American Journal of Animal and Veterinary Sciences 5 (4): 249-257, 2010
ISSN 1557-4555

Monitoring the Chemical and Microbiological Changes During Ripening of Iranian Probiotic Low-Fat White Cheese

N. Sabbagh, H. R. Gheisari and M. Aminlari
Department of Food Hygiene, School of Veterinary Medicine, Shiraz University,
P.O. Box 71345-1731, Shiraz, Iran

Abstract: Problem statement: The objective of this experiment was to manufacture an Iranianlow fat probiotic cheese. **Approach:** Iranian white brine cheeses (4 trials) were made by varyingprocesses, i.e., lowering the fat content and use of probiotic adjunct culture on separate days. All types of cheeses were ripened at 13°C for 2 weeks and at 6°C to the end of ripering period. Cheeses were analyzed for the compositional, microbiological, color and sensory characteristics and also lipolysis and organic acid profile. The Cheese of each trial was sampled at 1, 15, 30, 45 and 60 days during ripening. **Results:** Decreasing the fat level resulted in significant increases ($p<0.05$) in the level of moisture, protein and pH of whey. The results show that probiotic cheeses had higher moisture and pH than cheeses with bacteria ($p<0.05$). There were no significant differences ($p>0.05$) between the concentration of *L. acidophilus* of cheese groups when the fat content of samples was reduced. The rate and extent of lipolysis in the full-fat cheese was higher than in the low-fat control cheese ($p<0.05$). Results also showed decreasing fat content and addition of adjunct culture to the cheese treatments decreased the acetic and lactic acid contents ($p<0.05$). Decreasing the fat content of cheese samples and use of both factor in the treatments increased the a* value in the samples. Low fat cheeses received higher flavor and odor scores than full fat cheeses. Also addition of adjunct culture significantly ($p<0.05$) decrease the texture score of manufactured cheeses. **Conclusion:** Therefore the results of this study showed that the Iranian probotic low fat cheese is a functional food. It has better flavor and odor than normal cheese and can be used in many cases like as heart disease and obesity.

Key words: Iranian white cheese, probiotic low-fat, *Lactobacillus acidophilus*, ripening period, microbiological changes, Acid Degree Value (ADV), Total Free Fatty Acid (TFFA), Analysis Of Variance (ANOVA), adjunct culture, cheese samples, lactic acid contents, probiotic bacteria

INTRODUCTION

Probiotics are living micro-organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition (Guarner and Schaafsma, 1998). Foods containing such bacteria fall within the "functional foods" category and these are described as foods claimed to have a positive effect on health (Anuradha and Rajeshwari, 2005). Functional foods should contain at least 10^7 cfu g^{-1} probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to have positive effects on health (Picard *et al.*, 2005).

Many different strains and species of lactobacilli and bifidobacteria have been used commercially as probiotics. It is well known that *lactobacillus acidophilus* has health promoting effects and antagonistic activity against food-borne disease agents (Gilliand and Speck, 1977). *Lactobacillus acidophilus* is a probiotic microorganism available in conventional foods and dietary supplements. On the other hand, addition of adjunct cultures has shown great promise in manufacture of foods and improve their sensory quality (Mohebbi and Ghoddusi, 2008). Fermented dairy products are the best choice for probiotic microorganisms. Cheese may offer certain advantages over other products in terms of delivery of viable probiotics, such as the higher pH of the cheese, the higher fat content and more solid consistency of cheese which may offer protection to the probiotic in the gastrointestinal tract (Ong *et al.*, 2006; Pouch Downes, and Ito, 2001; Mazahreh *et al*., 2009). Dairy products especially cheese have high fat content, so many people

Corresponding Author: H.R. Gheisari, Department of Food Hygiene, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, P.O. Box: 71345-1731

on fat-restricted or health- oriented diets limit or avoid consumption of ripened cheese. Nowadays, fat reduction in the diet is important based on the scientific evidence linking diets that are high in fat to obesity, arteriosclerosis, coronary heart disease, elevated blood pressure, tissue injury and certain types of cancer (Guinee and Law, 2002).

In Iran, white brined cheese is a major item in the diet and Iranian white cheese is a close-textured brined cheese, resembling Beyaz Peynir (Turkish white cheese) and feta but differs from feta in the way it is made. No research has been conducted on the manufacture of an Iranian dietary cheese that has functional and probiotic effects. Thus, the objectives of the present study was to manufacture a low fat and probiotic cheese.

MATERIALS AND METHODS

Treatments, cultures and rennet: Four types of cheeses were produced. Control full fat cheese made with regular starter cultures (A). Full fat cheese made with regular starter cultures and probiotic adjunct culture (B). Two remained cheese treatments were like the ones that has described but were low fat, (C, D). Cheese batches manufactured using 10 kg of standardized milk for each treatment cheeses were manufactured in duplicate; each replicate was produced in one day. Two freeze- dried direct-to-vat cultures (YC-280 and La-5; Chr. Hansens Dairy cultures, Denmark) were used as starter. Culture YC- 280 contained *Streptococcus thermophilus* and *lactobacillus delbrueckii subsp. bulgaricus.* Culture La-5 contained *L. acidophilus*. As a coagulant, chymosin, derived by fermentation [Standard rennet, Chy-Max; Chr. Hansen Inc., Denmark; 2235 international milk clotting units (IMCU) ml^{-1} or 1-3gr/100 l milk] was used.

