

Levels of Some Microbial Contaminants in Domestic Soft Cheese in Governorate of Salahuddin, Iraq

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ABSTRACT

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The level of contamination with microorganisms in domestic soft cheese in the governorate of Salahuddin made mostly by rural families and sold in nearest towns and cities was investigated. Eighty-four random samples of domestic soft cheese commercialized in some towns of the governorate "Tikrit, Samaraa, Tuz and Balad" were collected from various local market places in sterile and cold storing conditions and examined for microbial counts and some chemical tests. The average total plate count, yeasts and molds, psychrotrophic, thermophilic, spore forming bacteria, lipolytic, proteolytic, coliforms and *Staphylococcus aureus* counts calculated as Log₁₀ were: 9.26, 6.08, 5.72, 4.28, 3.72, 6.08, 5.92, 6.11 and 5.93, respectively. In addition, *Salmonellae* was identified only in eight samples out of 44 (15.91%). Chemical analyses revealed no consistency in main contents of the tested cheese samples. The results showed the critical risks of the domestic soft cheese, and suggest inflexible hygienic measures must be followed.

مستويات بعض الملوثات الميكروبية في الجبن الطري المحلي في محافظة صلاح الدين، العراق

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جامعة تكريت / كلية الزراعة / قسم علوم الأغذية

الخلاصة

أجري هذا البحث لدراسة مستوى التلوث بالأحياء المجهرية في الجبن الطري المحلي في محافظة صلاح الدين والذي ينتج معظمه من قبل الأسر الفلاحية ويبيع في المدن والمناطق القريبة. تم جمع 84 عينة عشوائية من الجبن الطري المحلي من بعض مدن المحافظة "تكريت وسامراء وطوز وبلد" من مختلف الأسواق المحلية في ظروف تخزين معقمة وباردة وفحصت مستويات التلوث المايكروبي وأجريت بعض الاختبارات الكيميائية. بلغ متوسط مجموع العد المايكروبي الكلي والخمائر والأعفان وبكتريا الـ psychrotrophic وبكتريا المحبة للحرارة وأعداد البكتريا المكونة للسبورات والبكتريا المحللة للدهون وكذلك المحللة للبروتينات والكوليفورم والمكورات العنقودية الذهبية محسوبة على الأساس اللوغاريتمي (Log₁₀): 9.26 و 6.08 و 5.72 و 4.28 و 3.72 و 6.08 و 5.92 و 6.11 وأخيراً 5.93 على التوالي. وبالإضافة إلى ذلك وجدت بكتريا *Salmonellae* في ثمان عينة فقط من أصل 44 (15.91%). وكشفت التحاليل الكيميائية عدم ثبات نسب المحتويات الرئيسية لعينات الجبن. وأظهرت النتائج المخاطر الحرجة التي يتعرض لها المستهلك عند تناول الجبن الطري المحلي وضرورة إتباع تدابير صحية صارمة لمواجهة المخاطر الصحية.

الكلمات المفتاحية:

الجبن الطري المحلي ،

التلوث المايكروبي

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Introduction:

Soft cheese is an important worldwide foodstuff. In Iraq it is generally consumed within 3-4 weeks after manufacturing. Traditional unripened soft cheese is made from non-pasteurized milk in rural regions and remote villages (Ali *et al.*, 2013).

Raw milk contains about 30% of undesirable micro-organisms in total microbial count, therefore, this problem suggests inflexible hygienic measures must be followed in cheese making (Melkamsew *et al.*, 2012; Pazakova *et al.*, 2001).

Despite improvements in dairy processing, domestic soft cheeses are still very popular. This type of cheese is usually made from raw milk with insufficient hygienic quality in rural regions. Hence, raw milk can be primarily considered the main source of Microbial contamination (García and Díaz, 2011). In addition, worker's hand, packaging, transportation and marketing can be the secondary

cause in poor conditions of the soft cheese. Also, non-hygienic water rather than tap water mostly used in the cleaning of the utensils used in cheese making as well as general daily uses (Swai and Schoonman, 2011; Hill *et al.*, 2012; Mhone *et al.*, 2011; Uyttendaele *et al.*, 2015).

Temelli *et al* (2006) investigated the possible sources of the cheese contamination and found that starter culture was a possible contamination source for coagulase positive staphylococci, enterococci and psychrophilic bacteria, while floor and packaging material were as the contamination source of psychrophilic bacteria.

