THE EFFECTS ON CONTAGIOUS MASTITIS PATHOGENS IN BULK TANK MILK ON PHYSICOCHEMICAL PROPERTIES OF IRANIAN WHITE CHEESE

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ABSTRACT
The objective of this experiment was to study contagious mastitis pathogen effects on Iranian white cheese properties. Iranian white brine cheeses (2 trials) were made by healthy and containing pathogen bulk tank milk. All types of cheese were ripened at 13 °C for 2 weeks and at 6 °C to the end of ripening period. Cheeses were analyzed for the composition, color, lipolysis and proteolysis. The cheese of each trial was sampled at 1 and 60 days during ripening. No significant differences were observed between the fat, protein, ash and moisture contents of manufactured cheeses. Color parameters, lipolysis and proteolysis indices were not significantly different in two cheese groups. Therefore, incidence of *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacteria spp* and *Mycoplasma spp* in bulk milk tank does not change the physicochemical properties of Iranian white cheese. Despite the potential of these pathogens to cause health problems for consumers, they do not alter the characteristics of the cheese.

1. Introduction

Milk nature as complex biological liquid provides an excellent growth medium for many micro-organisms. It is unavoidable of milk contamination with micro-organism during its production procedure. So, the total microbial count of raw milk indicates the rate of its quality (Robinson, 2005). The source of bacterial contamination of raw milk can categorize into: air, milking equipment, feed, soil, feces and grass. Many factors can effect on the number and types of micro-organisms in milk immediately after milking such as animal and equipment cleanliness, season,

feed and animal health (Coorevits et al., 2008). Quality of milk is deteriorated by the presence of subclinical mastitis and reduces milk production. Mastitis definition refers to inflammatory reaction of mammary tissues towards an infection; this inflammation is characterized by an influx of white blood cells into the mammary gland, followed by an increase in endogenous milk proteases. Mastitis causes economic losses for dairy farmers due to reduced milk yields (Heringstad et al., 2003) and it is the most expensive common diseases on dairy farm. Understanding the prevalence and

distribution of mastitis is a basic key to the prevention of disease. Microbial culture from bulk tank milk can be used as monitoring tool in the control and evaluation of clinical and subclinical mastitis. The most prevalent pathogens causing mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacteria spp.* and *Mycoplasma spp.*

Microbial methods for detection and numbering of specific micro-organism is an critical part of quality control and quality assurance plan and it can be used for raw materials, intermediate samples, finished products or environmental/equipment sites. Surveillance system of several reported that about Milk borne and milk-product borne outbreaks is 2-6% of food-borne outbreaks (De Buyser et al., 2001). One dairy product, which is so popular among Iranian people, is Iranian White cheese. This product is close-textured brined cheese, being similar to Beyaz Peynir (Turkish White cheese) and Feta but the way of Feta making procedure is different from Iranian White cheese. In Iranian cheese production, the period of dry salting of curd and slime formation on the curd surface before brining, which is essential for the development of the characteristic Feta flavor during ripening, does not exist (Sabbagh et al., 2010). Iranian cheese milk originated from cow's milk, sheep's milk or mixtures of them and the main flavor of it created due to acidity and saltiness (Khosrowshahi et al., 2006).

The objective of the present study was to study the composition and physicochemical properties of manufactured Iranian White cheeses from bulk tank milk containing contagious mastitis pathogens.

2. Materials and methods
2.1. Milk sample collection
A total of 30 bulk tank milk samples were collected randomly from industrial dairy herds of Fars, IRAN in 2011. First, the clean and dry teat was scrubbed with cotton soaked twice in 70% ethyl ethanol and the first squirt of milk was discarded. All collected samples were immediately put in an insulated container with ice pack and transferred to the laboratory without delay to perform bacterial culturing, PCR assay and to detect physiochemical parameters. According to the PCR results, samples were divided into healthy and contaminated group. Then, the mentioned assays were done on produced white brine cheese (Iran, 1999) from 2 groups on zero and 60- day.

2.2. Bacteriological culture
Milk samples were brought to room temperature and the mentioned microorganisms causing mastitis were isolated and identified according to previous methods reported by Carter and Cole (2012).

