



Microbial and Chemical Parameters of Traditional Siahmazgi Cheese Produced in Zanzan Province, Iran

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Abstract | One of the most ancient forms of manufactured food is traditional dairy products, especially cheese. Siahmazgi is an Iranian traditional cheese produced which is getting increasingly popular. In this study, chemical and microbial quality of Siahmazgi cheeses (76 samples) are collected randomly from different regions of Zanzan province was studied. The aim of this study was to evaluate some chemical parameters (pH, acidity, salt) and undesirable microbial populations (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Coliforms*, molds and yeasts). Average results of chemical parameters: pH (4.79±0.27), titratable acidity (0.86±0.22%) and NaCl (4.56±1.2%) were determined. The results indicated that out of the total samples of Siahmazgi cheese in terms of *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts in cheese were 3, 21, 23 and 23 (in term of CFU/g) in samples were not agree with the Iranian national standard (ISIRI), respectively. Also, contamination with *Salmonella* spp. (in term of CFU/25g) was not observed in all analyzed samples. Our findings indicated that in addition to chemical characteristics (especially in terms of NaCl), the most microbiological (especially in case of *Escherichia coli*, *Coliforms*, molds and yeasts) loads of Siahmazgi cheese produced in Zanzan province are not agree with the Iranian National Standard, however continues supervision, sampling and controlling is required during production of their products from microbial and chemical contamination point of view.

Keywords | Siahmazgi cheese, Chemical characteristic, Microbial quality, Traditional dairy, Zanzan province-Iran

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INTRODUCTION

The traditional dairy products, especially cheese, have an important place in Iranian food culture. The amount of cheese production in the United States is 4.8 to 8.6 million tons in Western Europe (Ledenbach & Marshall, 2009). Traditional cheese has got great interest by consumer for its nutritional quality among other cheeses (Blažić et al., 2017) as a result because of the consumers

demand for this type of product, it's necessary to provide hygiene certificate for them. Since the shelf-life span for dairy products such as cheese is depend on their micro floral contamination in the product. Siahmazgi cheese is one of the traditional cheeses in Iran, which is producing from cow's milk with a small amount of common starter, which means that the ripening process is carried out mostly by the natural flora of the milk during the storage for a few months (Fontán et al., 2001).

The origin of this product is belong to rural area in the Siahmazgi village, suburbs of Shaft, Guilan province-Iran (northern Iran) but currently the main province that produces this products is Zanzan province-Iran (northwestern Iran) and according to the health guidelines of Iran Food and Drug Administration (IFDA, 2014), the cheese should be keep in disposable polyethylene packaging for a 3-4 months before distribution in the market. In old days, this product was produced from the mixture of ewe's and goat's milks by kept in sheepskin during ripening. This kind of cheese has got extremely firm texture at the end of repining period with some pea-sized holes on it, yellowish appearance and fermented taste. All these characteristics depend on special storage condition and ripening process (Farahani et al., 2014). The schematic of Siahmazgi cheese producing is shown in Figure 1.

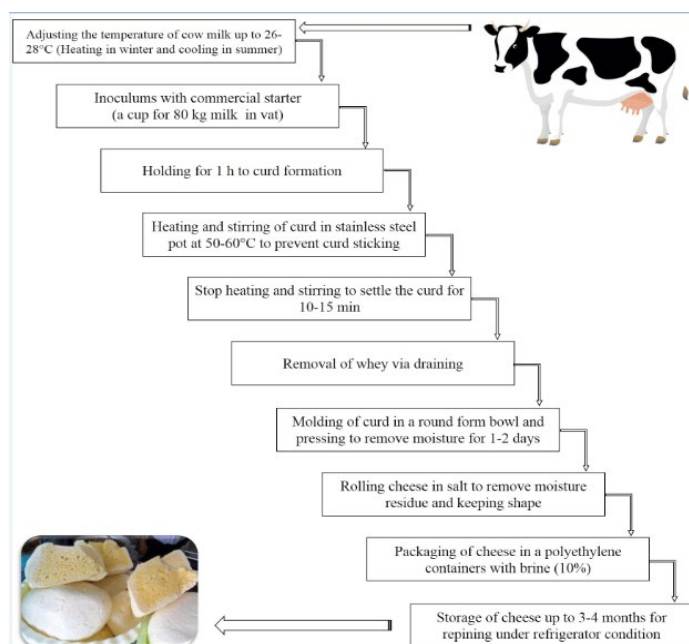


Figure 1: Flowchart of the Siahmazgi cheese making process.

