricus* in yogurt samples. Man-Rogosa Sharpe Agar (Oxoid CM361) was used in the counting process of *lactobacilli* in kefir and after 48 hours incubation at 30°C, gray white colonies of 0.5-2.5 mm diameter were evaluated. M17 Agar (Oxoid CM785) was used in the counting process of *lactic streptococci* and they were evaluated after 48 h incubation at 37°C [28].

Statistical analysis: A factorial arrangement was set up to study the influence of colostrum treatments (3) and using 2 replicates for yogurt and kefir separately. A total of 12 samples were investigated for microbiological and physical and chemical properties. All analyses were conducted twice. Data obtained from analysis of the samples were evaluated by variance analysis, and the differences among means were detected by Duncan's multiple range tests [29].

RESULTS AND DISCUSSION

Physical and chemical properties of yogurt and kefir samples were given in Table (3). According to the results, pH was not affected from colostrum addition in both yogurt and kefir samples. Protein and fat contents in samples increased slightly with increasing amount of colostrum. In fact, fat content of

colostrum is less than mature milk [30]. The increase of fat in colostrum added samples was thought to be due to addition as dry powder of colostrum. However, pH decrease was not affected adversely from the dry matter.

Fat content of kefir in the study of Satir, Guzel-Seydim [31] was determined higher than this study, with the value of 3.37 %. In another study, 3.08 % and 3.27 % fat contents were determined for bovine milk based kefir. Crude proteins were determined in the same study as 3.17 % and 3.39 % nearer to this study [32]. The viscosity of yogurt and kefir was increased appreciably by increasing colostrum concentration ($p < 0.05$). High protein and fat content in colostrum powder can be the reason of viscosity changes. In general, viscosity increases with increasing solids in liquid food (Table 3).

During incubation, LAB converts lactose of the milk to obtain energy for their maintenance and growth, and released metabolites, primarily lactic acid, result in a decrease in the pH of yogurt and kefir. Longer incubation time means more time available for the LAB to metabolize the lactose of the milk, and so that more lactic acid produced, which contributed to much lower pH surrounding the LAB. However, drop in pH below the optimum level, affect the intracellular pH of the LAB, which inhibit the enzyme activity, ion transport and nutrient uptake, and so that retard the growth and then the counts of the LAB.

Some microbial counts of colostrum added yogurt and kefir samples were given in Table (4). According to the results, in both yogurt and kefir samples, microbial counts showed no significant change with the colostrum addition ($p > 0.05$). The total mesophilic aerobic bacteria counts in kefir were determined higher than yogurt.

According to the studies on yogurt, the proportion of *Str. thermophilus* in the total acidifying microflora is higher than

Table 3: Physical and chemical properties of colostrum added yogurt and kefir samples.

Group name	pH	Non-fat dry matter (%)	Protein (%)	Fat (%)	Viscosity (P)
YC	4.67 a	8.27±0.02 a	3.21±0.01 a	2.80±0.0 c	13.2±0.4 c
Y1	4.68 a	8.36±0.03 a	3.25±0.02 a	3.00±0.0 b	18.5±0.4 b
Y2	4.69 a	8.59±0.16 a	3.35±0.06 a	3.10±0.0 a	25.5±0.1 a
KC	4.73 a	8.43±0.16 b	3.41±0.07 a	2.70±0.0 c	10.2±0.2 c
K1	4.72 a	8.58±0.15 a	3.35±0.03 a	3.00±0.0 b	15.6±0.1 b
K2	4.71 a	8.60±0.11 a	3.47±0.04 a	3.20±0.0 a	18.2±0.2 a

YC: Control yogurt, Y1: 8 % Colostrum added yogurt, Y2: 16 % Colostrum added yogurt, KC: Control kefir, K1: 8 % Colostrum added kefir, K2: 16 % Colostrum added kefir. "a, b, c" means separately within yogurt (YC, Y1, Y2) and kefir (KC, K1, K2) columns, data are significantly different ($p < 0.05$) according to the Duncan's Multiple Range Test.

Table 4: Some microbial counts of colostrum added yogurt and kefir samples in the 3rd day of storage.

Group name	Total mesophilic aerobic bacteria (log CFU/g)	<i>Str. Thermophilus</i> (log CFU/g)	<i>L. bulgaricus</i> (log CFU/g)
YC	6.13±0.12	7.28±0.03	6.77±0.49
Y1	6.24±0.37	7.57±0.03	7.15±0.16
Y2	6.06±0.08	7.54±0.18	7.44±0.39
		<i>Lactic streptococci</i> (log CFU/g)	<i>Lactobacilli</i> (log CFU/g)
KC	9.25±0.12	8.61±0.07	8.37±0.04
K1	9.27±0.13	8.53±0.31	8.50±0.15
K2	9.24±0.06	8.61±0.00	8.35±0.15

YC: Control yogurt, Y1: 8 % Colostrum added yogurt, Y2: 16 % Colostrum added yogurt, KC: Control kefir, K1: 8 % Colostrum added kefir, K2: 16 % Colostrum added kefir.