2.3. DNA extraction
DNA extraction was performed using a Cinnapur DNA kit (Cinagen, Iran). The specimens were centrifuged at 12000 rpm for 10 min. The supernatant was discarded and the pellet was vortexed and transferred into a 1.5 ml microtube. 200 µl of lysis buffer and 40 µl of proteinase K were added and incubated at 65 °C for 15 min. The DNA was further purified and re-suspended in 30 µl elution buffer according to the manufacturer's instruction, and kept at -20°C for further use. The concentration of DNA was subsequently estimated by absorbance at 260 nm and the purity of the DNA was checked by taking the ratio of O.D. reading at 260 and 280 nm using a spectrophotometer.

2.4. PCR assay
Four pairs of primers were used as previously described: species specific 225 bp, fragments 220-230 bp, 650 bp and 300 bp. which were subjected to *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacteria spp* and *Mycoplasma spp*.

respectively (Table 1). Multiplex PCR was carried out on 5 µl of the DNA template in a final reaction mixture of 50 µl containing 10 × PCR buffer, 1.5 mM MgCl2 (50 mM), 0.2 mM dNTP (10 mM), 1 µM of each of forward and reverse primer, 2.5 U Taq DNA polymerase (5 U/µl) (Cinna Gen, Iran). PCR cycling was performed in a gradient thermocycler (Eppendorf, Germany) with an initial denaturation step of 95°C for 5 min followed by 30 PCR cycles under the following conditions: denaturation at 95°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 30s. After the final cycle, the preparation was kept at 72°C for 5 min to complete the reaction. (BIOR XP, China) (Jin et al., 2009). The amplified products were subsequently electrophoresed in a 2% agarose gel, stained with ethidium bromide and photographed under UV light.

Species specific fragments of 225, 220-230, 650 and 300 bp (Figure 1), corresponding to *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacteria spp* and *Mycoplasma spp*. were then amplified. Seven of these samples were used, and three negative samples for cheese making. For each trial, 3 kg of milk was used. Different types of cheese were produced according to the national standard for white brine cheese (Iran, 1999). Each types of the produced cheeses were ripened at 13 °C for 2 weeks and at 6 °C to the end of ripening period (60 days). Cheese of each trial was sampled at 1 and 60 days during ripening. All experiments on milk and cheese samples were done in triplicate.

2.5. Chemical analysis of milk and cheese

Titrable acidity of milk was determined by Dornic method. Digital milk scan (Lactostar, Funke Gerber, 230V) was used to determine fat, protein, solid nonfat, lactose and freezing point. 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. Salt content was determined by Volhard method and fat content by the Gerber method. The ash content of cheese samples was determined by dry ash method and total protein content was determined by measuring total nitrogen using Kjeldahl method and converting it to protein content by multiplying by 6.38 (Sabbagh et al., 2010).
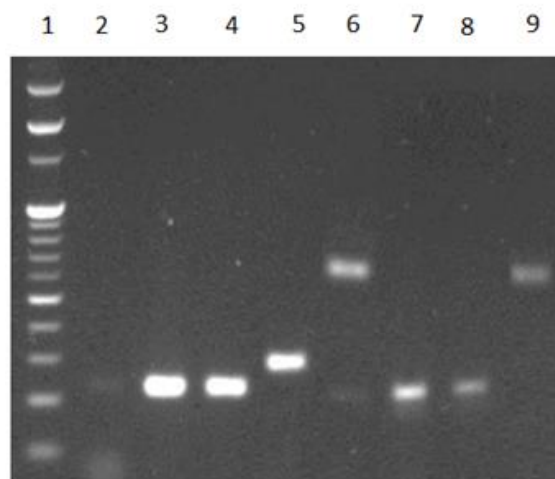
Figure 1. Representing 2% agarose gel electrophoresis for detection of *Staphylococcus aurous, Streptococcus agalactiae, Corynebacteria* spp and *Mycoplasma* spp, using multiplex PCR assay. Lane 1: 100 bp ladder, Lane 2: Negative control, Lane 3: 225 bp positive control, Lane 4: 220-230 bp positive control, Lane 5: 300 bp positive control, and Lane 6: 600 bp positive control, Lane 7: positive sample, Lane 8: positive sample, Lane 9: positive sample.