Cheese-making procedure: Iranian white brine cheeses were made on separate days at school of veterinary medicine of shiraz university. Pasteurized (72°C×15 sec) cow milk that was used for production of cheeses were obtained from choopan dairy company. For each trial, 10kg milk was used. Milk with 3% fat was used for full fat cheeses and with 1.5% fat was used for low fat cheese trials. The milk compositional characteristics are shown in Table 1. All types of cheese were made according to Iranian standard for white brinecheese. For cheese making, the milk was 35°C and pH was adjusted to 7.0. $CaCl_2$ was added at a level of 20 g 100 kg^{-1} of milk, followed by the addition of starter cultures 45 min before renneting. For conventional cheese, starter cultures (*S. thermophilus* and *L. delbrueckii subsp. bulgaricus*) were used at a level of 0.01% (w/v) and for probiotic ones milk was inoculated with starter cultures that has described and *L. acidophilus* at a level of 0.02% (w/v). Chymosin was added to each cheese vat at a level sufficient to coagulate the milk in 60 min (1-3 g 100 kg^{-1}). The curd was cut crossways into cubes of 3 cm and left for 10 min, followed by stirring and whey drainage. The curd was pressed in the vat using weights (10 kg) for 1 h and then cut to suitable size and packed in plastic containers. According to the methods that has described for each cheese trial, the cheese were soaked in 20% (w/v) sterile brine (the pH was adjusted to 7.0±0.2) for 16 h and then replaced with 11% brine for 2 months.

All types of cheese were ripened at 13°C for 2 weeks and at 6°C to the end of ripering period. Cheese of each trial were sampled at 1, 15, 30, 45 and 60 days during ripening.

Chemical analysis: Titrable acidity of milk was determined by the Dornic method. Its fat, protein, solid non fat, lactose and freezing point were determined by a digital milk scan (Lactostar, FUNKE GERBER, 230V). The pH of milk and cheese samples was measured using a digital pH-meter (CG 824, Germany). Cheese was analyzed for moisture and dry matter content by vacuum-oven (AOAC, 2010). Salt content by Volhard and fat content by the Gerber method. The ash content of cheese samples was determined by dry ash method and their total protein contents were determined by measuring total nitrogen using the kjeldahl method and converting it to protein content by multiplying by 6.38. All chemical measurements were done in quadruplicate (AOAC, 2010).

Microbiological analysis of milk: Milk samples were analyzed for aerobic mesophilic bacteria and coliform bacteria using standard methods (Vanderzant and Splittoesser, 1992).

Table 1: Chemical and bacteriological analysis of milks[a]

	Composition								
Cheese code	Fat (%)	Protein (%)	Lactose (%)	SNF (%)	FPP (%)	pH (%)	Acidity (%)	Coliform (log cfu ml^{-1})	Total count (log cfu ml^{-1})
A[b]	3.03±0.12	3.51±0.05	5.21±0.03	9.68±0.04	0.54±0.00 -	6.81±0.04	16.54±0.74	0.19±0.27	3.48±0.29
B	1.55±0.03	3.58±0.06	5.32 ± 0.08	9.69±0.15	0.54±0.02 -	6.81±0.02	16.71±0.71	0.27±0.31	3.32±0.17

Plate count agar (Merck, Germany) was used for enumeration of aerobic mesophilic bacteria. Plates were incubated aerobically at 30°C for 72 h. For the count of coliform bacteria, violet red bile agar (LAB M, Lancashire, BL96AU, UK) was used and incubated aerobically at 37°C for 48 h.

Microbiological analysis of cheese: From each cheese type, l0 g cheese was transferred in to a sterile bag under aseptic conditions and homogenized in 90 mL sterile diluent contained (0.1% peptone and 0.9% NaCl in distilled water, adjusting the pH to 7.0±0.2) for 3 min using a lab blender 400 stomacher (Model No, BA6021, 230-250 volts, 50 Hz). Serial dilutions were prepared by adding 1-9 mL sterile peptone and NaCl (0.1% and 0/9%). Samples were tested for counts of starter cultures (*S. thermophilus* and *L. delbrueckii sub*sp. *bulgaricus*) and adjunct culture (*L. acidophilus*) using standard methods. *S. thermophilus* was enumerated on Streptococcus thermophilus agar (Merck, Darmstadt, Germany) and incubated at 37°C under aerobic condition for 48 h and *L. delbrueckii sub*sp. *bulgaricus* was enumerated on pH-modified MRS agar [(pH 5.2) Merck, Germany] and incubated at 45°C in an anaerobic jar with a Gas Generating Kit (Merck) for 72 h. *L. acidophilus* was enumerated on MRS-sorbitol (MRS-S) agar and incubated anaerobically at 37°C for 72 h. MRS-sorbitol agar used for the selective enumeration of *L. acidophilus* was prepared by adding 10 ml of membrane filtered sterile 10% solutions (w/v) of sorbitol (Merck) to 90 ml of molten MRS agar just before pouring.

Lipolysis: The level of lipolysis was assessed in samples of 1, 15, 30, 45 and 60 day-old cheese by measuring the Acid Degree Value (ADV) and determination of total free fatty acids. The ADV was determined with a modified procedure developed by Park and Lee (2006). Approximately 20 g of sample was grated, homogenized and mixed with 6 g Na_2 SO_4 and adequate amount of chloroform and filtered through whatman study No.1. The ADV was determined on 25 mL of this extract that was titrated against the standard alcoholic 0.1N NaOH solution.

