

## Full Length Research Paper

# Microbiological properties of Turkish Beyaz cheese samples produced with different probiotic cultures

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In this study, Turkish Beyaz cheese samples were produced by adding commercial lactic culture [*Lactococcus lactis* and *Lactococcus cremoris* (A)] and different probiotics [*Bifidobacterium bifidum* BB-12 (B), *Bifidobacterium bifidum* BB-12+*Lactobacillus acidophilus* LA-5 (C), *Bifidobacterium bifidum* (D) and *Bifidobacterium longum* (E)]. Probiotic cultures were added to cheese milk at least  $10^7$  cfu/ml levels. Some microbiological properties of this cheese samples were investigated during ripening periods of 2, 15, 30 and 60 days. As a result, at beginning of the ripening period, the yeast-mould and coliform bacteria counts of all Beyaz cheese samples were higher than the standard values but the counts decreased during ripening period and reached standard values. It was determined that lactic acid bacteria counts (MRS agar) of probiotic cheese samples were lower than the control cheese. While the lactic acid bacteria counts growth on M17 agar of the cheese samples produced with C culture were the highest, the lactic acid bacteria counts of cheese produced with E culture were determined as the lowest. In addition, the probiotic bacteria counts of experimental cheeses decreased during the ripening period. The number of *Lactobacillus acidophilus* LA-5 in cheese C showed a decrease of 3 logarithmic units at the end of the ripening period.

**Key words:** White cheese, probiotic culture, survival, *Bifidobacterium bifidum*, *Bifidobacterium longum*.

## INTRODUCTION

Probiotics are live microbial food supplements, which are beneficial for the health of consumers by maintaining, or improving their intestinal microbial balance (Fuller, 1989; Mattila-Sandholm et al., 2002). There are different studies on human beings that provide evidence about health effects of probiotics. Foods containing probiotic bacteria belong to the category of functional foods, which are claimed to have several health specific benefits (Gupta and Abu-Ghannam, 2012; Pitino et al., 2012). Various parameters must be considered when adding probiotic bacteria. Probiotics can be described as dietetic and therapeutic products obtained by fermentation. A number of therapeutic benefits have been attributed

including control of diarrhea, improvement in lactose utilization in lactose malabsorbers, and improvement in host immune responses (Tharmaraj and Shah, 2004). products obtained by fermentation. A number of therapeutic benefits have been attributed including control of diarrhea, improvement in lactose utilization in lactose malabsorbers, and improvement in host immune responses (Tharmaraj and Shah, 2004). Probiotic organisms to be beneficial in food systems, cultures should maintain viability in the food until the time of consumption and be present in significant numbers, at levels of  $10^7$  viable cells per g<sup>-1</sup> or mL<sup>-1</sup> of a product (Ishibashi and Shimamura, 1993). Milk, yogurt, fermented