According to the standards of Codex Alimentarius Commission (CAC), this kind of cheese is classified as extra hard and medium fat cheese (Partovi et al., 2015). In other words, high quality, firm texture and special taste have made it extremely favorite among the native consumers especially in Zanzan province and North West of Iran. Although Siahmazgi cheese has been produced for a long time in Iran, but there is no published data on chemical and microbial characterization of this type of traditional cheese.

A factor that determines the status of chemical quality of cheeses were pH, acidity, NaCl content, and the characteristics of contaminating microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Coliforms*, molds and yeasts. A number of researches have indicated

quite far variables to affect deteriorative reactions. It is no doubted that cheeses vary widely in spoilage characteristics. Cheeses which commonly have the highest pH values, along with the lowest salt to moisture ratios, may spoil more quickly. In contrast, aged, ripened cheeses retain their desirable eating qualities for long periods because of their comparatively low pH, low water activity, and low redox potential (Ledenbach & Marshall, 2009).

The objective of this work was studied and evaluated the quality of traditional Siahmazgi cheese produced in Zanzan province. Aspects of the chemical and microbiological parameters of Siahmazgi cheese and their significance with respect to the quality and safety of the final products were assessed in this work.

MATERIALS AND METHODS

SAMPLING

Totally 76 Siahmazgi cheese samples were collected randomly from different regions of Zanzan province-Iran during the years of 2017 to 2018 (Figure 2). The method of choosing a sampling place was based on the highest level of factories and the highest amount of cheese production. According to the Institute of Standards and Industrial Research of Iran (ISIRI) NO.326, at least 100 g of each sample were collected in sterile bag (Persian ZipKeep Co., Tehran, Iran) and kept under adequate refrigeration condition (1-5 °C) until analysis (ISIRI, 2009b). Each sample was divided to microbiological and chemical analyses. All the experiments were carried out in triplicate (Nespolo & Brandelli, 2012).



Figure 2: Map of study area indicating the location of site where Siahmazgi cheeses were collected.

CHEMICAL ANALYSES

The pH value was recorded using a pH-meter (pH-Meter E520, Metrohm Herisau, Switzerland). Briefly, cheese samples (10 g) were homogenized with 10 mL of distilled water and the pH of dispersion was measured with pH-meter according to standard NO.2852 (ISIRI, 2007c). Acidity was determined by titration against a solution of 0.1 N NaOH with 0.1% Phenolphthalein solution as indi-

Table 1: The result of chemical analysis of Siahmazgi cheese products in Zanjan province

Characteristics	N ^a	S.V ^b	Freq & Perc ^c	Min ^d	Max ^e	Mean	S.D ^f
pH	76	$X < 4.8$	64 (84.2%)	4.20	6.50	4.79	0.273
Acidity (% lactic acid)	76	$X > 4.8$	12 (15.8%)				
		$X > 0.8$	72 (94.7%)	0.14	1.90	0.86	0.227
NaCl (% cheese weight)	76	$X < 0.8$	4 (5.3%)				
		$X < 3$	3 (3.9%)	1.70	8.10	4.56	1.228
		$X = 3 - 4.5$	40 (52.6%)				
		$X > 4.5$	33 (43.4%)				

^a Number of sample analyzed; ^b Standard value; ^c Frequency and percent; ^d Minimum; ^e Maximum; ^f Standard deviation

Table 2: The result of microbial parameters of Siahmazgi cheese products in Zanjan province

Microbial Quality	<i>S. aureus</i> (Cfu/g)	<i>E. coli</i> (Cfu/g)	<i>Salmonella spp.</i> (Cfu/25g)	<i>Coliforms</i> (Cfu/g)		Mold & Yeast (Cfu/g)	
N ^a	76	76	76	76		76	
S.V ^b	Positive	Positive	Positive	X<10	X>10	X<10 ²	X>10 ²
F ^c	3	21	0	53	23	53	23

^a Number of sample analyzed; ^b Standard value; ^c Frequency

cator (ISIRI, 2007c). The pH and acidity value were analyzed according to Iranian national standard NO.2852 (milk and milk products - determination of titrable acidity and value pH). Furthermore, cheese samples were analyzed for NaCl content by Volhard method according to standard NO.1809 (ISIRI, 2007a). All chemicals were purchased from MerckKGaA, Darmstadt, Germany.