Production of organic acids: Production of lactic acid and acetic acid was determined using High Performance Liquid Chromatography (RP-HPLC) with a modified procedure developed by Akalin *et al*. (2002). Grated cheese samples (7.0 g) were added to 50 mL of buffer-acetonitrile mobile phase, homogenized, extracted for 1 h and centrifuged at 7000 g at 4°C for 5 min. The supernatant was filtered twice through a 0.2 µm membrane filter (Organe, Gryodisc CA-PC 1520012) and approximately 1 mL aliquot from each sample was stored in HPLC vials at-20°C until analyzed.

The HPLC system consisted of a liquid chromatography shimadzu LC-6A, equipped with a UV-Vis detector, shimadzu SPD-6AV, a column oven model CTO-6A, a system controller model SCL-6A and computerized analyzing chroinatopack model C-R6A and a shimadzu reverse phase C_{18} column model (CLC-ODS) shim-pack. The system were used at room temparature (25°C). UV-Vis detector was set at 214 nm. Organic acids were eluted from the column at flow rate of 1.2 mL min^{-1}. The chart speed was maintained at 1 cm min^{-1}. A mobile phase of HPLC grade $(NH4)_2HPO_4$, 0.5% w/v and acetonitrile, 0.4% v/v (at pH 2.24 with H_3PO_4) was run through the column. HPLC grade reagents were used as standards (Merck Chemical Co). Solvents were degassed under vaccum. Both solvents and standard solutions were filtered through 0.2 and 0.45 µm membrane filters (Orange, CA-PC).

Sensory evaluation: An acceptance sensory panel evaluated randomly coded cheese samples. The panel consisted of 40 members, with an age range from 20-50 years. Consumer panelists were the students of faculty of veterinary medicine and the employees of food hygiene and public health department of shiraz university. All types of cheeses were evaluated for texture, flavor, odor, color and appearance by the consumer panel on a 5-point hedonic scale (1 = least liked to 5 = most liked). Cheese blocks were cut into standard, bite- sized pieces; each piece measured 1.5×1×1 cm. Cheese pieces were placed into airtight plastic containers and conditioned at room temperature for 2 h before evaluation. Crackers and water were offered without limit to panelists during testing for cleaning the palates (Foegeding *et al*., 2003).

Color analysis: The color of cheese samples at 1, 15, 30, 45 and 60 days of ripening period was quantitatively determined using a Hunter lab colorimeter system (Hunter lab, DP-9000, Hunter Associates laboratory, Inc., Reston, VA), in which L, a and b values correspond to whiteness, redness and yellowness, respectively. Color measurements were performed in triplicate for each treatment at different sites.

Statistical analysis: Data were analyzed using Analysis Of Variance (ANOVA) of the General Linear Models procedure of the Statistical Analysis System software (SAS., 2005). Duncan's multiple range test was used to determine if significant differences existed among treatments. $p<0.05$ was considered as a level of significance.

RESULTS

Compositional and physico-chemical properties: The mean values for the compositional and physicochemical

Table 2: Compositions of Iranian white brine cheeses [a]

	Composition (%w/w)						
Cheese code	Fat	Protein	Ash	Moisture	Salt	pH CH [d]	pH WH [d]
A [c]	16.45±2.62[b]	13.61±4.15 [a]	3.70±0.63[c]	52.62±3.64[c]	3.21 0.63	5.80±0.17[d]	5.96±0.29[a]
B	19.05±3.33[c]	16.90±2.64[bcd]	3.22±0.51[ab]	52.10±1.72[c]	2.93±0.52	5.98±0.27[d]	6.26±0.2[bc]
C	10.10±2.48[a]	18.98±2.24[d]	3.09±0.60[a]	52.67±2.29[c]	2.80±0.71	5.69±0.16[bc]	6.14±0.16[b]
D	11.10±3.09[a]	18.47±2.44[cd]	3.23±0.50[ab]	49.47±1.75[b]	2.79±0.62	5.35±0.50[a]	5.97±0.22[a]

[a]: Each value in the table is the mean ± SD of four replications; [a-d]: Means within the same column with different superscripts differ significantly ($p<0.05$); [c]: A = Full fat cheese without probiotic; B = Full fat cheese with probiotic culture; C = Low fat cheese without probiotic; D = Low fat cheese with probiotic culture; [d]: pHCH = pH of cheese, pHWH = pH of whey

Table 3: Effect of ripening time on composition of Iranian white brine cheeses [a]

	Ripening time				
Composition (%w/w)	0	15	30	45	60
Fat	18.50±4.25[d]	15.56±3.60[c]	13.87±3.85[bc]	12.34±3.72[b]	10.22±3.51[a]
Protein	21.61±2.59[c]	16.95±3.32[b]	15.55±3.38[b]	13.92±2.40[a]	13.50±2.65[a]
Ash	2.62±0.54[a]	3.11±0.38[b]	3.49±0.43[c]	3.83±0.34[d]	3.89±0.48[d]
Moisture	48.29±2.45[a]	50.20±0.56[b]	51.11±2.68[bc]	52.06±2.66[c]	53.56±2.74[d]
Salt	2.04±0.42[a]	2.65±0.58[d]	3.19±0.32[c]	3.37±0.36[c]	3.60±0.26[d]
pH cH	5.49±0.25[a]	5.52±0.31[a]	5.71±0.38[b]	5.76±0.38[b]	5.98±0.25[c]
pH wH	6.17±0.48[b]	5.97±0.35[a]	6.17±0.23[b]	6.20±0.25[b]	6.34±0.41[b]

[a]: Each value in the table is the mean ± SD of four replications. (n = 32); [a-d]: Means within the same row with different superscripts differ significantly ($p<0.05$); [d]: pHcH = pH of cheese; pHwH = pH of whey

