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

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Abstract: Problem statement: The objective of this experiment was to manufacture an Iranian low fat probiotic cheese. Approach: Iranian white brine cheeses (4 trials) were made by varying processes, 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 ripening 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 probiotic 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...
Table 1: Chemical and bacteriological analysis of milks

| Cheese | Code | Fat (%) | Protein (%) | Lactose (%) | SNF (%) | FPP (%) | pH (%) | Acidity (%) | Coliform (log cfu ml⁻¹) | Total count (log cfu ml⁻¹) |
|--------|------|---------|-------------|-------------|---------|---------|--------|-------------|--------------------------|---------------------------|
|        | A    | 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, 10 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 subsp. 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 subsp. 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 homogenized and mixed with 6 g Na₂SO₄ 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 C₁₈ column model (CLC-ODS) shim-pack. The system were used at room temperature (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)₂HPO₄, 0.5% w/v and acetonitrile, 0.4% v/v (at pH 2.24 with H₃PO₄) was run through the column. HPLC grade reagents were used as standards (Merck Chemical Co). Solvents were degassed under vacuum. 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 coted 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...
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 subsb bulgaricus and L. acidophilus concentrations were in the range of 7.54-8.71, 5.74-6.87 and 7.10-8.21 (log10 cfu g-1), respectively.

The concentration (log10 cfu g-1) of S. thermophilus, Lactobacillus delbrueckii subsb. 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

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

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

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

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

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

*: Each value in the table is the mean ± SD of four replications; 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

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

*: 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); 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 of 73.55-78.05, -1.20 to -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 fat probiotic 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
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 syneresis of the curd and 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 probiotic adjunct 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 10⁷ 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). 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 \(10^5\) and \(10^6\) cfu g\(^{-1}\). As indicated earlier, it is necessary to maintain the viability of \(L.\) acidophilus at >\(10^5\) 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; Kasmoglu 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 the amount 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 decrease the L* value
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