Table 5: Color properties of colostrum added yogurt and kefir samples

Group name	<i>L*</i>	<i>a*</i>	<i>b*</i>
YC	76.45±0.00 a	-0.54±0.00 c	3.58±0.01 c
Y1	75.96±0.01 b	-0.40±0.01 b	4.23±0.01 b
Y2	76.19±0.00 b	-0.10±0.01 a	5.40±0.01 a
KC	76.17±0.10 b	-0.89±0.03 c	2.43±0.08 c
K1	76.53±0.11 a	-0.64±0.02 b	3.44±0.06 b
K2	76.70±0.01 a	-0.51±0.01 a	4.01±0.01 a

YC: Control yogurt, Y1: 8 % Colostrum added yogurt, Y2: 16 % Colostrum added yogurt, KC: Control kefir, K1: 8 % Colostrum added kefir, K2: 16 % Colostrum added kefir. *L** (brightness: 100 – white, 0 – black), *a** (+ red; – green) and *b** (+ yellow; – blue). “a,b,c” means within columns are significantly different ($p < 0.05$) according to the Duncan’s Multiple Range Test.

that of *L. bulgaricus*, even if they were inoculated in identical proportions [33]. We have obtained results consistent with this requirement. In a study on yogurt, the number of the microflora characteristic for yogurt after 21 d of storage in relation to the degree of condensation of processed milk was counted as lactobacillus 5.50, streptococcus 5.15 log CFU/g [34]. Bielecka, Majkowska [33], determined in a natural yogurt 9.15 log CFU/g of *Str. thermophilus* bacteria and 8.99 log CFU/g of *L. bulgaricus* bacteria, despite the fact that the milk was inoculated with a culture of identical proportion of these bacteria. Similar results were reported by Broussalian, Westhoff [35] investigating yogurts obtained from processed milk which was inoculated with a traditional culture consisting of *Str. thermophilus* and *L. bulgaricus* at 1:1 ratio, after 5 h of incubation at 42°C. In a study investigating the microbial properties of yogurt available for consumption in Turkey, the lowest and highest number of streptococci and lactobacilli were counted that, 6.00 log CFU/g to 8.00 log CFU/g for streptococci and 5.00 to 8.00 log CFU/g for lactobacilli [36]. *Str. thermophilus* and *L. bulgaricus* were researched to be present in commercially available yogurts in the Philippines. The highest *Str. thermophilus* count was obtained with 10.20 log CFU/ml and lowest with 8.18 log CFU/ml. The highest *L. bulgaricus* count was also found with 9.96 log CFU/ml while also had the lowest count of 8.15 log CFU/ml [37]. All the results obtained in different studies, on the activity of kefir and yogurt lactic cultures have been shown to be effective such factors as the using cultures, the raw milk, production technologies, additives, packaging materials and storage conditions.

The microbial population found in kefir grains has been

cited as an example of a symbiotic community; this symbiotic nature has made problematic the identification and study of the constituent microorganisms within kefir grains [38]. The microbial population in kefir grain was found to consist primarily of lactobacilli (65–80%) [39], with lactic streptococci and yeasts comprising the remainder. In this study, *Str. thermophilus* and *L. bulgaricus* counts in colostrum added yogurt samples increased more than lactic streptococci and lactobacilli in kefir samples (Table 4). In a study, kefir was prepared using Bali cattle milk; samples were analyzed after 24, 48 and 72 h incubation periods. MRS and M17 LAB counts changed from 9.51 to 8.99 and from 9.49 to 9.28 log CFU/ml during 24 h to 72 h incubation, respectively [40]. These results are near to the counts determined in this study. In another study, after 30 h of incubation, the number of lactobacilli in goat milk kefir was counted as 7.00 log CFU/g [41].

According to the results, *L** value showed a significant reduction with colostrum addition in yogurt, while showed a significant increase in kefir. The most significant increase occurred in both *a** and *b** values of colostrum added samples ($p < 0.05$). Colostrum is yellowish color which has increased the yellow color in both the samples of yogurt and kefir (Table 5).

CONCLUSION

Colostrum is the lacteal secretion produced after parturition and plays an important role in post-natal health as an immune booster. In addition to nutrients such as proteins, carbohydrates, fats, vitamins and minerals, bovine colostrum contains several

biologically active molecules that are essential for specific functions. Bioactive components like growth factors promote the growth and development of the newborn while antimicrobial factors provide passive immunity and protection against infections during the first weeks of life. Recently, the colostrum is getting increasing attention due to its special nutritional values. Therefore, there are many studies on functional properties of colostrum and fermented colostrum. However effects of colostrum on food products are not examined in detail.

The obtained results in this study showed that, colostrum have not a negative effect on the development of LAB in yogurt and kefir. The effect of colostrum on total mesophilic aerobic bacteria counts of yogurt and kefir were negligible. *Str. thermophilus* and *L. bulgaricus* counts were higher in colostrum added yogurt samples. *Lactic streptococci* and *lactobacilli* counts in kefir were higher than *Str. thermophilus* and *L. bulgaricus* counts in yogurt; however, they weren't affected significantly from colostrum addition. As a result, colostrum can be added to yogurt and kefir to increase their nutritional and protective properties. It is necessary to supply more hygienic production conditions and to improve the preservation parameters of colostrum. For future studies, it will be useful to extract bioactive components of colostrum and to investigate their addition in food supplements.

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