Table 4: Concentration (Log_{10} $cfug^{-1}$) of Iranian white brine cheese starter culture and probiotic adjunct culture [a]

	Bacteria		
Cheese code	ST [d]	LB	LA (La5)
A [c]	8.71±0.76[c]	5.58±1.23[a]	
B	8.59±1.18[c]	5.74±1.81[a]	8.21±1.08[b]
C	7.54±0.56[ab]	5.78±0.51[a]	
D	7.89±0.59[b]	6.87±1.48[b]	7.10±1.13[a]

[a]: Each value in the table is the mean ± SD of four replications; [a-c]: Means within the same column with different superscripts differ significantly ($p<0.05$); [c]: A = Full fat cheese without probiotic; B = Full fat cheese with probiotic culture; C = Low fat cheese without probiotic; D = Low fat cheese with probiotic culture; [d]: ST = *Streptococcus thermophilus*; LB = *Lactobacillus delbrueckii* subsp. bulgaricus; LA = *Lactobacillus acidophilus*

properties of various Iranian white brine cheeses are given in Table 2. The fat, protein and ash contents of manufactured cheeses were in the range of 10.10-19.05, 13.61-18.98 and 3.09-3.70, respectively. The moisture of all cheeses met the Iranian standard values of a maximum of 60% (ISIRI, 2002). There were no significant differences between cheeses for the levels of salt. As it is shown in Table 2, the pH contents of cheeses and their wheys were in the range of 5.35-5.98 and 5.97-6.26, respectively.

The fat and protein contents of manufactured cheeses decreased during ripening, while the ash, moisture, salt, pH of cheese and pH of whey increased. Of course, the pH of whey decreased during the first 15 days of ripening and then increased to the end of ripening period (Table 3).

Microbiological analysis: Concentration of Iranian white brine cheeses starter culture and probiotic adjunct culture (*L. acidophilus*) are shown in Table 4. *S.thermophilus*, *Lactobacillus delbrueckii* subsp *bulgaricus* and *L. acidophilus* concentrations were in the range of 7.54-8.71, 5.74-6.87 and 7.10-8.21 (log_{10} cfu g^{-1}), respectively.

The concentration (log_{10} cfu g^{-1}) of *S. thermophilus*, *Lactobacillus delbrueckii subsp. bulgaricus* and *L. acidophilus* (La-5) decreased during ripening (Table 5).

Production of organic acids: The metabolic activity of the microorganisms in cheese was monitored by estimating the metabolic products, lactic and acetic acids (Table 6). The lactic and acetic acid contents of manufactured cheeses were in the range of 3.11-7.62 and 2.93-12.31, respectively.

The lactic acid contents of cheeses decreased rapidly during the first 30 days of ripening and then increased. The acetic acid contents of manufactured cheeses decreased during 45 days of ripening and then increased to the end of ripening period (Table 7).

Lipolysis: The extent of lipolysis in the manufactured cheese, expressed Acid Degree Value (ADV), is shown in Table 6. The ADV of cheeses were in the range of 1.34-1.88. The Acid Degree Value (ADV) of cheeses increased during ripening period (Table 7).

Table 5: Effect of ripening time on concentration (log_{10} cfu g^{-1}) of Iranian white brine cheese starter culture and probiotic adjunct culture [a]

	Ripening time				
Bacteria	0	15	30	45	60
ST[c]	8.76±1.07[c]	8.01±0.57[b]	7.59±0.82[b]	7.66±1.00[b]	6.95±0.89[a]
LB	7.39±0.95[d]	6.64±1.30[c]	5.97±0.84[b]	5.78±1.04[b]	4.91±0.83[a]
LA (la5)	8.32±0.91[c]	7.94±1.02[bc]	7.29±0.99[ab]	7.29±1.08[ab]	6.63±0.45[a]

[a]: Each value in the table is the mean ± SD of four replications; [b]: a-d Means within the same row with different superscripts differ significantly ($p<0.05$); [c]: ST = *Streptococcus thermophilus*; LB = *Lactobacillus delbrueckii* subsp. *bulgaricus*; LA = *Lactobacillus acidophilus* (la-5).

Table 6: Effect of treatment on organic acid concentrations (mg g^{-1}) and acid degree value of Iranian white brine cheeses [a]

	Cheese code			
	A[c]	B	C	D
Lactic acid	3.11±1.78 [a]	7.62±3.44 [b]	5.23±3.36[a]	4.55±2.03 [a]
Acetic acid	5.32±0.99 [b]	12.31±2.92 [d]	2.93±1.9 [a]	5.19±2.39 [b]
Acid degree value	1.88±0.68[bc]	1.34±0.39 [a]	1.87±0.38[bc]	1.69±0.48 [b]

[a]: Each value in the table is the mean ± SD of four replications; [b]: a-d Means within the same row with different superscripts differ significantly ($p<0.05$); [c]: A = Full fat cheese without probiotic; B = Full fat cheese with probiotic culture; C = Low fat cheese without probiotic; D = Low fat cheese with probiotic culture.