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milk, desserts, fruit juice and various kinds of cheese are among the major recent probiotic products (Souza and Saad, 2009). A variety of probiotic dairy products, including cheeses with particular functional properties, is available in market worldwide (Gomes et al., 1995; Gardiner et al., 1998; Songisepp et al., 2004; Ross et al., 2005; Ibrahim et al., 2010; Songisepp et al., 2012). Especially, among different food products able to deliver probiotics, cheese has been claimed as a good carrier of probiotic bacteria because it enables their passage as viable cells throughout the gastrointestinal tract (Kılıç et al., 2009; Madureira et al., 2008; Rodrigues et al., 2012). As pointed out by Cruz et al. (2009), cheese is one of the most versatile food products, offering opportunities for many marketing strategies, new technology developments, and for adding health benefits, including the possibility of transforming it into a probiotic food carrier (dos Santos et al., 2012). Cheese contains a complex combination of microorganisms that changes with time; initially containing large numbers of starter lactic acid bacteria (SLAB) and then with maturation, an increasing number of nonstarter lactic acid bacteria (NSLAB) (Darukaradhy et al., 2006). To enumerate probiotic bacteria in such a mixed population, selective media should be employed that would allow the growth of the organisms of interest and inhibit other microorganisms encountered in a particular food product. Alternatively, differential media that will allow easy identification of probiotic colonies in the presence of other colonies can be used, provided the probiotic bacteria are in sufficient numbers. In the absence of standard selective or differential media, reliable enumeration of probiotic bacteria in the less complex microbial populations found in yoghurt has been reported to be difficult (Talwalkar and Kailasapathy, 2004). Turkish Beyaz cheese is most widely consumed in Turkey. Turkish Beyaz cheese is a soft or semi-hard type cheese which is produced from sheep's or cow's milk or their mixture (Kılıç et al., 2009). Previous studies have shown that there are significant strain differences in the viability of probiotic bacteria during storage of cultured dairy products (Ross et al., 2002). An adequate strain selection must be carried out to produce probiotic dairy products (Vinderola and Reinheimer, 2003). In addition to good viability in the intestine, technological properties are a prerequisite for potential use of the strains as probiotic culture in cheese (Kask et al., 2003). Most studies on probiotic cheeses have focused on maintaining a high probiotic population during the shelf life of the food. Results have shown that several cheese varieties, example, Cheddar, Gouda, Canestrato Pugliese, Fresco, etc., are able to carry high numbers of different strains of probiotic bacteria for variable periods (Phillips et al., 2006; Roy et al., 1998; Vinderola et al., 2000).

This study was to evaluate success in maintaining the viability of different probiotic strains (*Bifidobacterium bifidum* BB - 12, *Bifidobacterium bifidum* BB - 12+ *Lactobacillus*

*acidophilus* LA-5, *Bifidobacterium bifidum* and *Bifidobacterium longum*) in Turkish Beyaz cheese samples.

## MATERIALS AND METHODS

### Cultures

Freeze-dried cultures of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* obtained from DSM Food Specialties Pty. Ltd. (Moorebank, NSW, Australia) were used for cheese starter culture preparation. Besides, the different strains of probiotic bacteria were used as adjunct cultures: this cultures, *B. bifidum* BB-12 and *B. bifidum* BB-12+*L. acidophilus* LA-5 were obtained from Peyma Hansen (Gayrettepe, Istanbul, Turkey) while *B. bifidum* and *B. longum* were obtained from Christian Hansen (Christian Hansen, Valinnhos, Brazil). The organism were activated with the methods in the study of Martensson et al. (2002).

### Cheese Producing

In this study, five groups of Turkish Beyaz cheeses were produced. A control batch was produced using 1 mL/100mL commercial culture mix consisting of *Lactococcus lactis* and *Lactococcus cremoris* and four batch [B (*Bifidobacterium bifidum* BB-12), C (*Bifidobacterium bifidum* BB-12+*Lactobacillus acidophilus* LA-5), D (*Bifidobacterium bifidum*) and E (*Bifidobacterium longum*)] was produced using equal concentrations of the probiotic and commercial mixes. Cheese producing trials were performed initially with 100 L of raw milk. The milk was pasteurized at 65°C for 30 min and cooled to 35°C, and CaCl<sub>2</sub> (20g/100 L) was added into the milk. A commercial culture mix inoculum (1mL/100mL) of the cheese starters (*Lactococcus lactis* and *Lactococcus cremoris*) was added to each of the vats. Probiotic strains were inoculated into samples (B, C, D and E) at levels high enough to attain 10<sup>7</sup> cfu/mL in cheese milk. A 12 ml of chymosin (Peyma Hansen, Turkey) dispersed in 100 mL water was added to each cheese vat at a level sufficient to coagulate the milk in 90 min. After curdling, the curds were cut into small cubes, approximately 1 cm<sup>3</sup> and the curds were allowed to rest. After pressed, the cheeses were cut into cubes about 8x8 cm and the cubes salted with pasteurized brine (12% w/v, NaCl) for 6 h. Then brine-salting, cheese was held at room temperature for 12 h and cheese blocks were then packed in bags containing brine and all cheese was ripened at 4±1°C for 60 days. The cheese production was performed in triplicate (Demirci and Şimşek, 1997). Cheese samples were analysed in 2, 15, 30 and 60 days of storage.

