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Microbial and Physicochemical Quality of Kurdish Soft Cheese in Retail Markets in Erbil

ABSTRACT

Dairy products is considered the most complete foodstuff that provide human with most of their vital needs. The aim of this study to evaluate some chemical parameters and bacteriological quality of traditional white soft cheese. Our finding indicates that, the pH ranged between 5.72 to 7.25 with an average of 6.53, moisture percentage ranged between 37.76 % to 54.95 % with an average of 45.03%, fat percentage between 1.2 % to 4.3 % with an average of 2.36 %, protein percentage ranged between 16.3 % to 23.16 % with an average of 19.55 %. Ash percentage ranged between 1.7% to 2.8% with an average of 2.20. Total solid percentage ranged between 25 % to 37.7 % with an average of 31.26 %. Acidity ranged between 1.3 % to 2.5 % with an average of 1.70 %. Salt percentage ranged between 1.4 to 4.3 with an average of 2.89 %. The standard plate count of soft cheese was detected to be the interval of 1.2×10^4 - 4.7×10^6 cfu/g. In the all cheese samples the coliform bacteria count was detected to be between 4.0×10^3 to 8.6×10^5 cfu/g. The yeast and mold count was found to be 9.6×10 to 3.2×10^5 cfu/g. The results found 20 % of *Staphylococcus aureus* contaminations, 13.33 % of *Salmonella spp.* contaminations. Cheese produced in Erbil are not agree with the Iraqi Standard.

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INTRODUCTION:

Milk and cheese have high nutritive value due to its high content of protein, fat, minerals especially calcium, phosphorous, iron, and vitamins (Badawi, 1996: Food composition tables, 1998: and McGee, 2004). These components make it an important source of nutrient required for growth in infants and children and for maintenance of health in adults (Nicolaou *et al*, 2011; Torres-Vitela *et al*, 2012). Cheese is made from milk through souring of milk (Miller *et al*, 1999). The used milk either raw or pasteurized. (Al-Ashmawy *et al*, 1994). The most important reasons that affect total aerobic mesophilic bacteria (TAMP) count is not pasteurizing the milk, not complying with hygienic and presenting cheese in fresh form for consumption without maturation (Hayaloglu and Kirbag, 2007).

The extensive consumption of milk and dairy products makes these foot stuffs targets for potential adulteration with financial gains for unscrupulous producers (Nicolaou *et al*, 2011). Microbiological analysis is critical for the assessment of quality and safety, conformation with standards and specifications and regulatory compliance (Verga, 2007). The presence of *Staphylococcus aureus* in raw milk or its products could occur due to accidental contamination by handlers (Aung *et al*, 2017). In the same regards subclinical mastitis is considered as a source of *Staphylococcus aureus*, that induce food poisoning (Sahu, *et al*, 2014). Enteric pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella spp.* are also the causes of concern

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on public health is relation to the consumption of cheese worldwide (Aoust, 1985; Baylis, 2009, and Kousta *et al*, 2010).

MATERIALS AND METHODS:

A total of 15 samples of local white soft cheese were purchased randomly from markets of Erbil city during the period from January to April 2018. All the samples were labeled and transported quickly as possible in ice box to the laboratory of Food Technology department. In our area white soft cheese was manufactured originally from sheep's, goat's, or cow's milk. Soft cheese was produced from raw unpasteurized milk by adding rennet enzyme at 40 °C and remove the whey immediately after coagulation and subjected to selling. As a control procedure, each sample was examined in parallel triplicates in order to confirm the same density of bacterial colonies each time of testing the same sample.

Bacteriological analysis:

Preparation of samples for bacterial count:

All samples were diluted up to 10^{-6} in 0.1 % sterile peptone water and microbiological analysis were performed. Eleven grams of cheese were added to 99 ml of sterile distilled peptone water in a flask and shaken well to make 10^{-1} dilution. Further dilutions were prepared in sterile distilled peptone water. Prepared samples were serially diluted 10^{-6} in sterile water and used to enumerated bacteria in specific culture medium (Heikal *et al*, 2014).

Standard plate count (SPC): Standard plate count was executed using plate count agar by pour plate method according to Laird *et al*, (2004). This dishes were incubated at $32\pm 1^\circ\text{C}$ for $48\pm 3\text{h}$. The number was calculated and expressed as cfu/gm cheese (Haddad and Yamani, 2017).