Table 7: Effect of ripening time on organic acid concentrations (mg g^{-1}) and acid value of Iranian white brine cheeses[a]

	Ripening time				
	0	15	30	45	60
Lactic acid	10.43±4.38[c]	3.61±1.49[ab]	3.12±1.10[a]	3.75±2.05[ab]	4.66±2.27[b]
Acetic acid	8.51±2.48[b]	5.59±2.97[a]	6.16±5.31[a]	4.75±2.73[a]	6.61±3.62[ab]
Acid degree value	1.32±0.32[a]	1.74±0.42[b]	1.75±0.36[b]	2.04±0.38[c]	2.43±0.51[d]

[a]: Each value in the table is the mean ± SD of four replications; [b]: a-d Means within the same row with different superscripts differ significantly ($p<0.05$)

Table 8: L, a and b scales changes of Iranian white brine cheese treatments [a]

	Scales		
Cheese code	L	a	b
A [c]	77.68±4.20[b]	-1.26±1.32 [b]	8.84±5.96
B	78.05±3.73[b]	-1.20±1.19 [b]	10.60±6.84
C	77.44±4.34[b]	-2.33±0.90 [a]	7.44±5.18
D	73.55±4.58[b]	-1.86±0.98 [ab]	8.95±5.15

[a]: Each value in the table is the mean ± SD of four replications; [b]: a-c Means within the same column with different superscripts differ significantly ($p<0.05$); [c]: A = Full fat cheese without probiotic; B = Full fat cheese with probiotic culture; C = Low fat cheese without probiotic; D = Low fat cheese with probiotic culture.

Cheese opacity: Table 8 shows the results of color analysis of Iranian white brine cheese treatments. The L, a and b values of manufactured cheeses were in the range of73.55-78.05, -1.20-(-2.33) and 7.44-10.60, respectively. There were no significant differences in the l and b values of manufactured cheeses.

Ripening time affects L, a and b values of cheese treatments, in the manner that L value decreased during the ripening period, but a and b values increased (Table 9).

Sensory characteristics: The results of the sensory panels assessment of cheese groups are given in Table 10. No significant differences were seen between odor and color scores. The treatment that was full fat without probiotic (A) received the highest (4.40) and treatment that was full fat with NaCl and probiotic culture (B) received the lowest (3.57) scores for texture.

The highest score for flavor (3.5) belonged to the groups of A and D (full fat without probiotic and low fatprobiotic cheese) and the lowest one (3.17) belonged to group B (full fat probiotic cheese. The highest score for appearance (4.20) belonged to the group A and the lowest one (3.85) belonged to group B.

DISCUSSION

Compositional and physicochemical properties: Results of this study showed that reducing the fat level resulted in significant increases ($p<0.05$) in the level of moisture, protein of cheeses and pH of whey and decreases in pH of cheeses. In agreement with the literature (Guinee *et al*., 2000; Michaelidou *et al*., 2007), the moisture and protein contents increased significantly ($p<0.05$) when the fat content of samples was reduced. Water in cheese is found as either free or bound to the protein since fat, the other major component, is hydrophobic . Hence, the high protein contents of low fat cheeses probably caused retention of more water in the cheese samples.There were no

Table 9: Effect of ripening time on L, a and b scales changes of Iranian white brine cheeses [a]

	Ripening time				
Scales	0	15	30	45	60
L	82.06±2.59[d]	79.50±2.85[c]	76.67±2.84[b]	73.50±3.26[a]	74.04±3.65[a]
a	7.35±0.66[b]	-0.75±1.39[c]	-2.28±0.65[a]	-2.37±1.03[a]	-2.33±0.73[a]
b	0.87±0.76[a]	4.87±1.49[b]	11.92±2.99[c]	11.64±2.31[c]	14.42±2.42[d]

[a]: Each value in the table is the mean ± SD of four replications; [b]: a-d Means within the same row with different superscripts differ significantly ($p<0.05$)

Table 10: Sensory evaluation of different Iranian white cheese groups [a]

	Cheese code			
	A[c]	B	C	D
Texture$_{(5)}$	4.40±0.67[d]	3.57±0.87[a]	4.04±0.83[bcd]	3.95±0.71[bc]
Flavor$_{(5)}$	3.50±0.87[abc]	3.17±0.74[a]	3.29±1.11[bc]	3.50±0.96[abc]
Odor$_{(5)}$	3.50±0.87	3.50±0.71	3.70±0.90	3.92±0.76
Color$_{(5)}$	4.25±0.74	3.97±0.69	4.04±0.91	4.04±0.86
Appearance$_{(5)}$	4.20±0.72[ab]	3.85±0.80[a]	3.97±0.90[ab]	3.97±0.80[ab]

[a]: Each value in the table is the mean ± SD; [b]: a-d Means within the same row with different superscripts differ significantly ($p<0.05$); [c]: A = Full fat cheese without probiotic ; B= Full fat cheese with probiotic culture; C = Low fat cheese without probiotic; D = Low fat cheese with probiotic culture

significant differences between salt and ash contents of cheese groups when the fat content of samples was reduced. These results are consistent with other studies (Katsiari *et al*., 2002).

As may be seen from Table 2, no significant differences in the ash and salt contents were observed among the cheeses. No significant differences were observed among the cheeses with and without the addition of probiotic bacteria for the salt, fat, ash and protein content. The results show that cheeses without probiotic bacteria have higher moisture and pH than cheeses with bacteria. The lower content of pH in cheese manufactured with probiotic culture causes synersis of the curdand decreases the moisture content of cheese samples. The composition of the cheeses was within the suggested ranges of Iranian White Brine cheeses with good quality and texture parameters (ISIRI, 2002). The results thus show that addition of probiotic microorganisms into white brine cheese has no direct effect on cheese composition. The comparison of cheese manufactured with low fat milk and probioticadjunct culture with control one show that there were no significant differences among the cheeses for the protein, salt and pH content, but there were significant differences in fat, ash and moisture content. The results show that addition of adjunct culture resulted in significant decrease in the level of moisture. Michaelidou *et al.* (2003) have studied on proteolysis of feta cheese. Their results showed that Decreasing the concentration of milk fat resulted in significant ($p<0.05$) increases in the levels of cheese moisture and protein and a significant decrease ($p<0.05$) in the content of fat in dry matter.