2.6. Cheese color measurement

The color of cheese samples at days 1 and 60 of ripening period was quantitatively determined using a simple digital imaging method described by Yam and Papadakis (2004), with a slight modification. Color values L*, a* and b* were determined.

2.7. Lipolysis

The acid degree value ADV was determined with a modified procedure developed by Park and Lee (2006).

2.8. Proteolysis

Water-soluble nitrogen (WSN) was determined in aliquots of water-soluble cheese extract (WSE) prepared as described by Fox et al., (2004). Total nitrogen (TN) of cheese samples and water-soluble nitrogen (WSN) of extracts were determined using the micro-kjeldahl method with an automatic digester model 430 and distillation unit model 322 (Buchi, flawil, hysic land). Index

of maturation (IM) or Nitrogen Solubility Index (NSI) was expressed as a percentage of WSN of the cheese TN (WSN$\times$100/TN) and it was used to follow the proteolysis degree during ripening. Analysis of the free amino groups in the WSE of the cheeses was determined according to the method of Fox et al., (2004). Data were analyzed by independent t test and Mann-Whitney U test (SPSS software, version 16, p<0.05).

Table 1. Primers that used for specification of gap gene, 16s–23s rRNA, 16s rRNA, deoxyribodipyrimidine photolyase (UVrC) gene amplicons corresponding to *Staphylococcus aurous*, *Streptococcus agalactiae*, *Corynebacteria spp* and *Mycoplasma* spp.

Primers	Oligonucleotide sequence (5' 3')	Product size (bp)
Au-F	TTCGTACCAGCCAGAGGT	225
Au-R	TTCAGCGCATCACCAAT	
SU-F2	AGCCGCCTA AGGTGGGAT	220–230
SU-R	ATGGAGCCTAGCGGGATC	
Cb-F2	CGTGCTTTAGTGTGTGCG	650
Cb-R3	GGCACGGAAATCGTGGAAG	
Mb-F	GCTTCAGTATTTTGACGG	300
Mb-F	GGTTTAGCTCCA TAACCAGA	

3. Results and discussions
3.1. Microbial and PCR analysis

Thirty raw bulk tank milk and their produced cheese samples were analyzed by means of both classic microbiological and molecular techniques, to detect *Staphylococcus aureus, Streptococcus agalactiae, Corynebacteria spp.* and *Mycoplasma spp.* In agreement with microbiological tests: 5 milk samples were contaminated with Staphylococcus aurous and Mycoplasma bovis, 2 samples with *Streptococcus agalactiae* and one with *Corynebacterium bovis*.

PCR results of the various cheeses in day zero indicated that 3 samples were contaminated with *Staphylococcus aurous*, and 2 samples with *Mycoplasma bovis*. After 60 days of cheese ripening, pathogen contaminations in cheese samples were

reduced and *Staphylococcus aurous* was detected in one sample. So, *Staphylococcus aurous* and *Mycoplasma bovis* have been transferred from contaminated milk to zero-day cheese. During cheese ripening, *Mycoplasma bovis* was destroyed, but *Staphylococcus aurous* was shown to be resistant to ripening conditions.

Microbial culture from bulk milk samples can be an effective tool for milk quality evaluation compared with the individual cow milk samples collection and examination, and it may be a useful method for indicating prevalence level of contagious mastitis pathogens in herd. The contagious pathogens settling in cow's udder are *Staphylococcus aureus, Streptococcus agalactiae,* and *Mycoplasma spp*; therefore, the presence of these mastitis causing organisms in bulk milk are strong indicator of

the occurrence of intramammary infections in the herd (Fox et al., 2005). In this research, *Staphylococcus aurous* was detected from 5 bulk tank milk samples, *Mycoplasma bovis, Streptococcus agalactiae* from 2 samples and *Corynebacterium bovis* from one sample. There is some concern that bacteria in raw milk may transfer to the raw dairy products and raise a risk of food poisoning to consumers (Headrick et al., 1998). As regarding the mentioned information, some samples of white Iranian cheese were positive as *Staphylococcus aurous* and *Mycoplasma bovis* in the first day of ripening, but *Mycoplasma bovis* was not diagnosed 60 days later.