MICROBIOLOGICAL ANALYSES

Microbiological analyses of Siahmazgi cheese samples were determined after 90 days of ripening. They were analyzed for the detection of the following microorganisms: *Coliforms* (CFU/g) were performed on Violet Red Bile Lactose Agar (VRBL) and another appropriate culture media, according to the Colony-count technique and the most probable number technique on 3-replicate, 3 dilution tube MPN procedure, at 35°C (ISIRI, 2008a, 2009a). Positive *Escherichia coli* (CFU/g) were counted on MPN method: aerobic incubation at 44°C during 18 to 24 h (ISIRI, 2016). *Salmonella* spp. (25 CFU/g) were determined according to the ISIRI4413 standard (ISIRI, 2010). For the detection of *Salmonella* in Siahmazgi cheese, samples prepared with dilution and Lactose broth was used as pre-enrichment medium. The methods of isolation of *Salmonella* spp. can be done by inoculating samples on Tetrathionate broth than subculture by using brilliant green agar as selective enrichment cultures and incubated at 42-43 °C overnight. All suspected colonies were submitted to the standard biochemical reactions which are Triple Sugar Iron (TSI) Agar, Lysine Iron Agar, Indole, Urease, Methyl Red, Lysine Decarboxylase (LIA) and Simmon Citrate utilization to confirm whether they belong to *Salmonella* spp. *Staphylococcus aureus* (CFU/g) was counted on Baird-Parker agar

(BPA): Aerobic incubation at 37°C during 24 h. In case of presumed *S. aureus* colonies, the presence of the coagulase was checked by exposing a 24 h culture (Brain Heart Infusion Agar) to rabbit plasma (ISIRI, 2007b). Molds and yeasts (CFU/g) were counted on yeast extract/dextrose/oxytetracycline/agar/medium and Oxytetracycline hydrochloride solution: Aerobic incubation at 25°C for 5 days (ISIRI, 2008b; Sarbazi et al., 2015). Types of culture media were purchased from MerckKGaA, Darmstadt, Germany. All the microbial experiments were done in duplicate.

STATISTICAL ANALYSES

Statistical analysis of data was performed using SPSS (Version IBM SPSS Statistics 25 for Windows; SPSS Inc.). The data was submitted to the analysis of variance (One-Way ANOVA). Quantitative findings were reported as mean (\pm) standard deviations. All statistical analysis were based on a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

EVALUATION OF CHEMICAL QUALITY

The results of chemical analysis of the 76 cheese samples are shown in Table 1. According to the findings, the average pH of all samples was 4.79 throughout the studied period. The maximum and minimum value of pH was 6.5 and 4.2, respectively. However, quantitative evaluation of pH has indicated that 84.2% (64 samples) was in standard ranges ($X < 4.8$). Determination of cheese acidity was shown that, the mean values of acidity were $0.86 \pm 0.2\%$ and 72 out of 76 samples were within the standard range ($X > 0.8$). The results have shown that, Nacl was 1.7 to

8.1% and the mean content of it was $4.56 \pm 1.2\%$. However the quantitative content of NaCl showed that in Siahmazgi cheese, 33 out of 76 samples (43.4%) were above, 3 samples (3.9%) were below, and 40 samples (42.6%), were within the standard range of Iran ($X = 3 - 4.5$).