The fat and protein contents of manufactured cheeses decreased during ripening, while the ash, moisture, salt, pH of cheese and pH of whey increased. Of course, the pH of whey decreased during the first 15 days of ripening and then increased to the end of ripening period. When cheese placed in brine, there is a net movement of NaCl molecules, as Na^+ and Cl^-, from the brine into the cheese because of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese containing dissolved materials such as acids and minerals diffuses out through the cheese matrix with a flux approximately twice that of NaCl so as to restore osmotic pressure equilibrium. The establishment of these dynamic phenomena increased the salt content of the treatments as they ripened. The ash content of cheese samples increased as the salt content increased respectively.

The fat content of cheese treatments decreased during ripening due to slightly increasing of moisture content and also lipolysis of fat. Hayaloglu *et al.* (2002) related the decrease in protein content of Turkish white cheese (Beyaz peynir) during ripening to diffusion of some proteolysis products from the curd into the brine. In agreement with the findings in this study, Ehsani *et al.* (1999) reported that the amount of total N and NPN in the brine was increased during ripening of Iranian white cheese.

According to the literature (Lucey *et al*., 2003), the slow solubilization of colloidal calcium phosphate during ripening causes a slow increase in pH. The pH of whey increase due to the liberation of free amino acids and short peptides from the cheese curd.

Microbiological analysis: As it is described, Functional foods should contain at least 10^7 cfu g^{-1}

probiotic bacteria and should be consumed at levels higher than 100 g day^{-1} to havepositive effects on health (Picard *et al.,* 2005). All of the probiotic cheese treatments have the desired concentration for the microorganisms.

There were no significant differences between the concentration of *L. acidophilus* of cheese groups when the fat content of samples was reduced. These results are consistent with other studies (Ryhanen *et al*., 2001). As the results show, decreasing the fat level resulted in significant decreases in the concentration of *S.thermophilus* and *L.bulgaricus*. In agreement with the other studies, the higher fat content may offer protection of microorganisms (Ong *et al.*, 2006).

As it is shown, *L. acidophilus* had a synergistic effect on the concentration of *L. bulgaricus* with a significant effect. This may be because of the pH of samples with probiotic adjunct culture that is lower than samples without this culture.

The concentration (log_{10} cfu g^{-1}) of *streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. Bulgaricus and *Lactobacillus acidophilus* (La-5) decreased during ripening. Although *L. acidophilus* decreased until the end of the ripening period, it did not decrease below 10^7 and 10^6 cfu g^{-1}. As indicated earlier, it is necessary to maintain the viability of *L. acidophilus* at $>10^7$ cfu g^{-1} of cheese, to call the cheese probiotic (Picard *et al.,* 2005). All probiotic-containing cheeses developed in this study thus satisfied the criteria for a probiotic food product. *L. acidophilus* counts in all cheeses however decreased by one to two log cycle probably due to unfavourable conditions in the cheese such as high salt in moisture, high pH (It is well known that lactobacilli grow best under acidic conditions, lack of fermentable carbohydrate and low ripening temperature (Ong *et al*., 2006; Kasımoglu *et al*., 2004). *L. bulgaricus* counts in all cheeses however decreased more (about one three log cycle) than *S. thermophilus* (about two log cycle) due to increasing the pH (Ong *et al*., 2006).

Production of organic acids: The main organic acids of all Feta and Fetalike-type cheeses throughout ripening were lactic and acetic acids. In this study, significant differences observed between cheese samples with probiotic adjunct culture and without probiotic adjunct culture in lactic acid content that it is higher in the samples with probiotic culture. These results are consistent with other studies. This maybe due to the presence of *L. acidophilus* that produce more lactic acid in the treatments. Low fat content of cheese samples cause higher content of lactic acid that it is due to the higher moisture content of these treatments (Katsiari *et al*., 2002).

Acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria. It contributes greatly to the final flavor of Feta cheese (Abd El-Salam and Alichanidis, 2004; Kandarakis *et al*., 2001; Kondyli *et al*., 2002).In this study, acetic acid decreased significantly when the fat content of samples was reduced. This is due to the moisture content of the samples (Katsiari *et al*., 2002). Acetic acid content of cheese samples with probiotic culture was significantly higher than the samples without culture. This is due to the higher amount of free amino acids in cheeses with adjunct culture (Michaelidou *et al*., 2003), which might have served as precursors for the formation of acetic acid.

The lactic acid contents of cheeses has a slight increase during the first day of ripening due to the use of residual lactose trapped in the curd and decreased rapidly during the first 30 days of ripening. These results are in agreement with other studies (Ong *et al*., 2006). The acetic acid contents of manufactured cheeses decreased during 45 days of ripening and then increased to the end of ripening period. Acetic acid is considered as a product of several biochemical pathways, such as fermentation of lactate and citrate or metabolism of amino acids by bacteria. It contributes greatly to the final flavor of Feta cheese (Abd El-Salam and Alichanidis, 2004; Kandarakis *et al*., 2001; Kondyli *et al*., 2002).The variation of acetic acid contents of cheese treatments could be due to theamount of free amino acids in cheeses which might have served as precursors for the formation of acetic acid.