Coliforms count: Coliforms count was done as described in the International Standard, ISO 21528-2:2004. Pour plate was applied using violet red bile glucose agar (VRBG). Plates were incubated at 37°C for $24\pm 2\text{h}$ (Haddad and Yamani, 2017).

Mould and yeasts count: Moulds and yeasts were counted on Sabourate Dextrose agar with aerobic incubation at 25°C for 5 days (Haddad and Yamani, 2017).

Enumeration of *S. aureus* and *E. coli*: *S. aureus* and *E. coli* counts were determined on Mannitol salt agar and MacConkey agar plates by surface plated as described by Harrigan and McCance 1990: Struijk *et al*, 2001: Kongo *et al*, 2008. The colony forming units per ml of the original samples were obtained by multiplying the counts obtained with the dilution factor.

Identification of bacteria: In case of presumed *S. aureus* colonies, the presence of the coagulase was checked by exposing a 24 h culture to rabbit plasma. Gram stain, catalase test, and anaerobic utilization of glucose and mannitol were used for more assurance. Positive control of confirmed *S. aureus* ATCC strains was used for comparison. The typical red colonies, being 1-2 mm in diameter, which developed in the Violet Red Bile agar culture media, were Gram negative stained and these bacteria were identified using the other required (ICMSF, 1982). For isolation of *E. coli* (EMB) Eosin Methylene Blue agar was used and the plates were incubated for 24 hours at 37°C . The IMVIC test was applied to the colonies with greenish metallic luster (Alper and Nesrin, 2013).

Preparation of samples for detection of *Salmonella spp.*:

Isolation of *Salmonella spp.* was carried out following the procedures indicated in US Food and Drug Administration (FDA). Briefly 25 gm of samples was preenriched in 225 ml buffered peptone water and incubated at 35°C for 18 h. Next, 0.1 ml of each sample homogenate was enriched into 10 ml of Rappaport-Vassiliadis broth (RV) and incubated at 42°C for 18 h. Then, a loop full of RV culture was streaked on CHROM, Xylose Lysine Deoxycholate (XLD) agar, and Salmonella and Shigella Agar (SSA), and then incubated at 37°C for 1 day. Three to five typical (pink to red with or without dark center on XLD) colonies of *Salmonella spp.* were streaked on nutrient agar slant and incubated at 37°C for 18-24 h for further biochemical identification (Quinee *et al*, 2007: Omar *et al*, 2018).

Biochemical confirmation tests including slant of triple sugar iron agar (TSI), lysine iron agar (LIA), and urea agar, three slants were incubated at 37°C for 24 h (ISO 6785, 2001). Positive control

of confirm *Salmonella enterica* subsp. *enterica* serovar Typhimurium was purchased and used for comparison (Haddad and Yamani, 2017).

Physical analysis:

pH: pH was measured according to AOAC method (2000), with some modification. An amount of 50 ml of distilled water was added to a 10 gm sample and homogenized using a Stomacher so that a potentiometer Orion could be used later.

Moisture: Determining the moisture content was performed according to AOAC method. An amount of 3gm of cheese was placed in an aluminum tray of constant weight, and the drying of the sample was carried out at 105 °C for 12 hours in a forced air convection oven (AOAC, 2000).

Chemical analyses:

Protein determination: The total nitrogen content in white soft cheese (TN %) was determined by Kjeldahl method according to IDE (1993). The total protein content was calculated by multiplying in the (TN %) by 6.38.

Fat determination: It was measured according to AOAC method (1990).

Ash and salt: Ash and salt content of cheese were determined according to Hooi *et al*, (2004).

Acidity: Acidity was determined by titration, 10 gm of white cheese were weighed and placed in a conical flask. Distilled water at 40 °C was added to the sample until the volume in the flask rose to 105 ml. The flask was vigorously agitated and filtered through Whatman No. 43 filter paper. Twenty-five milliliters of the filtrate were pipetted in a 75 ml beaker and then five drops of phenolphthalein indicator were added to the filtrate and titrated against NaOH (0.1 mol equi/L) till a faint pink color that lasted for 30 s was obtained. The titratable acidity was then calculated as described in the official method (AOAC,1990) (Elsamani *et al*, 2014)

Total solid: Total solids content of the samples was determined to AOAC (1990) methods. About 5 ml of each sample and 2 gm of cheese were weighed into three pairs of pre-weighed aluminum dishes. The weight of each sample and the dishes was recorded. The dishes were put in an air oven at 100 °C for 3 h, then placed in a desiccator to cool for 30 min and weighed. Heating, cooling, and weighing were repeated several times to get a find weight of less than 0.5 gm.