In present study, *Staphylococcus aureus* was detected from the primary sampling of bulk tank milk and cheese at various stages of production. Jørgensen et al., (2005) isolated *Staphylococcus aureus* from 10 of 11 cows, and the farmer, equipment, the environment, and the cheese and *Staphylococcus aureus* concentrations in the cheese were not at significant level as a risk of staphylococcal food poisoning. Active lactic starter cultures in cheese usually inhibit *Staphylococcus aureus* growth. Lactic acid as a weak organic acid produced in the fermentation period of dairy products, and can interfere in pH homeostasis of bacteria resulting in stressed cells. In the undissociated form lipophilic form of lactic acid and other weak organic acids can freely diffuse across the bacterial cell membrane. Then, the acid can dissociate and release protons inside of cell and finally acidify the cytoplasm. Cell energy is used to maintain internal pH, and hence, cell growth is reduced or inhibited, but in our study, *Staphylococcus aureus* was detected in 60 day of cheese maturation. The reason can be that *Staphylococcus aureus* is one of the most halotolerant, non-halophilic bacteria, and can grow in the presence of up to 3.5 M NaCl. It has been proofed that high concentrations of

NaCl inhibit growth (Armstrong-Buisseret et al., 1995).

In agreement with some researches, *Staphylococcus aureus* detection has been reported from cheese and raw milk. The prevalence of 20 to 38% *Staphylococcus aureus* in Norwegian raw milk products has been mentioned (Jørgensen et al., 2005). In a Swedish study, coagulase-positive *staphylococci* were detected in 38% of on farm manufactured raw goat cheeses (Armstrong-Buisseret et al., 1995). In France, *Staphylococcus aureus* is reported to be the most frequent cause of foodborne disease from raw milk cheeses (De Buyser et al., 2001) and in Norway, outbreaks of *Staphylococcus aureus* food poisoning have been attributed to raw goat cheese (Watkinson et al., 2001), raw cow cheese and potato-mash made with raw milk. According to the PCR and microbiological tests in our study, 16.6% bulk samples were contaminated to *Staphylococcus aureus.* In favorable conditions, *Staphylococcus aureus* may grow and produce enterotoxins in foods, and because the toxins are stable with respect to heat and storage they may be present in foods where viable *Staphylococcus aureus* are absent. Dairy animals are probably the main source of contamination of raw milk with *Staphylococcus aureus* (Vautor et al., 2003). In particular, dairy animals with subclinical *Staphylococcus aureus* mastitis may shed large numbers of *Staphylococcus aureus* into the milk. Jørgensen et al., (2005) reported that the bacteria were spread with the milk and product material to the equipment and the environment during milking and cheese production (Jørgensen et al., 2005). The sanitation process seemed effective in removal of *Staphylococcus aureus*.

Mycoplasma bovis as a causative agent of mastitis is responsible for considerable economic losses to the dairy industry. Because of the highly virulent and

pathogenic nature of *Mycoplasma bovis*, it is so vital to identify the infected animals at early stage of infection and cause lower overall impact on the herd. *Mycoplasma bovis* is an important mastitis agent that was detected in bulk tank milks and first day of cheese ripening in our study. It appears ripening situation inhibit mycoplasma growth in the 60 day of cheese ripening. It was found that osmotic lysis of Mycoplasma organisms depend on the temperature of incubation and the pH of the suspending medium, being lowest at pH values near neutrality. The organisms were resistant to osmotic shock at 0 °C, but lysed rapidly at 37 °C (Feenstra et al., 1991).

Streptococcus *agalactiae* and *Corynebacterium bovis* were noticeable in bulk tank milk, but they were not detected in the various times of cheese production. *Streptococcus agalactiae* infection in dairy cattle has a considerable role in reduction of milk quality and milk products. Milk from cows with subclinical mastitis decreases the quality of cheese and other manufactured milk products (Politis and Ng-Kwai-Hang, 1988).