Although soft cheese is a nutritious food, it may act as a good means for pathogenic microorganisms (Araújo *et al.*, 2002). This risk is not limited to a single region, but it is a dilemma worldwide; Food and Drug Administration, FDA (2005) announced some cheeses made with raw milk are probable source for health risk. The consumption of contaminated cheese accounted for the 0.4% of the total foodborne outbreaks in Europe (European Food Safety Authority “EFSA”, 2008). In fact, during last 35 years, there were about 53 poisoning outbreaks due cheese consumption. Enteropathogenic *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* were among the microbial contaminants. (Fox *et al.*, 2017). Being made from non-pasteurized milk, soft cheeses contain high microbial counts and exceeds beyond the upper limits maintained by rules and regulations practiced in many countries and indicate the inadequate processing conditions of soft cheese.

Enterotoxins of *Staphylococcus aureus* is a common food-borne disease worldwide (Kadariya *et al.*, 2014). Araújo *et al.* (2002) showed that 95.5% of cheese samples in the city of Rio de Janeiro had high levels of faecal coliforms. In addition, the isolation of *Staphylococcus aureus* and serogroups of enteropathogenic *Escherichia coli* (EPEC) suggested the soft cheese commercialized in Rio de Janeiro may affect consumers' health. Cremonesi *et al.* (2007) tested 33 samples of raw milk cheese and found all the samples were positive for *Staphylococcus aureus* contamination. *Escherichia coli* was isolated from 76 samples out of 77 random samples, and 19.48% of isolates were belonged to EPEC serogroup in Kerman, Iran (Najand and Ghanbarpour, 2007). Similarly, 60 samples of Karish cheese in Egypt were examined and *Escherichia coli* were detected in 75% of the samples. In Iraq, Abbar and Kaddar (1991) reported 40.5% of soft cheese samples were contaminated by EPEC strains. Coliform bacteria, *Escherichia coli*, *Staphylococcus aureus* and mold-yeast counts were detected in some dairy products in Kirklareli, Turkey (Çetin *et al.*, 2015). Many works showed inconsistency in the proportions of some components of soft cheese. Abou-Ghorrah (2010) reported that the average percent of fat, humidity and NaCl were 24 ± 0.2 , 48 ± 0.88 and 11.42 ± 0.54 respectively. Meanwhile Haddal (2010) found the values of the same components were: 18.8 ± 0.4 , 54 ± 0.81 and 4.2 ± 0.14 respectively. Al-Manhal (2013) tested ten samples of soft cheese in Basrah, Iraq and found the ranges of the same tests mentioned earlier were 10.54-15.91, 55.33-67.13 and 1.52-4.20 respectively.

This work investigated the level of contamination in marketed domestic soft cheese made mostly in rural regions which lack proper sanitation conditions and without pasteurization of raw milk.

Materials and methods:

General:

Analyses of 48 samples of Iraqi soft cheese made from bovine raw milk were microbiologically quantified in the period between March and June 2010. Forty-eight random samples of domestic soft cheese commercialized in main cities of the governorate; Tikrit, Samaraa, Tuz and Balad were collected from various local market places in sterile and cold storing conditions by crush ice and examined for microbial plate counts within 4-6 hours. Some chemical tests were done to evaluate the general chemical content. Plate dilution method was used for the microbiological analysis of cheeses. Each sample was macerated well according to Duncan *et al.* (2004) with a sterile spatula and needed amount of each sample was weighted carefully in sterile conditions.

Basic dilution (10^{-1}) was obtained by mixing 11 g of the sample and 99 ml of warmed (40 to 45 °C), sterile, 2% sodium citrate solution ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) (Laird *et al.*, 2004), and then followed by homogenization of the sample twice for 2 minutes and left for 30 min before the completion of the work. Cell forming units (cfu) were counted of respective groups of microorganisms in 1 g of cheese.

Determination:

Total microbial count:

Plate Count Agar was used for determine of Total Counts in the samples (Zimbrow, 2003). Up to dilutions of 10^{-5} and 10^{-6} were prepared. Petri dishes were put upside-down in a Laboratory incubator set to $32 \pm 1^\circ\text{C}$ for 48 hours. Plates with 30 to 300 colonies were counted with the aid of colony counter.