The results showed that the average pH values of the cheese were similar to those described by Farahani et al., which work on 18 samples of Siahmazgi cheese produced in Rasht province-Iran (Farahani et al., 2014). In our study when the value of pH was 4.2, we had no microflora found in cheese samples, on the other hand when pH value reached to the 5.1, *Staphylococcus aureus*, *Escherichia coli*, *Coliform*, molds and yeasts were observed in samples. Therefore pH factor playing an important role on presence of microorganisms in cheese samples (Farahani et al., 2014). The results of study have shown a relative increase in level of microorganisms at the end of the storage period of Siahmazgi cheeses. The findings have indicated that, when NaCl and acidity content were in 4.6% and 1.2%, respectively, no microflora had found in cheese samples. But in 1.7% of NaCl and 0.3% of acidity, we observed some microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts in some samples. The final status of the traditional cheese depends on the salt concentration, due to its specific effect on the growth of the lactic acid bacteria, enzyme activity and biochemical relationships during the course of the ripening period (Tayyar, 1995). Generally, proximate compositions include pH, moisture, ash, NaCl, fat, and protein contents of food sample of Siahmazgi cheese were determined in other study by Partovi et al. (2015). The results have indicated that, the pH of the samples was ranged from 4.76 to 5.18 and salt content was between 3.76 to 5.22%. By comparing these reports with the results of our study, we can conclude that the range of pH is similar, but the mean of salt content was a slightly different. Study by Farahani et al. (2014) on the chemical properties of Siahmazgi cheese such as pH, titratable acidity, dry matter, fat, protein, ash, salt content, water soluble nitrogen in total nitrogen, and non protein nitrogen in total nitrogen, have shown significantly increased during ripening ($P < 0.05$). The results indicated that Siahmazgi cheese contained high values (5.65%) of salt content. The rate of spoilage of dairy foods include cheese is slowed by the application of following chemical treatments like as: reducing the pH by fermentation and conversation of lactose to lactic acid, by addition of edible organic acids or other approved preservatives, adding sugar or salt to reduces the water activity (aW), removing of water, packaging under the available oxygen, and freezing process (Ledenbach & Marshall, 2009).

EVALUATION OF MICROBIAL QUALITY

The wide array of available dairy foods challenges the mi-

crobiologist, engineer, and technologist to find the best methods to prevent the contamination with microorganisms, destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Some spoilage microorganisms include Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odors, off-flavors and visible changes in color or texture. Therefore, it can have effects on food safety and quality, nutrition and consumers' acceptance (Ledenbach & Marshall, 2009). The development of the different microbial group counts throughout the manufacture and ripening (after 120 days) of Siahmazgi cheese are shown in Table 2. Totally 76 cheese sample, the values of *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts of samples were 3, 21, 23 and 23 in term of CFU/g, respectively. Although, *Salmonella* spp. (in term of CFU/25g) were not detected in the cheeses manufactured.

According to the results, counts of total *Coliforms*, molds and yeasts were high and possible contamination of raw milk during milking, unrefrigerated storage and transportation condition may occurred (Mirzaei et al., 2008). The major problems that raise the number of bacteria in traditional cheese are the use of contaminated raw milk, unpasteurized, uncontrolled natural fermentation and improve ripening and storage conditions (Şengül, 2006). Study by Albenzio et al. (2001) have reported that the mold and yeast amount had not significantly decline the Turkish white cheese in 105 days of ripening time. The presence of yeasts in cheese during the storage period is due to the persistence to decreased moisture and pH due to the ability to metabolize the acid, as well as the proteolytic and lipolytic activity of these microorganisms. This result was in agreement with our results. The microflora of Serra cheese was monitored during ripening period (35 days) was studied. The results have reported that after 7 day, the numbers of microorganisms reached their maximum and lactic acid bacteria and *Coliforms* were the predominant groups (Macedo et al., 1995), but in our study the mold and yeasts were the dominant group in Siahmazgi cheese samples. Studies of Freitas and Malcata (2000) showed that the presence of *Escherichia coli*, fecal *Coliforms* and total *Coliforms* in Portuguese and Spanish ovine cheeses were extremely variable, ranging from 2 to 8 log CFU/g. When cheese is produced following traditional procedures from raw milk, the environmental microflora plays an important role in fermentation and this is one of the most significant parameters affecting the cheese quality (Broadbent & Steele, 2005). In addition, the biodiversity of bacteria involved in cheese production can be considered as an essential factor for the maintenance of the typical features of traditional cheese products. Recent studies have shown that the indigenous microflora of raw milk influence the biochemical characteristics and flavor of cheeses. Lactic

acid bacteria (LAB) are widely used in food fermentation including dairy products. They are known to produce various antibacterial substances including bacteriocins, which can prevent the growth of various unwanted gram-positive bacteria in the genera *Bacillus spp.*, *Enterococcus spp.*, *Listeria spp.*, *Clostridium spp.* and *Staphylococcus spp.* (Batdorj et al., 2007). Contamination of cheeses is especially susceptible to growth of *Coliforms*, so great care must be taken to monitor the quality of water used in these processes. A high incidence of contamination of mold species is particularly well adapted to the cheese-making environment and can be difficult to eradicate from a production facility. Chemical sanitizers may be rendered ineffective by bio-films leaving viable bacteria to be dislodged into the milk product (Ledenbach & Marshall, 2009). The percentage of compliance or non-compliance results with the Iran national standard values in microbial parameters of Siahmazgi cheese products in Zanjan province are shown in Figure 3. Our findings are indicating that, the percentage of *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts as contaminants in cheese samples were 3.95%, 27.63%, 30.26% and 30.26%, respectively. The results indicated that all of samples were in recommended standard value in *Salmonella spp.* (negative).