### Microbiological analysis methods

A pour plate method was employed for the determination of microbial groups. Eleven gram of samples cheese were homogenized in 99 ml of a sterile solution (0.85% NaCl) using a Stomacher (Lab. Stomacher Blander 400 BA 7021, Swardmedical). Further decimal dilutions were prepared with the same diluent (Diliello, 1982). Analyses were carried out using the following procedures:

1. Total aerobic mesophilic bacteria were enumerated on plate count agar (Oxoid) and with aerobic incubation at 30 ± 1°C for 48 h (Diliello, 1982).
2. Coliform counts were determined by the Violet Red Bile Agar (Oxoid) with plate incubation at 35 ± 2°C for 48 h (Diliello, 1982).
3. LAB counts were determined by MRS-agar (Oxoid) and incubated anaerobically at 30° C for 48 h (Diliello, 1982).

**Table 1.** Counts of the microbial groups found in probiotic Beyaz cheese samples (log cfu/g).

Beyaz Cheese Samples	Ripening Period (day)	TAMB	LAB grown on MRS Agar	LAB grown on M17 Agar	Coliform Group Bacteria	Yeast and Mould
A (Control)	2	7.63±0.56	7.15±0.59	6.82±0.32	3.80±0.31	2.01±0.43
	15	7.07±0.63	7.39±0.70	7.10±0.68	2.63±0.29	1.34±0.12
	30	6.72±0.26	6.90±0.41	6.55±0.71	1.05±0.12	<1
	60	7.13±0.37	7.73±0.52	6.23±0.50	<1	<1
B	2	8.36±0.21	7.84±0.46	6.26±0.46	2.56±0.56	2.70±0.09
	15	7.17±0.32	7.27±0.34	7.34±0.23	3.83±0.41	2.07±0.22
	30	7.74±0.74	6.79±0.26	6.04±0.18	2.35±0.20	<1
	60	6.82±0.61	5.71±0.35	5.51±0.39	<1	<1
C	2	8.27±0.35	8.07±0.43	7.34±0.60	1.44±0.16	2.72±0.32
	15	8.18±0.29	7.20±0.64	8.43±0.77	2.56±0.21	1.17±0.15
	30	7.63±0.32	7.57±0.56	7.20±0.35	2.25±0.10	2.30±0.10
	60	6.75±0.47	6.18±0.34	5.34±0.41	1.03±0.16	2.02±0.24
D	2	7.61±0.24	6.15±0.17	6.83±0.38	3.87±0.39	2.03±0.12
	15	7.87±0.50	7.17±0.43	7.63±0.20	1.65±0.21	2.37±0.30
	30	6.72±0.38	6.73±0.25	6.03±0.49	1.37±0.13	<1
	60	7.17±0.42	7.66±0.39	7.23±0.41	<1	<1
E	2	7.40±0.63	7.19±0.31	6.90±0.38	2.85±0.34	2.01±0.32
	15	6.88±0.28	6.14±0.47	6.24±0.67	1.73±0.18	2.31±0.24
	30	7.14±0.37	7.02±0.40	5.60±0.52	1.26±0.10	<1
	60	6.70±0.45	6.85±0.53	6.01±0.70	<1	<1

\*\* The values presented are the average of three recurrences

A(Control): Only lactic culture; B: *B. bifidum* BB-12+lactic culture; C: *B. bifidum* BB-12+*L. acidophilus* LA-5+lactic culture

D: *B. bifidum*+lactic culture; E: *B. longum*+lactic culture; TAMB: Total aerobic mesophilic bacteria; LAB: Lactic acid bacteria

4. LAB counts were determined by M17-agar (Oxoid) and incubated aerobically at 30±1°C for 48 h (Diliello, 1982).

5. Yeasts and moulds were enumerated on Potato Dextrose Agar (PDA) (Oxoid) and incubated at 25°C for 5 - 7 days (Koburger and Marth, 1984).