Yeasts & molds count:

Potato dextrose agar (PDA) medium was used. Petri-dishes were incubated at 25 °C for 5 days (Freitas *et al.*, 1995).

Psychrotrophic bacteria:

Psychrotrophic microbial count was done on plate count agar. After pouring the culture medium over the inoculum, the petri-dishes were incubated at $7 \pm 1^\circ\text{C}$ for 10 d (Frank and Yousef, 2004).

Thermophilic bacteria:

Thermophilic microbial count was done on plate count agar. After pouring the culture medium over the inoculum, the petri-dishes were incubated at $55 \pm 1^\circ\text{C}$ for 48 hours (Frank and Yousef, 2004).

Spore-forming bacteria:

Tests were done as described in Harrigan and McCance (1976); tested dilutions heated for 15 min at 80 °C and then cooled immediately to 10°C. 1 ml was taken and put in a petri-dish for each dilution and starch milk agar was poured. All petri-dishes were incubated at 32 °C for 72 h.

Lipolytic bacteria:

Lipolytic bacteria count was conducted as described in Harrigan and McCance (1976) on medium consisted of 100 ml of nutrient agar plus 1 g of vegetable oil . Petri dishes were put upside-down in an incubator at 21 °C for 5-7 days. After incubation time, copper sulphate solution (20%) was used to wet the colonies for 5 min. and then washed gently with distilled water. Bluish green colonies were counted.

Proteolytic bacteria:

Proteolytic bacteria count was done as described in Harrigan and McCance (1976) on milk agar (100 ml of nutrient agar plus 10 ml skim milk). Petri dishes were put upside-down in an incubator at 21 °C for 2-3 days. After incubation period, hydrochloric acid solution (1%) was poured onto bacterial growth for one min. Colonies with clear zone were counted.

Coliform bacteria

Coliform bacteria count in cheese samples was done on Violet red bile agar medium. Dilutions of 10^{-3} and 10^{-4} were used to determine Coliform Bacteria. Petri dishes were cultivated upside-down in an incubator at 32 °C for 24 h. colonies with violet colour and 1-2 mm in diameter was counted (Davidson *et al.*, 2004).

Staphylococcus aureus:

Mannitol salt agar was used to count it. The plates were left for 15 min and then incubated upside down at 32 °C for 48 h. Yellow colonies with yellow zones were counted (Power and McCuen, 1988).

Salmonellae:

Salmonella spp. was done for 44 samples and detected in a two-stage enrichment procedure. Twenty-five ml of cheese was pre-enriched in 225 ml of buffered peptone water at 37 °C for 24 h. Ten millilitres of the pre-enrichment sample was then incubated in 100 ml of tetra-thionate broth base at 37°C for 24 h. Enrichments were then streaked onto brilliant-green agar. The selective media were incubated at 37°C for 24 h. Light pink zones with shiny red halo were checked. (Al-Delaimy, 1979)

Chemical tests:

Twenty four samples were tested in order to reveal some contents of the cheese samples that have undergone microbial counts. All chemical tests were conducted according to “Standard Methods for the Examination of Dairy Products” (Hooi *et al.*, 2004).

Statistics:

Data from microbial enumeration were analysed using Complete random design (CRD) software, version 9, 2002 (SAS Institute Inc., Cary, NC). Significance of results was inferred at $\alpha < 0.05$.

Results and Discussion:

Simple sensory evaluation for cheese samples collected were done in order to exclude any sample undergone obvious deterioration in quality by eliminating samples with obvious rancid flavour and bitter taste.

Results of microbial plate counts (shown in logarithmic scale in Figures 1) revealed prevalence of microbial contamination in domestic soft cheese in some regions in Salahuddin governorate, especially in levels of coliform and *Staphylococcus aureus* during the study.

