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The Microbiological and Chemical Quality of Traditional Lighvan Cheese (White Cheese in Brine) Produced in Tabriz, Iran

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Abstract: The aim of this study, was to determine the hygienic condition of processing and chemical characteristics of Lighvan cheese, a white cheese traditionally produced from raw ewe's milk and occasionally made from either raw goat's milk, raw cow's milk or from mixed of them in Tabriz. In this study, 178 cheese samples were collected randomly from retailers in different regions of Tabriz and examined. The results indicated that the mean value of coliforms bacteria, *Staphylococcus aureus*, molds and yeasts of cheese were $(4.14\pm1.27)\times10^2$, $(4.53\pm0.32)\times10^3$, $(4.54\pm2.37)\times10^3$ and $(92.08\pm38.86)\times10^3$ CFUg^{-1}, respectively. Also, the number of coliforms, *Staphylococcus aureus* and molds in 10.1, 10.1 and 34.2% of the samples were higher than the limits allowed by the national standard for Iranian industrial white ripened cheese, respectively. Furthermore, *E. coli*, faecal coliforms and positive coagulase *Staphylococcus aureus* were isolated from 58.4, 62.9 and 18% of the samples, respectively. No Salmonella was found in the samples. Chemical parameters such as pH (4.68±0.30), titrtable acidity (1.41±0.44%), dry matter (41.56±3.51%), protein (20.13±2.25%), fat (19.1±3.14%), NaCl (4.35±1.38%) and ash (5.92±1/26%) were also determined. It can be concluded that Lighvan cheese is one of the promising traditional white cheese in view of its high nutritional value and unique aroma and taste but hygienic condition of its processing is not very satisfactory.

Key words: Traditional lighvan cheese, microbiological and chemical quality, Tabriz

INTRODUCTION

In spite of advancement and development of industrial equipments and techniques and despite the increase of the production and variety in industrial cheese in Iran, several traditional cheese types are being produced and consumed in different regions of the country. The traditional white cheese in Tabriz markets are commonly called Lighvan cheese. It was traditionally and commonly made from raw ewe's milk and occasionally made from either raw goat's milk, raw cow's milk or a mixture of them. Because of its pleasant organoleptic properties, this type of cheese is popular and widely consumed all over Iran and is enjoying high economical and nutritional value.

In its manufacture raw milk is heated to approximately 32°C and rennet is added. After coagulation, the curd is cut into small pieces by primitive equipment and covered with a cotton cloth and pressed to exclude whey as much as possible. Then the curd is cut into slices and salted on the surface with coarse-grained salt. At the end, the product is put into tins, covered with brine and ripened in natural cells where the constant temperature and humidity create the optimal condition for 2-8 months of cheese ripening. All steps of manufacturing are manual.

Cheese is currently considered as one of the safest foods consumed, however, pathogenic bacteria that can be transmitted through the dairy products, including cheese, are important from public health point of. Historically there have been outbreaks of infection associated with the consumption of cheese and the predominant organisms responsible have included salmonella, Listeria monocytogenes, verocytotoxin producing *Escherichia coli* (VTEC) and *Staphylococcus aureus* (Razavilar, 2002; Karim, 2006; Tamagnini *et al*., 2005).

Detailed investigations have demonstrated that the sources of contamination were raw milk, inadequately pasteurized milk, or post-pasteurization contamination with organisms originally derived from raw milk or from manufacturing's environment (Tamagnini *et al*., 2006).

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The aim of this research, was to study the hygiene condition during the cheese processing and chemical characteristics of Lighvan cheese, a traditional white cheese in brine, in Tabriz markets.

MATERIALS AND METHODS

Sampling: In this study, 178 cheese samples (300±50 g) were collected from retailers in different regions of Tabriz between 24 June and 22 October 2007. The samples were transported to laboratory in sterile and cold containers 4°C and preserved at this temperature. Each sample was divided into 2 parts, one for microbiological and the other for chemical analysis. The samples were kept not >12 h from sampling to microbiological and chemical analysis.

Microbiological analysis: Cheese samples 25 g were homogenized for 1 min in 225 mL of a sterile solution of 2% (w/v) sodium citrate using a stomacher, Lab-Blender (PBI International, Milan, Italy). Further, decimal dilutions were prepared with 1/4 diluted Ringer solution. All of the analysis were performed in duplicate. Microbial examinations were carried out.