6. Probiotics strains, the count of *L. acidophilus* LA-5, was enumerated on MRS<sub>D</sub>-Sorbitol agar and for the count of *Bifidobacterium* strains were enumerated on MRS-NNLP agars and incubated aerobically at 37°C for 72 h (Dave and Shah, 1997).

### Statistical analysis

All statistical analysis was performed on a computer running SAS for windows. Analysis of variance was performed using the routine Proc ANOVA. Significant treatment was separated using Duncan's multiple range test (Yildiz and Bircan, 1994).

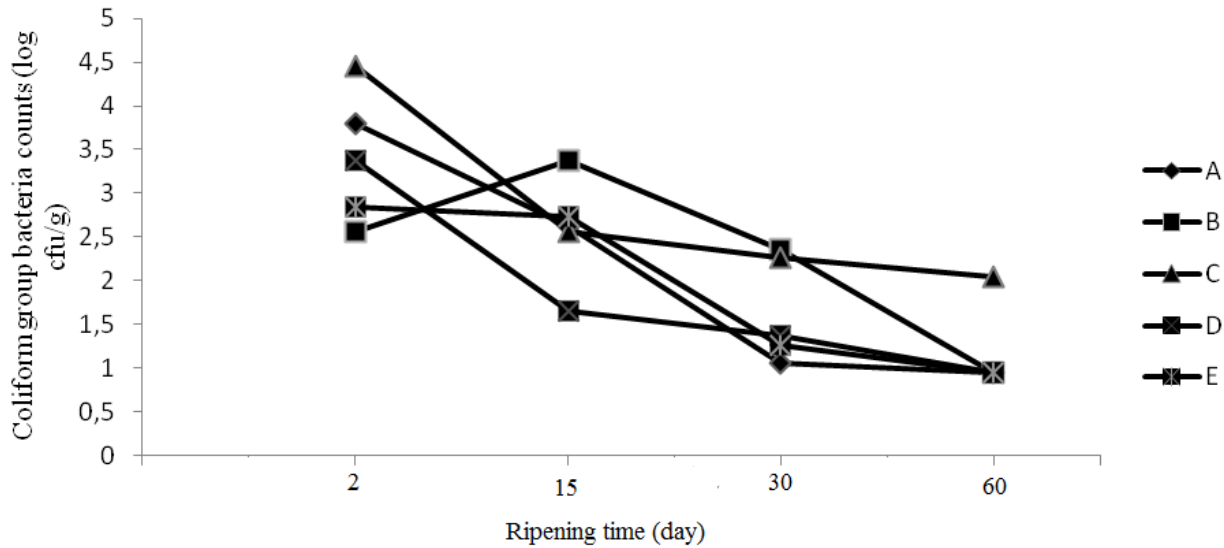
## RESULTS AND DISCUSSION

Results of some microbiological analysis of Beyaz cheese samples during ripening are given in Table 1.