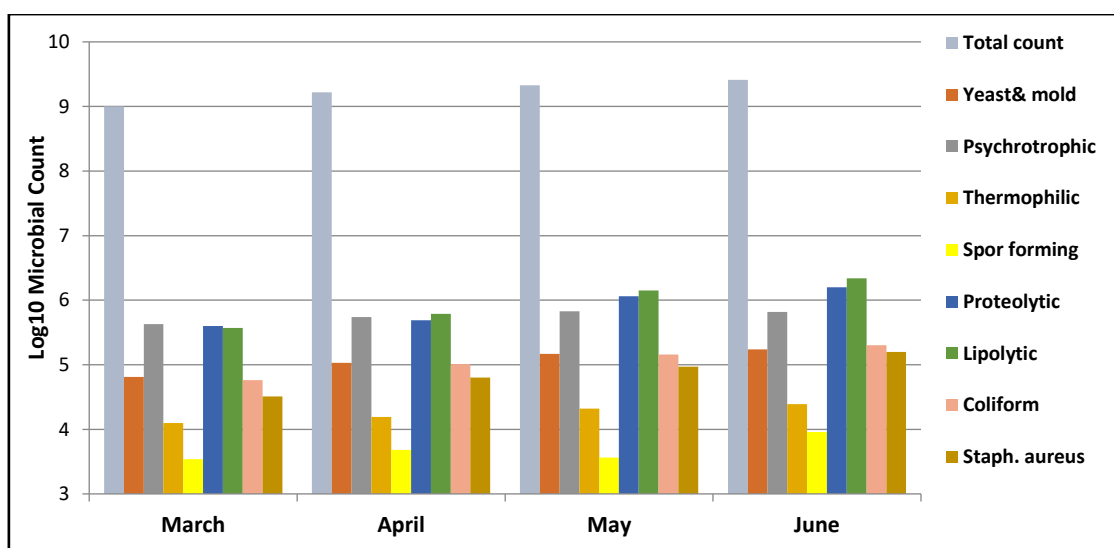


Figure 1 Logarithmic scale for microbial plate counts for the soft cheese samples tested.

Generally, higher levels of contaminants were observed in June which is the beginning of summer in the area. Coliforms and *Staphylococcus aureus* were observed in all the samples. High microbial counts suggests the bad hygienic conditions which domestic cheese is made in homes and villages because raw milk is usually used in making soft cheese without any heat treatment to remove pathogenic microorganisms.

The average total plate count, yeasts & molds, spore forming bacteria, coliforms and *Staphylococcus aureus* counts were 9.26, 6.08, 3.72, 6.11 and 5.93, respectively (Figure 2). *Salmonella* was identified only in eight samples out of 44 (15.91%).

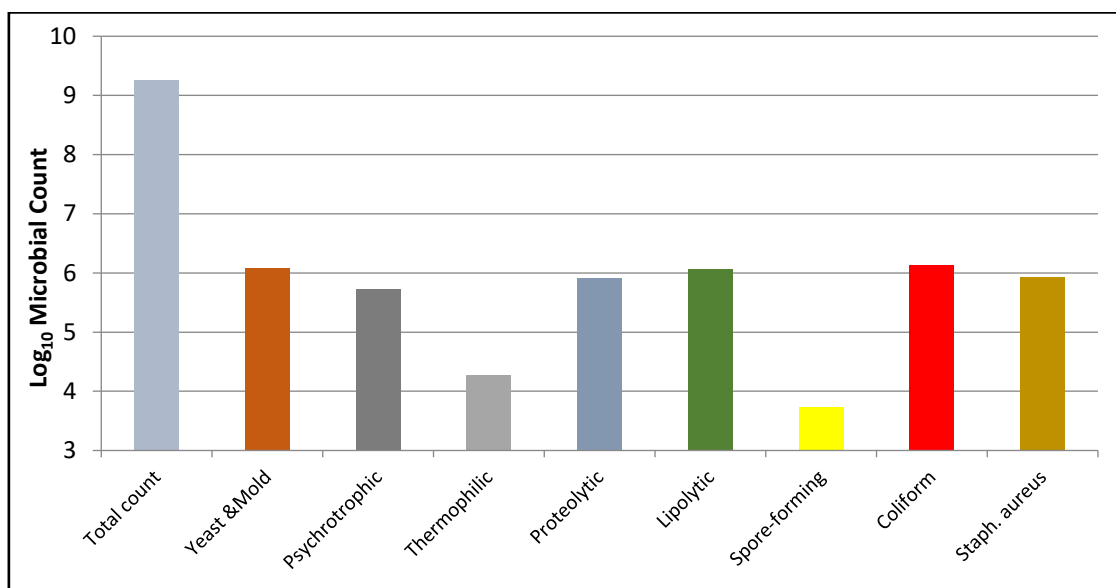


Figure 2 Logarithmic scale for the average of the microbial plate counts during four months of the study

These results are higher than Iraqi Quality Standards (Microbiological Limits in Food IQS: 2270/5) which accepts not more than 10^3 cfu/g of coliform and *Staphylococcus aureus* as well. And *Salmonella* should be absent. Also the results are higher than Egyptian and Indian microbiological criteria for soft cheese (ES: 1008/11/2005 and FSSAI: 2011). Indian standards for total plate count, yeasts & molds, coliforms and *Staphylococcus aureus* counts are 5×10^5 per g, absent in 1g, absent in 0.1 g and absent in 1 g respectively meanwhile Egyptian for yeast, fungi and coliform are 400, 10 and 10 per g respectively.