According to EU Regulations (Regulation 853, 2004) the total mean of microorganisms should not exceed 100.000 per ml of raw cow's milk after production. In this study we aimed to investigate the microbiological quality of 30 bulk milk samples. We determined the total bacterial count higher than 100.000 cfu/ml in both healthy and contaminated tested sample, while Aaku et al., (2004) calculated 5.5×10^6 cfu/ml of the total mean of micro-organisms in pooled raw milk, which is higher than in our study.

Coliform counts higher than 100 cfu/ml are regarded by some authorities as unfavorable hygiene production. High coliform counts may be attributed to unrecognized coliform mastitis, mostly by *E. coli*. The coliform micro-organisms are also found on the surface of the under washed or moisture milking equipment.

In present study, coliform count and total bacterial count in contagious mastitis group were higher than healthy group.

3.2. Compositional and physicochemical properties

The milk and cheese compositional characteristics are shown in Tables 2 and 3 and two groups do not show any significant differences. The fat and protein contents of manufactured cheeses decreased significantly during ripening, while the ash, moisture, salt and pH of cheese increased ($p < 0.05$).

Table 2. Chemical and bacteriological analysis of bulk tank milks that used for cheese making (Mean±SD)

Composition	Fat (%)	Protein (%)	Lactose (%)	SNF [a] (%)	FPP [b] (%)	pH (%)	Acidity (%)	Coliform count (log cfu ml^{-1})	Total count (log cfu ml^{-1})
A [c]	2.49 ± 0.80	3.09± 0.27	4.77 ± 0.89	8.84 ± 0.64	-0.49 ± 0.06	6.79 ± 0.16	18.48 ± 0.17	4.21 ± 0.27	6.11 ± 2.29
B [d]	2.62 ± 0.86	3.22 ± 0.23	4.89 ± 0.42	9.02 ± 0.69	-0.51 ± 0.06	6.67 ± 0.15	18.03 ± 0.14	4.87 ± 0.31	6.32 ± 3.17

[a] SNF= solid non fat; [b] FPP= freezing point
[c] A= healthy milk; [d] B= milk containing pathogens

Table 3. Composition of Iranian white cheese in brine during the 60 days storage (Mean±SD)

Treatment	Day	Fat (%)	Protein (%)	Ash	Moisture	Salt	pH CH	pH WH
A [a]	0	18.50 ± 4..25	16.95 ± 1.32	3.11± 0.38	48.29± 2.45	2.81± 0.25	5.49± 0.25	6.17± 0.48
A	60	10.22± 3.51*	13.5± 1.65*	3.89± 0.28*	53.56± 2.74*	3.70± 0.33*	5.98± 0.18*	6.34± 0.41
B [b]	0	17.75±3.16	15.37± 1.04	3.09± 0.30	47.77± 1.95	2.69± 0.34	5.35± 0.11	5.97± 0.35
B	60	10.80±2.80 *	12.47± 1.44*	3.64± 0.15*	53.19± 3.12*	3.52± 0.27*	5.85± 0.28*	6.20± 0.29

*indicates $p<0.05$ between different days of each group
[a]A= cheese made with healthy milk   [b]B= cheese made with milk containing pathogen

A slight increase in fat, lactose of milk was seen from healthy animal to infected groups, but no significant decrease was observed in protein, SNF, pH of milk. The exact composition of milk varies with the breed, species, feeding regimes and udder health (Ahmad et al., 2007). Subclinical mastitis changes the milk composition and any variety in its percentage affects the suitability of milk processing and the quality of its products (Sharif and Muhammad, 2009).

The degree of these changes depends on infecting agent and the inflammatory response. In present study, fat percentage in cow's increases from (2.49 ± 0.80) in milk from uninfected cows to (2.62 ± 0.86) in milk from infected cows, these results are in agreement with Schmitz et al. (2004), but disagree with Lehloenya et al., (2008). Some research reported that milk from infected animals with sub- clinical mastitis had significant increase in the activity enzyme called lipase that causes milk fat breakdown (Uallah et al., 2005), which is not compatible with our result about fat percentage. Also, cow's protein content was lower in milk from infected animals than in uninfected animals. These results were in agreement with Lehloenya et al., (2008), though disagree with Ullah et al., (2005). In this study, it

appeared that solid nonfat (SNF) in infected animals was higher than SNF in healthy cows. The results are in accordance with the finding of Merin et al., (2004) in cows but disagreed with Hussain et al., (2012). Lactose content indicates a slight increase which supports Hussain et al., (2012) and is not in agreement with Dhillon et al., (2000). The pH was lower in the infected animals' milk. pH results proved (Hussain et al., 2012) and do not underscore (Ogola et al., 2007).