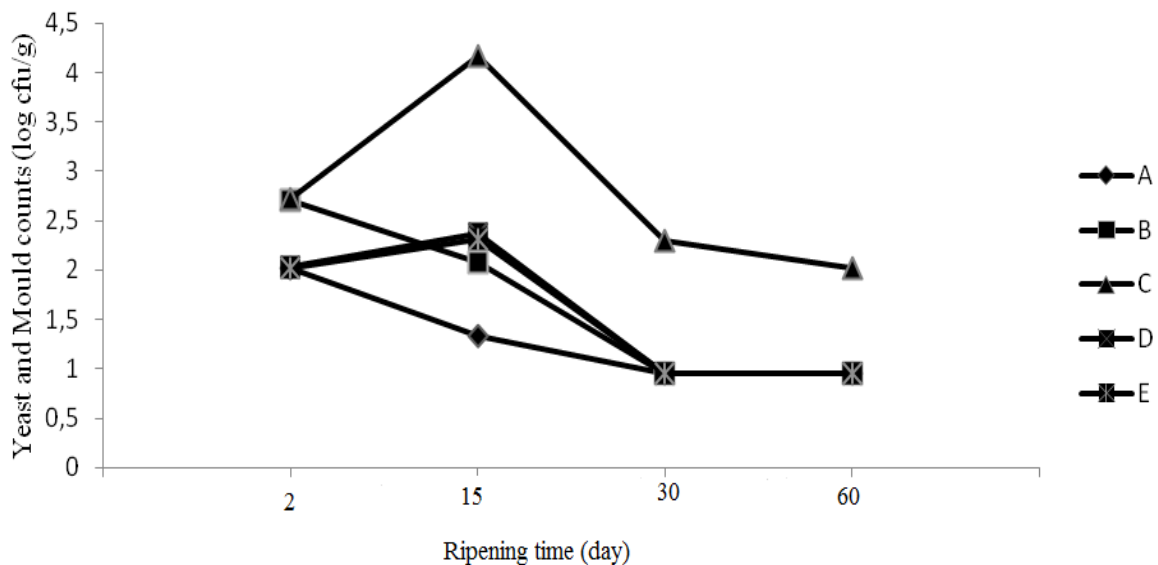
The TAMB counts of samples were around 6.70 cfu/g and 8.36 cfu/g. Generally, as the ripening periods increased, the acidity had an inhibiting effect on the total number of microorganisms and therefore, the TAMB counts of samples decreased. In addition, the studies

presented that the quality of the raw material, the conditions under which cheese is produced and ripened, the thermal operations applied and the cultures added had an effect on the TAMB numbers of sample white cheese (Fontecha et al., 1990).

Similarly Nikolaou et al. (2002) found that the TAMB counts of low-fat Batzos cheese decreased at the end of the ripening period. The penetration of salt into cheese generally decrease the bacteria counts. The lactic acid bacteria counts on MRS agar in Beyaz cheese samples added probiotic bacteria were lower than that of unadded. As ripening periods increased, LAB count of samples decreased. This can stem from increasing of salt ratio during ripening. Laleye et al. (1987) reported that the increasing of salt ratio in cheese decreased the lactic acid bacteria numbers. Same result was found by Sousa and Macata (1996). The lactic acid bacteria counts on M17 agar of cheese samples increased at 15 days of ripening, but counts decreased at 30 and 60 days of ripening. Thus, throughout ripening, *B. lactis* Bb-12 was present in high numbers and the most predominant flora in Beyaz cheese (Brearty et al., 2001). Kılıç et al. (2009) reported that lactic acid bacteria counts on M17 agar of the Beyaz cheese samples were 8 logcfu/g at the



**Figure 1.** The changes of coliform bacteria counts of probiotic Beyaz cheese samples during ripening



**Figure 2.** The changes of of yeast and mould counts of probiotic Beyaz cheese samples during ripening.

beginning of ripening period and at ripening period for 120 days cheese samples had 5 logcfu/g lactic acid bacteria counts. Breatry et al. (2001) found similar results in Cheddar cheese samples. These findings were parallel to our findings.

The coliform group bacteria counts of samples were found around <1 and 3.87 logcfu/g. At Turkish Beyaz Cheese standard, the coliform counts were limited to maximum 2 logcfu/g (TS591). During ripening of Beyaz cheese samples, coliform counts decreased (Figure 1). The coliform counts of all samples ripened for 60 days were under standard value (TS591). Kılıç et al. (2009) reported the coliform counts of probiotic Beyaz cheese

samples were lower than standard value (<2log cfu/g).