Lipolysis: The rate and extent of lipolysis in the full-fat cheese was higher than the low-fat control cheese. This observation is in agreement with the results of Kondyli *et al.* (2002) for Feta cheese. Other workers noted a reduction in the Total Free Fatty Acid (TFFA) level in other cheese varieties as the fat content was reduced (Aly, 1994). The low fat cheeses supplemented with the adjunct culture showed slightly higher levels of ADV compared to the untreated low-fat cheese. This increase in the ADV could be due to the formation of simple nitrogen compounds, especially free amino acids, which might serve as precursors for the formation of volatile fatty acids (Aly, 1994).

The Acid Degree Value (ADV) of cheeses increased during ripening period. This is due to the lipolysis that occur to some extent. The ADV may be a good index of cheese ripening (Katsiari *et al*., 2001).

Cheese opacity: Decreasing the fat content of cheese samples increase the a* value and decreasethe L* value

in the samples. Ripening time affects L*, a* and b* values of cheese treatments, in the manner that L* value decreased significantly during the ripening period, but a* and b* values increased. In the study of Marchesini *et al.* (2009), Asiago cheese ripening significantly influenced colour, resulting in a linear decrease of L* through the time of aging. In the case of b* it was observed a major decrease during the first year and after 18 month. Redness (a*) resulted in a significant reduction but only in the first year. According to the literature (Dufosse *et al*., 2005; Pinho *et al*., 2005), cheese ripening led to a decrease of L*, but to an increase of b*; the difference in the results was probably due to the ripening time and to the degree of lipolysis that seemed much higher, like in other hard cheeses. L* showed a negative correlation with the protein percentage, meanwhile there wasn't a correlation with the fat content. But a* and b* were both negatively related to fat content.

Khosrowshahi *et al.* (2006) reported that the progressed ripening significantly ($p<0.05$) decreased the whiteness of Iranian white cheese.

Sensory characteristics: Decreasing the fat content has no significant effect on texture, color and appearance of samples but there were significant differences between groups for odor and flavor scores. Low fat cheeses received higher flavor and odor scores than full fat cheeses. Also addition of adjunct culture significantly decrease the texture score of manufactured cheeses and this culture had no significant effect on flavor, odor, color and appearance scores of cheeses. Katsiari *et al.* (2002) reported that in Kefalograviera-type cheese, the appearance of the experimental low-fat cheeses was considered good and did not significantly differ from that of the full-fat cheese. The body and texture scores for the control low fat cheese were, however, significantly lower than those of the full-fat cheese. These results may be attributed to the lower level of proteolysis found in the control low-fat cheese, as shown by the lower levels of soluble nitrogen fractions. Addition of adjunct cultures significantly improved the body and texture of the resultant low-fat cheese. The control full-fat cheese received significantly higher flavor scores than the control low-fat cheese. The differences in grading scores may be attributable to differences in proteolysis, the levels of which showed the same trend as the grading scores.

CONCLUSION

In conclusion the results of this study showed that the Iranian probotic low fat cheese is a functional food. It has better flavor and odor than normal cheese and can be used in many cases like as heart disease and obesity.

REFERENCES

Abd El-Salam, M.H. and E. Alichanidis, 2004. Cheese varieties ripened in brine (3rd ed.. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan and T. P. Guinee (Eds.). Cheese: chemistry, physics andmicrobiology. Elsevier Academic Press, London, pp: 227-249.

Akalin, A.S., S. Gonc and Y. Akabast, 2002. Variation in organic acids content during ripening of pickled white cheese. J. Dairy Sci., 85: 1670-1676. http://www.ncbi.nlm.nih.gov/pubmed/12201516

Aly, M.E., 1994. Flavour enhancement of low-fat Kashkaval cheese using heat or freeze-shocked Lactobacillus delbrueckii var. helveticus cultures. Die Nahrung, 38: 504-510. DOI: 10.1002/food.19940380506

Anuradha, S. and K. Rajeshwari, 2005. Probiotics in Health and Disease. JIACM., 6: 67-72. http://medind.nic.in/jac/t05/i1/jact05i1p67.pdf

AOAC, 2010. Official Methods of Analysis. 18th Ed., 3rd rev. Association of Official Analytical Chemists, Arlington, VA.

Dufosse**,** L., P. Galaup, P. Carlet, C. Flamin and A. Valla, 2005. Spectrocolorimetry in the CIE L*a*b* color space as useful tool for monitoring the ripening process and the quality of PDO red-smear soft cheeses. Food Res. Int., 38: 919-924. DOI: 10.1016/j.foodres.2005.02.013

Ehsani, M.R., S. Azarnia and A.R. Allameh, 1999. The study of the transfer of nitrogen materials, phosphorus, calcium, magnesium and potassium from the curd into brine during the ripening of Iranian white brine cheese. Iranian J. Agric. Sci., 30: 11-17.

Foegeding, E.A., J. Brown, M.A. Drake and C.R. Daubert, 2003. Sensory and mechanical aspects of cheese texture. Int. Dairy J. 13: 585-591. DOI: 10.1016/S0958-6946(03)00094-3

Gilliand, S.E. and M.L. Speck, 1977. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. J. Food Protect, 40: 820-823. http://agris.fao.org/agris-search/search/display.do?f=1978/US/US78014.xml;US7863356

Guarner, F. and G.J. Schaafsma, 1998. Probiotics. Int. J. Food Microbiol., 39: 237-238.

Guinee, T.P. and B.A. law, 2002. Role of milk fat in hard and semihard cheeses, in Fats in Food Technology. 1st Edn., K.K. Rajah (Eds.). Sheffield Academic Press Ltd., UK, pp: 275-331.