Salmonella identification was carried out after pre-enrichment of the sample in buffered peptone-water and enrichment in selenit cystine broth and incubated at 37°C for 18-24 h. After the enrichment step, the cultures were surface streaked onto brilliant green agar and Hecktoen agar (Difco). These plates were incubated at 37°C for 24-48 h (Olerta *et al*., 1999).

The enumeration of total coliforms was performed on Violet Red Bile Agar (VRBA) incubated at 31±1°C for 24±2 h (Karim, 2002). Faecal coliforms were detected from those colonies considered as total coliforms in VRBA, transferred to Brillinant Green Bile Broth (BGBB) and incubated at 44±0.5°C for 24-48 h. From positive cultures, sub-cultures were made on eosin methylene blue lactose sucrose (EMB) agar (EMBA) and incubated at 35±1°C for 24 h. *E. coli* isolates were biochemically characterized by IMVIC tests (Karim, 2002).

Coagulase positive Staphylococcus enumeration on Baird's Parker Agar (BPA) incubated at 35±1°C for 48 h. Colonies were examined by Gram stain, catalase test, anaerobic utilization of glucose and manitol and coagulase test (ISIRI 1194, 1994).

Yeasts and molds were enumerated on Potato Dextrose Agar (PDA) acidified with 10% tartaric acid (Merck, Darmstadt, Germany) following the surface-plate method and incubated at 22-25°C for 5-7 days (ISIRI 997, 1994).

Chemical analysis: Moisture, NaCl content and pH value were determined according to the International Dairy Federation methods (Gobbetti *et al*., 1999). Total Nitrogen (TN) level was determined by the kjeldahl method (Parvaneh, 1996) and multiplication of TN by 6.38 gave the protein content (Olerta *et al*., 1999). Acidity was determined using 20 g of sample in 1902 of distilled water and blended in a Stomacher for 2 min. The supernatant liquid was filtered through Whatman N 0.1 filter paper. Acidity was determined on the supernatant liquid by titration against a solution of 0.1N NaOH with 0.1% phenolphthalein solution as indicator. Titratable acidity was expressed as per cent of lactic acid (Miriam and Rosalia, 2004). The fat content was determined according to the Gerber method (Parvaneh, 1996).

RESULTS

The results obtained from the microbiological analysis of the cheese samples are shown in Table 1 and in Fig. 1 and 2.

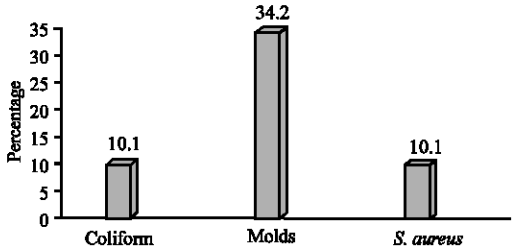

Fig. 1: Prevalance rate of samples in those contamination was above limits allowed by the Iranian standard

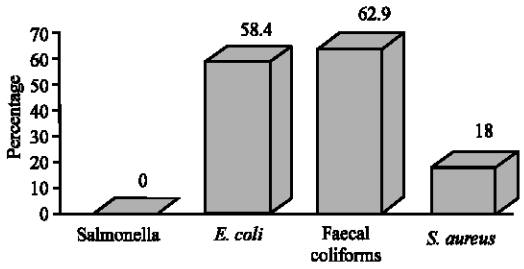

Fig. 2: Prevalance rate of zero tolerant microorganisms in traditional chees presented in Tabriz markets

Table 1: Descriptive statistics for hygienic indicator microorganisms of lighvan cheese

Microorganism groups	Statistical parameters					
	n[a]	x[b]	Min[c]	Max[d]	S.D[e]	S.E[f]
Coliform	178	423.78	3	2400	846.40	63.44
Staphylococcus aureus	178	4529.21	0	14000	02127.32	0159.45
Mold	178	4540.51	0	200000	23696.82	1885.22
Yeast	178	92078.48	0	3080000	38859.32	3091.48
Fungi	178	96618.99	0	3080000	39085.29	3109.46

[a]Number of sample analyzed; [b]Mean; [c]Minimum; [d]Maximum; [e]Standard deviation; [f]Standard error of mean