The lowest yeast and mould count was found in samples added only lactic starters. At 15 days of ripening period, yeast and mould counts increased (Figure 2). Then, the counts decreased at 30 and 60 days of ripening. Guizani et al. (2006) reported that yeast and mould can be found in cheese at all ripening periods. This state can result from the fact that yeast and moulds can grow at different water activity ( $a_w$ :0.65-0.90), low pH (min.3) and different temperature degrees (Montville and Matthews, 2005).

The probiotic bacteria counts used in the production of Beyaz cheese samples during ripening are given in Table 2.

As seen from Table 2, all probiotic bacteria counts

**Table 2.** The probiotic bacteria counts of Beyaz cheese samples (logcfu/g).

Beyaz cheese sample	Ripening period (day)	Probiotic bacteria counts (log cfu/g)	
B ( <i>B. bifidum</i> BB12)	2	7.61±0.20	
	15	7.34±0.35	
	30	6.87±0.12	
	60	6.50±0.18	
C ( <i>B. bifidum</i> BB12 * + <i>L. acidophilus</i> LA-5** )	2	7.80±0.42 (*)	8.61± 0.21(**)
	15	6.74±0.16	7.60±0.32
	30	6.78±0.12	6.41±0.14
	60	6.33±0.33	5.13±0.12
D ( <i>B. bifidum</i> )	2	7.90±0.41	
	15	7.80±0.26	
	30	6.79±0.34	
	60	6.67±0.37	
E ( <i>B. longum</i> )	2	6.72±0.50	
	15	5.64±0.31	
	30	6.52±0.10	
	60	5.20±0.27	

\*\* The values presented are the average of three recurrences

\* *B. bifidum* BB12; \*\* *L. acidophilus* LA-5

decreased during ripening periods. But, ripening temperature also had an affect on the counts of probiotic in all cheeses. Although *B. bifidum* BB12 decreased at 1 log level, *L. acidophilus* LA-5 decreased at 3 log levels. Different probiotic bacteria can be tolerated at different level of acidity and salt. Ozer et al. (2009) made probiotic Beyaz cheese with *B. bifidum* BB12 and *L. acidophilus* and found that the probiotic bacteria decreased during ripening periods for 90 days at significant level. The result was parallel with our research results. But, Fritzen-Freire (2010) reported that counts of *B. Bifidum* BB12 in cheese did not decrease during ripening periods for 28 days. The limiting factor on the growth of *B. Bifidum* is soluble oxygen and salt. Gomes et al. (1995) found that there was an initial increase in *L. acidophilus* during production but then there was a 2 log decrease in 9 weeks. Bergamini et al. (2006) found that *L. acidophilus* counts in cheese samples increased during ripening periods for 60 days. But, Philips et al. (2006) found that *L. acidophilus* in Cheddar cheese decreased to 3 log cfu/g at during 7 months of ripening.

## Conclusions

The aim of this study was to investigate microbiological properties of Turkish Beyaz cheese which was probiotic cultures (*Bifidobacterium bifidum* BB-12, *Bifidobacterium bifidum* BB-12+ *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* and *Bifidobacterium longum*) and determine the viability of the probiotic strains. At the beginning of the ripening period, the yeast-mould and coliform bacteria counts of all Beyaz cheese samples

were higher than the standard values but the counts decreased during ripening period and were within the admissible range according to Turkish Standards for cheese (TS 591, 1995). All probiotic strains survived the cheese making process at a high level without any alteration of cheese production process. At the end of 2 months ripening at 4°C, these cultures maintained the levels of >6.0 log cfu g<sup>-1</sup>. In addition, the probiotic bacteria counts of experimental cheeses decreased during the ripening periods. While the population of *Bifidobacterium* species was reduced by 1 log, the number of *Lactobacillus acidophilus* LA-5 reduced by 3 log at the end of the ripening period, respectively. The results show that the probiotic culture strains can be used in Turkish Beyaz cheese production alone or in combination with commercial starter cultures successfully.

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