In this study as mentioned above, cheese was stored for 60 days at 6 °C. Madadlou et al., (2006) reported that Iranian white cheese in brine at 5 °C after 50 days changes chemically, biochemically and in opacity. As ripening progressed, moisture and protein contents continuously decreased, whereas their total ash and salt increased. Fat content and pH of cheeses remained stable during ripening (Madadlou et al., 2006). The results of that study about decreasing of moisture and protein content are in agreement with our results, although unchanging lipid and pH of cheese contradicts our findings, as in our study the fat decreased, whereas the level of pH increased. The results of Navidghasemizad et al., (2009) research indicated that the main cause for pH decrease in cheese is starters due to slow growth and activity of starter's lactic acid bacteria of

cheese that made from milk of cattle with mastitis, which are in competition with bacteria in milk, the level of pH increased, probably due to removal starters with the whey.

In our study, moisture and protein contents decreased (Table 3), while ash and salt increased in two groups. It possibly explained by net movement of NaCl molecules from the brine into the cheese texture as a result of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese including dissolved materials such as acids and minerals spread out through the cheese matrix with a flux approximately twice that of NaCl so as to obtain osmotic pressure equilibrium (Guinee et al., 2002), and this decreased the moisture content and increased their salt content of samples as ripening progressed. Lower pH at renneting period decrease the net charge on casein micelles and probably improved the activity of rennet (Guinee et al., 2002), going to greater protein recovery in the curd. This, therefore, increased the protein content of cheese treatments. Banks et al., (1987)

proved that the amount of CP in cheese from normally heat-treated (72°C for 16 s) and acidified milk (pH 5.8) was higher than unacidified milk. In the present study as shown in Table 2 and 3, the high level of pH could have an adverse effect on protein content.

3.3. Cheese opacity

The L^*, a^* and b^* values of manufactured cheeses are presented in Table 4. L^* and b^* values of samples do not differ significantly ($p>0.05$). Ripening time change L^*, a^* and b^* values of cheese treatments, such that L^* value decreased during the ripening period, but a^* and b^* values increased ($p<0.05$).

Regarding the scattering of light by any about opacity, it should be noted that system is related to its heterogeneity (Pastorino et al., 2002). In a solid texture of dairy products such as cheese, light can cross over the superficial layers and is scattered by milk fat globules (Lemay et al., 1994) and whey pockets (Paulson et al., 1998).

Table 4. Instrumental color analysis (mean ± S.D) on the surface of the studied cheeses at days of 0 and 60.

Treatment	Day	L^*	a^*	b^*
A	0	83.25 ± 1.33[a]	-1.33 ± 0.71[a]	1.72 ± 0.79[a]
A	60	75.78 ± 1.65[b]	-2.33 ± 0.83[b]	13.5 ± 1.15[b]
B	0	83.09 ± 1.57[a]	-1.84 ± 0.41[a]	1.78 ± 0.39[a]
B	60	74.35 ± 2.44[b]	-2.67 ± 0.95[b]	14.25 ± 1.41[b]

A= cheese made with healthy milk   B= cheese made with milk containing pathogen
L^*; 0 = black and 100 = white; a^*; -60 = green and +60 = red; b^*; -60 = blue and +60 = yellow.
In each treatment, means within the same column with different superscript letters differ significantly ($p<0.05$).

In this experiment, whey transferred from cheese texture into the brine as ripening

progressed and finally cheese moisture decreased (Table 3). The surface area

occupied by light-scattering centers was therefore decreased. Kaya (2002) reported that the ripening of Gaziantep cheese in the weakest brine caused highest moisture content and the highest L value compared with samples ripened in stronger brines. Color changes due to starter concentration were happened spontaneously to changes in the moisture content of the treatments at a given ripening time, which end in lower light scattering and L values.