Table 2: The results of some chemical parameters of lighvan cheese

Chemical composition	Statistical parameters					
	n[a]	x[b]	Min[c]	Max[d]	S.D[e]	S.E[f]
Protein (%)	178	20.13	13.58	24.92	2.25	0.27
Fat (%)	178	19.10	12.00	27.00	3.14	0.27
Salt (%)	178	4.35	2.19	9.95	1.38	0.10
Ash (%)	178	5.92	3.79	12.14	1.26	0.10
Acidity (%)	178	1.41	0.60	3.60	0.44	0.03
pH	178	4.68	4.22	5.59	0.30	0.02
Drymatter	178	41.66	31.50	81.42	3.51	1.34

[a]Number of sample analyzed; [b]Mean; [c]Minimum; [d]Maximum; [e]Standard deviation; [f]Standard error of mean

Table 3: The results of some chemical parameters in dry matter of lighvan cheese

Chemical composition (%)	Statistical parameters					
	n[a]	x[b]	Min[c]	Max[d]	S.D[e]	S.E[f]
Protein	178	49.55	23	57	9.60	1.30
Fat	178	45.o6	37	67	12.34	1.04
Salt	178	8.82	7.3	28	4.74	0.40
Ash	178	11.65	10	33	4.65	0.39

[a]Number of sample analyzed; [b]Mean; [c]Minimum; [d]Maximum; [e]Standard deviation; [f]Standard error of mean

As Fig. 1 displays, the number of coliforms, *Staphylococcus aureus* and molds in 10.1, 10.1 and 34.2% of samples were above limits allowed by national standard described for the Iranian industrial white ripened cheese (ISIRI, 2406, 1994), respectively.

As Fig. 2 displays, *E. coli,* faecal coliforms and coagulase positive *Staphylococcus aureus* were isolated from 58.4, 62.9 and 18% of samples, respectively and Salmonella was not found in the samples.

The results obtained from the chemical analysis of the cheese samples are shown in Table 2 and 3.

DISCUSSION

As Table 1 displays the average coliform count determined was 423.8±63.4 cfu g^{-1} (mean ±SEM) changing between 3 cfu g^{-1} and 2400 cfug^{-1}. There is no national standards for microbiological and chemical characteristics of traditional white cheese (Lighvan) in the country. Iranian standard described for industrial white ripened cheese (ISIRI, 2406, 1994) suggests that white cheese should not contain >100 cfu g^{-1} coliform bacteria. As Fig. 1 displays, the number of coliforms in 10.1% of the samples exceed maximum level (100 cfu g^{-1}). In related stududies, Bahrami *et al.* (2006) reported that *E. coli* was present in 80 (100%) and the number of coliforms in 80 (100%) of traditional cheese samples presented in Ilam city market was higher than standard limits. Nik Niaz *et al.* (2006) reported that *S. aureus* was present in 65 (68%) of traditional cheese samples presented in Tabriz market. The great variation was observed between samples, which could be related to the stage of ripening and production quality (Ceylen *et al.*, 2003). The higher total coliform counts implies risk that other enteric pathogens may be present in the same sample (Yucel and Ulusoy, 2006). It is well known that ripening acts as a natural selector, during which lactic acid bacteria normally inhibit pathogens (Caridi *et al.*, 2003; Cetinkaya and Soyutemiz, 2006). Recently, it has been demonstrated that strains of Lactobacillus paracasei subsp. Paracasei have an intense antagonistic activity against *E. coli* through bacteriocin production (Caridi *et al.*, 2003). According to our results, the number of indicator micro-organisms, coliforms, were high and this suggests that contamination of raw milk during milking has occured, also unrefrigerated storage and transportation and possible contamination of cheese during manufacturing seems to be the common way raw of ewe's cheese milk (Psoni *et al.*, 2003; Zarate *et al.*, 1997). Various factors contribute to the decline of these micro organisms during ripening, they include increase in the concentration of NaCl (Zarate *et al.*, 1997), inhibition of these bacteria by lactic acid bacteria (Nunez *et al.*, 1985) basically by causing a decrease in pH and an increase in lactic acid concentration (Zarate *et al.*, 1997) and low temperature of storage.