Guinee, T.P., M.A.E. Auty and M.A. Fenelon, 2000. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. Int. Dairy J., 10: 277-288. DOI: 10.1016/S0958-6946(00)00048-0

Hayaloglu, A., A.M. Guven and P.F. Fox, 2002. Review: Microbiological, biochemical and technological properties of Turkish white cheese Beyaz Peynir. Int. Dairy J., 12: 635-648. DOI: 10.1016/S0958-6946(02)00055-9

Institute of Standard and Industrial Research of Iran, ISIRI, 2002. Cheese in brine, specifications and test methods. ISIRI no, 2344-1. Iran.

Kandarakis, I., G. Moatsou, A.I.K. Georgala, S. Kamarinides and E. Anifantakis 2001. Effect of draining temperature on the biochemical characteristics of Feta cheese. Food Chem., 72: 369-378. DOI: 10.1016/S0308-8146(00)00245-4

Kasımoglu, A., M. Goncuoglu and S. Akgun, 2004. Probiotic white cheese with Lactobacillus acidophilus. Int. Dairy J., 14: 1067-1073. DOI: 10.1016/j.idairyj.2004.04.006

Katsiari, M.C., L.P. Voutsinas, E. Alichanidis and I.G. Roussis, 2001. Lipolysis in reduced sodium Kefalograviera cheese made by partial replacement of NaCl with KCl. Food Chem., 72: 193-197. DOI: 10.1016/S0308-8146(00)00218-1

Katsiari, M. C., L.P. Voutsinasand and E. Kondyli, 2002. Improvement of sensory quality of low-fat Kefalograviera-type cheese with commercial adjunct cultures. Int. Dairy J., 12: 757-764. DOI: 10.1016/S0958-6946(02)00066-3

Khosrowshahi, A., A. Madadlou, M. Ebrahim zadeh Mousavi and Z. Emam-Djomeh, 2006. Monitoring the chemical and textural changes during ripening of Iranian White Cheese made with different concentration of starter. J. Dairy Sci., 89: 3318-3325. http://www.ncbi.nlm.nih.gov/pubmed/16899664s

Kondyli, E., M.C. Katsiari, T. Masouras and L.P. Voutsinas, 2002. Free fatty acids and volatile compounds of low-fat Feta-type cheese made with a commercial adjunct culture. Food Chem., 79: 199-205. DOI: 10.1016/S0308-8146(02)00132-2

Lucey, J.A., M.E. Johnson and D.S. Horne, 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci., 86: 2725-2743. http://www.ncbi.nlm.nih.gov/pubmed/14507008

Marchesini, G., S. Balzan, S. Segato, E. Novelli and I. Andrighetto, 2009. Color traits in the evaluation of the ripening period of Asiago cheese. Ital. J. Animal Sci., 8: 412-413.

Mazahreh, A.S., J.M. Quasem, A.F. Al-Shawabkeh, I.A. Afaneh and A.S. Mazahreh *et al*., 2009. The effect of adjusting PH on stretchability and meltability to White brined nabulsi cheese. Am. J. Applied Sci., 6: 543-550. DOI: 10.3844/ajassp.2009.543.550

Michaelidou, A., M.C. Katsiari, L.P. Voutsinas, A. Polychroniadou and E. Alichanidis, 2007. Effect of multiple-species starters on peptide profile and free amino acids in low-fat Kefalograviera-type cheese. Food Chem., 104: 800-807. DOI: 10.1016/j.foodchem.2006.12.041

Michaelidou, A., M.C. Katsiari, E. Kondyli, L.P. Voutsinas and E. Alichanidis, 2003. Effect of commercial adjunct culture on proteolysis in low-fat Feta-type cheese. Int. Dairy J., 13: 179-189. DOI: 10.1016/S0958-6946(02)00148-6

Mohebbi, M and H.B. Ghoddusi, 2008. Rheological and Sensory Evaluation of Yoghurts Containing Probiotic Cultures. J. Agric. Sci. Technol. 10: 147-155. http://www.sid.ir/en/VEWSSID/J_pdf/84820080203.pdf

Ong, L., A. Henrikssonb, N.P. Shah, 2006. Development of probiotic Cheddar cheese containing *Lactobacillus acidophilus*, Lb. casei, Lb. paracasei and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. Int. Dairy J., 16: 446-456. DOI: 10.1016/j.idairyj.2005.05.008

Park, Y.W. and J.H. Lee, 2006. Effect of freezing on organic acid contents and lipolytic index of plain soft and Monterey Jack goat milk cheeses. Small Ruminant Res., 63: 58-65. DOI: 10.1016/j.smallrumres.2005.02.009

Picard, C., J., Fioramonti , A. Francois , T. Robinson , F. Neant and C. Matuchansky, 2005. Review article: bifidobacteria as probiotic agents-physiological effects and clinical benefits. Aliment Pharmacol Ther. 22: 495-512. http://www.ncbi.nlm.nih.gov/pubmed/16167966

Pinho, O., E. Mendes, M.M. Alves and I.M. Ferreira, 2005. Chemical, physical and sensorial characteristics of “Terrincho” ewe cheese: Changes during ripening and intravarietal comparison. J. Dairy Sci., 87: 249-257. http://www.ncbi.nlm.nih.gov/pubmed/14762067

Pouch Downes, F. and K. Ito, 2001. Compendium of methods for the microbiological examination of foods. Washington, DC: American Public Health Association.

Ryhanen, E.L., A. Pihlanto-Lepp.al.a and E. Pahkala, 2001. A new type of ripened, low-fat cheese with bioactive properties. Int. Dairy J., 11: 441-447. DOI: 10.1016/S0958-6946(01)00079-6

SAS, 2005. Statistical analysis system package. Cary, NC: SAS Institute, Inc.