Results suggest domestic cheese marketed in Salahuddin might be an important vehicle of transmission for pathogens which after consumption of defective cheese can cause serious infections to human body.

Other microorganisms; psychrotrophic and thermophilic bacteria gave good ideas about conditions in which the microbial contaminants could grow in both the cold and warm storage conditions. The average counts of psychrotrophic and thermophilic bacteria recorded as log₁₀ were 5.72 and 4.28, respectively. During the study, both showed the highest counts in June.

On the other hand lipolytic and proteolytic bacteria revealed the probability of fast deterioration of the domestic cheese. The average counts recorded were 6.08 and 5.92 respectively. These bacteria affect the quality of foodstuffs by enhancement rancid flavour and bitter taste which were slightly start to appear in some samples at the time of sensory evaluation.

Chemical tests were conducted to uncover the percentages of some contents of cheese samples during the period of the study (Table 1). The lowest ratio of fat was observed in April (17.30%) while the highest ratio was in June (27.30%). Although humidity affects the growth of microorganisms, which showed lowest level in June (50.10%) while the highest level was in April (60.70%), but the level of microbial growth was higher in June. These results revealed that the levels of humidity was not of great importance in microbial growth. All chemical tests in this work showed insignificant differences ($p \geq 0.05$).

Table 1 Mean and range of some contents of cheese samples tested each month during the period of the study.*

Contents (%)	March			April			May			June		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Min.	Max.		Min.	Max.		Min.	Max.		Min.	Max.
Fat	23.38	19.85	24.57	21.23	17.30	23.10	23.63	22.70	24.40	24.68	21.60	27.30
NaCl	5.80	3.76	6.40	6.08	5.20	7.20	6.13	5.50	6.80	6.08	5.20	7.20
Humidity	57.13	54.70	58.90	58.95	57.60	60.70	53.50	50.70	55.10	52.68	50.10	57.10
Total acidity	0.76	0.45	1.10	0.75	0.54	1.05	1.05	0.82	1.31	1.14	0.78	1.36
pH	6.00	5.50	6.40	5.90	5.60	6.20	5.63	5.30	6.00	5.33	5.10	5.70

* Mean of four samples tested monthly.

The average percent of fat, humidity and NaCl for four months were 23.23 ± 2.43 , 55.56 ± 3.55 and 5.93 ± 0.89 respectively (Table 2). The tests revealed wide range in the contents. Mostly, this maybe as a result of the individuality in raw milk used and the farmers experience in cheese making. The values of fat% were close to those that reported by Haddal (2010) and higher than Abou-Ghorrah's (2010) and Al-Manhal's (2013).

In this study, the percentages of NaCl and Total acidity were higher in comparison with the results reported by Haddal (2010) and Al-Manhal (2013). The differences in chemical composition may be as a result of poor handling of raw milk and traditional methods followed in soft cheese making in rural regions (Bachmann *et al*, 2011).

Table 2 Mean and range of some contents of all samples tested.*

Contents (%)	Mean	Standard deviation	Range	
Fat	23.23	2.43	17.30	27.30
NaCl	5.93	0.89	3.76	7.20
Humidity	55.56	3.55	50.10	60.70
Total acidity	0.92	0.28	0.45	1.36
pH	5.71	0.39	5.10	6.40

* Mean of 16 samples tested.

Conclusion:

Domestic soft cheese made from raw bovine milk by the villagers might be a source of contamination, especially coliforms and *Staphylococcus aureus*. This highlights the need to perform good hygienic practices and effective inspection from production through the delivery chain to the consumer. More studies are essential to uncover these dangerous contaminants with the possibility of accompanying enteric pathogens of which *E. coli* O₁₅₇:H₇ is one of the most serious of known coliform food borne pathogens and it needs to be studied thoroughly.

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