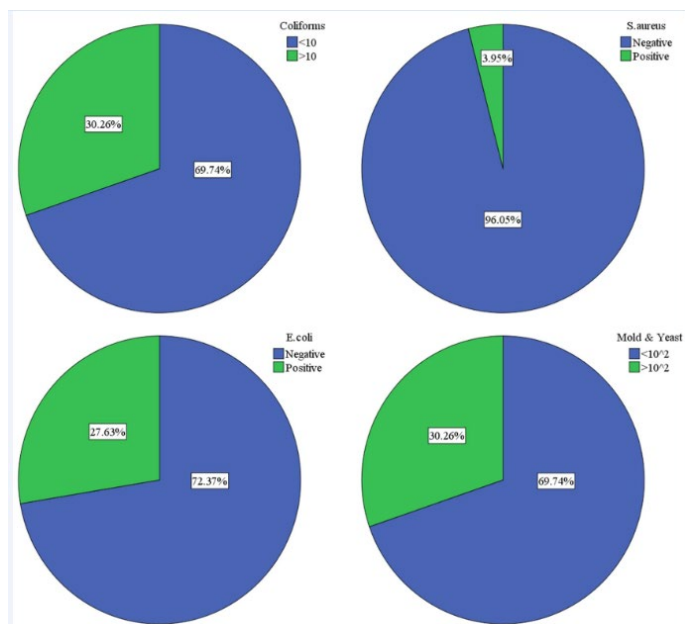


Figure 3: The percentage of microbial contamination of Siahmazgi cheese products in Zanjan province.

Previous studies about microbial contamination of traditional cheese produced in Markazi Province-Iran by Rezaei et al. (2014) have reported that all of the samples had *Coliforms*, *staphylococcus aureus*, mold, and yeast contamination greater than Iranian standard values ($P < 0.001$). They found 34% of *E. coli*, 24% of coagulase-positive staphylococcal and 8% of *Salmonella spp.* contaminations. It was difference microbial population (higher values) than our results.

The assessment of microbiological quality of food service companies revealed that more than 80% of the air in processing areas was not in good hygienic conditions (Andrade et al., 2003). While the introduction of pasteurization process has helped to ensure the safety of dairy products, but progress has been weak in preventing the microbial spoilage of cheese and dairy products. The type of spoilage microorganisms differs widely among dairy foods because of the selective effects of practices followed in production, formulation, processing, packaging, storage, distribution, and handling (Ledenbach & Marshall, 2009). The presences of microorganisms were possibly owing to deficient hygienic conditions maintained during the manufacturing and ripening of the cheese (Fontán et al., 2001). Cheese made from unpasteurized milk and traditional manufacturing procedures may possess a very diverse and rich microflora; the quality of the cheese depends to a great extent on the composition of that microflora (Şengül, 2006). Compared our findings with some researches (Delamare et al., 2012; Levkov et al., 2012; Vasek et al., 2013) have shown that, the traditional cheese products have similar microflora content and mostly it is related to the quality of raw milk, sanitary production facilities, Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP). However, because of Zanjan traditional Siahmazgi cheese produce from unpasteurized milk with less sanitation conditions, our results indicated that microbial and chemical factors of Zanjan traditional Siahmazgi cheese had less compliance with the Iran National Standards values than other dairy products such as pasteurized yoghurt (Mirza Alizadeh et al., 2014), yoghurt drink (Ayran or Doogh) and milk products (Mirza Alizadeh et al., 2017).

CONCLUSIONS

The results of this study showed that the considerable microbiological contamination of traditional Siahmazgi cheese was related to the flowing microorganisms like *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts were observed in cheese samples, and the correlation between the values of pH, salt content, and acidity. According to our findings, the measured values containing chemicals like percent of salt content and acidity affect on presence of *Staphylococcus aureus*, *Escherichia coli*, *Coliforms*, molds and yeasts of Siahmazgi cheese and change these values led to increased microflora. In our study *Salmonella spp.* not detected in Siahmazgi cheese samples at the end of ripening time.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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