3.4. Lipolysis

The ADV of cheeses are presented in the Table 5 and no significant differences are observed between two groups. The acid degree value of cheeses increased significantly during ripening period.

3.5. Proteolysis

High SCC milk usage also negatively influence on flavor, body and texture grades. For example, cheddar cheeses from high SCC milk samples have been indicated a "lipolytic" or "oxidized" flavor and milk for cheddar cheese production have been presented a higher concentration of free fatty acids, which can cause rancidity in dairy products. It has been reported that the flavor or texture defects in mozzarella, Prato or ewes cheeses made with high SCC milk, is due to higher levels of lipolysis or proteolysis in the cheese (Andreatta et al., 2007). These mentioned results were compatible with our data about acid degree value of cheeses which increased significantly during ripening period.

The TN, WSN, NSI and FAAs of cheeses did not show any significant difference between two groups (Table 5). TN and WSN of cheeses decreased significantly during ripening and NSI increased ($p<0.05$). FAA contents showed a non-significant increase during ripening period.

Cheese proteolysis in ripening period is the concerted action of proteolytic enzymes. At the initial stage of ripening, enzymes such as chymosin and plasmin (an indigenous proteinase in milk) affect intact casein in the cheese curd.

Proteinases and peptidases of lactic acid bacteria cause further breakdown of casein, large peptides, and oligopeptides into small peptides and amino acid and transfer to the secondary stage of ripening (Attaie, 2005).

Table 5. Acid degree value, total nitrogen (TN), water soluble nitrogen (WSN), nitrogen solubility index (NSI) and free amino acids (FAAs) of the studied Iranian white cheese in brine.

Treatment	Day	Acid degree value	TN%	WSN%	NSI	FAA(Ab 507 nm)
A[a]	0	1.32±0.28	3.38±0.40	0.05±0.01	1.59±0.50	1.46±0.81
A	60	2.43±0.51*	2.11±0.31*	0.14±0.06*	6.53±2.79*	1.67±0.64
B[b]	0	1.47±0.32	3.25±0.51	0.06±0.02	1.63±0.82	1.42±0.53
B	60	2.78±0.48*	2.14±0.39*	0.14±0.07*	7.11±3.49*	1.63±0.56

*indicates $p<0.05$ between different days of each group
aA= cheese made with healthy milk   B= cheese made with milk containing pathogen.

These changes cause flavor development through the release of free amino acid and texture (Sabbagh et al., 2010), which will increase the pH level. Primary proteolysis of caseins in mastitis milk brings about proteolysis increase of β-caseins during the

early stages of cheese ripening. Also, it causes an accelerated breakdown of αS_1-casein. As a consequence, protein losses in the whey are increased. In present study, a non-significant increase of FAA, a significant decrease of TN and WSN and significant increase of NSI were observed. In other research, cheese made with high SCC milk samples have been shown higher levels of proteolysis regardless the cheese type (Le Maréchal et al., 2011).

As Table 5, the FAAs in both the control and treatment (A, B) were increased after 60 days, and the amount of FAAs in control group was higher than treatment as a result of free AA movement into the brine. As the starter concentration in milk increased, the amount of free tyrosine-tryptophan in cheese increased at a known storage time. The strong explanation for starter cells increase retained at the curd is the higher initial cells used to inoculate the milk or pH decrease at wheeling or both and caused more production of proteinases and peptidases that were excreted into curds and resulted in more breakdown of casein molecules (Attaie, 2005) and larger peptides to smaller peptides and free AA. The lower pH values of the treatments with higher starter may lead to more hydrolysis of casein molecules. However, it may slightly improve the activity of retained rennet at the curd (Watkinson et al., 2001).

4. Conclusions

In this research the results didn't show any difference between physicochemical properties of the manufactured cheeses. The reason of this result is high total bacterial count in milk of Iranian dairy farms. Therefore, In Iran, the incidence of contagious mastitis pathogen is important for safety of dairy products and no for quality of them.

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