Iranian industrial white ripened cheese standard suggests that it should not contain feacal coliforms and *E. coli.* As Fig. 2 shows, *E. coli* and feacal coliforms were isolated from 58.4 and 62.9% of samples, respectively. *Escherichia coli* O157:H7 survived for 30-40 days at pH values of 4.0-4.5 (McIngvale *et al.*, 2000), 34-38 days at 5°C at pH value of 4.4 and 158 days when it is added to cheddar cheese milk at the rate of 103 cfu mL^{-1} (Reitsma and Henning, 1996). It is stated that salt stimulates the inactivation of micro orrganisms (Tsegaye *et al.*, 2004). Regarding only salt, *E. coli* O157:H7 can tolerant NaCl concentrations as high as 8.5% (Glass *et al.*, 1992). In other words, it is not possible to inactivate this bacterium only with increasing the amount of sodium chloride in white cheese. Besides, salt concentration is limited by standards. According to the Iranian standards for white ripened brine cheese, NaCl content should not exceed 5% in dry-matter. Therefore, the risk of *E. coli* infection is high.

The average *Staphylococcus aureus* count determined was (4.53±0.16)×103 cfu g^{-1} changing between 0 and 14000 cfu g^{-1} (Table 1). The number of *S. aureus* in

10.1% of samples was above limits allowed by Iranian standards for the industrial white ripened cheese (<100 cfu g^{-1}). According to our results, coagulase positive *Staphylococcus aureus* was isolated from 18% of the cheese samples (Fig. 2).

Staphylococcus aureus is often found in raw milk and in the environment of the cheese plants (equipment and personel). This organism is salt-tolerant and is able to grow under a wide range of conditions; low acid production may allow Staphylococci to grow and produce enterotoxins (Olerta *et al*., 1999).

Table 1 shows the average of yeast and mold counts (92.08±3.09)×103 and (4.53±1.88)×103 cfu g^{-1}, respectively. According to our results, 34.2% of the samples had mold counts higher than the limits of national standard (<100 cfu g^{-1}). A significant ($p<0.05$) negative correlation ($r = -0.548$) was found between yeast and mould counts with acidity % of the samples, which could be considered for the fact that yeast and mould count could metabolize lactic acid and lower acidity percent (Turkoglu *et al*., 2003). Yeasts may contribute to the ripening process by utilizing lactic acid or by their proteolytic and lipolytic activity (Psoni *et al*., 2003). Relatively high counts of yeasts are frequently observed in many soft, semi soft and surface ripened cheeses, probably originating from the processing equipment and dairy environment (Viljoen, 2001). It has been suggested that a significant source of yeast contamination is probably the brine solution. However, one should keep in mind that the relatively high counts of yeasts enumerated may vary between dairies and even between consecutive days in the same dairy. This can be attributed to pasteurization efficiency, variation in salt concentration, temperature, accidental occurrences of contaminating yeasts, as well as the standards of hygiene prevailing during cheese making (Eugenia *et al*., 2003; Estepar *et al*., 1999).

The average dry matter of cheese samples was 41.56% changing between 31.5 and 81.42%. The differences between samples are due the fact that this type of cheese relies upon individual dairies and there is no standard for cheese manufacturing method. Institute of Standards and Industrial Research of Iran (ISIRI) suggests that industrial white ripened cheese should not contain lower than 40% dry matter (ISIRI 2344, 2006). According to, chemical results obtained in this study, dry-matter content in 74(41.5%) samples was lower than limits allowed by ISIRI 2344. The average fat in dry matter content of cheese samples was 45.06% changing between 37 and 67%. According to these results, traditional lighvan cheese is a fat cheese (ISIRI 2344, 2006). The protein content of cheese samples changed from 13.58-24.92%, the average was 20.13% indicating that the cheese is a good protein source. The salt content of samples changed from 2.19-9.95%, the average was 4.35%. According to our results, 18(10.1%) and 32(18%) of samples had salt content higher and lower than limits of Iranian standards (3-5%), respectively (ISIRI 2344, 2006).

The average titratable acidity of samples was 1.41%. There was an apparent variation in titratable acidity, changing from 0.6-3.36%. The ISIRI 2344 limits the titratable acidity of industrial white ripened cheese (minimum 0.8%). The titratable acidity in 4(2.2%) of samples was lower than standard limits (ISIRI 2344, 2006). The lactic acid contributes not only in taste of cheese but it also helps cheese to maintain its convenient body, texture and protects it against some kinds of microbiological spoilage (Ceylen *et al*., 2003). The mean of pH value in samples was 4.68(D°) changing from 4.22-5.59 D°. According to our results the acidity of 120 (67.4%) out of 178 samples conformed with ISIRI 2344, 2006 (≤4.8 D°).

According to this study it can be concluded that Lighvan cheese is one of the promising traditional cheese types in view of its high nutritional value and unique and aroma but hygienic condition of it is not very satisfactory.

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