Total solids content of each three samples was calculated as follows: -

Total solids (gm/100 gm) = (weight of sample after drying/ weight of sample before drying) x100

RESULTS:

The data in table (1) summarize the chemical composition of local Kurdish white soft cheese. The pH ranged between 5.72-7.25 with an average of 6.53. Moisture percentage ranged between 37.76 %-54.95 % with an average of 45.03 %. Fat percentage between 1.2 %-4.3 % with an average of 2.36 %. Protein percentage ranged between 16.3 % -23.16 % with an average of 19.55 %. Ash percentage ranged between 1.7 %-2.8 % with an average of 2.20 %. Total solid percentage ranged between 25 %-37.7 % with an average of 31.26 %. Acidity (lactic acid) ranged between 1.3 %-2.5 % with an average of 1.70 %. Salt percentage ranged between 1.4-4.3 with an average of 2.89 %. The Iraqi standard (1/693:1988) for chemical properties of cheese for pH is 6.4±0.2, moisture is ≥ 50 %, protein is 13.51%-21.01%, fat is 1±16 %, and salt is 2 %±0.2 as shown in table 1.

In our study, the standard plate count (SPC) in the samples of soft cheese was detected to be the interval of 1.2x10⁴-4.7x10⁶ cfu/g. In the all cheese samples examined in this study the coliform bacteria count was detected to be between 4.0x10³ and 8.6x10⁵ cfu/g. In this study, the yeast and mould count was detected to be at the interval of 9.6x10 and 3.2x10⁵ cfu/g. The results found 20 % of *Staphylococcus aureus* at the interval of 8.3x10³ and 6.0 x 10⁴, 13.33% of *Salmonella spp.* contaminations and all of the samples had *Escherichia coli* contaminations at the interval of 4.1 x 10³ and 9.1 x 10⁶ as shown in table 2 and figures 1-5. The Iraqi standard (2270/5: 2006) for microbial content, the yeast and mould count less than 1x10² cfu/g, for coliform count less than 1x10³ cfu/g, and no *Salmonella spp.*

Table (1): The physical and chemical composition of local Kurdish white soft cheese

No.	pH Iraqi standard 6.4±0.2	Moisture Iraqi standard ≥ 50%	Protein Iraqi standard 13.51%- 21.01%	Fat Iraqi standard 1±16%	Ash mass	Salt Iraqi standard 2%±0.2	Acidity	Total solid
1	6	52.2%	23%	3%	2%	2.3%	1.3%	30.9%
2	7.1	38.12%	17.18%	1.5%	2.5%	1.4%	1.75%	33.4%
3	7	54.95%	21%	3.3%	2%	3%	1.46%	32.2%
4	6.75	39.94%	17.86%	4.3%	2.2%	3.9%	1.4%	26.25%
5	5.72	46.86%	21.26%	1.65%	2%	4%	1.3%	37.7%
6	5.8	38.42%	21%	2.9%	2%	2.4%	2%	33.5%
7	6.58	47.46%	16.58%	3%	2.7%	4.2%	1.3%	33.7%
8	7.25	42.22%	16.3%	2%	2.25%	3.1%	2.5%	30.5%
9	7	44.08%	17.18%	4.2%	2.24%	1.9%	1.76%	31%
10	6.67	47.46%	21%	1.5%	1.7%	2.2%	2%	25%
11	6.16	41.67%	22%	2%	2.23%	2.7%	1.7%	31.4%
12	6.61	37.76%	23.16%	2.3%	2.8%	1.8%	1.6%	33%
13	6.95	46.65%	18%	1.2%	2.2%	3.6%	1.9%	27.8%
14	6.6	44.59%	16.84%	1.4%	2.4%	2.6%	1.6%	34%
15	5.78	53.2%	21%	1.22%	1.87%	4.3%	2%	28.6%
mean	6.53	45.04%	19.55%	2.36	2.20%	2.89%	1.70%	31.26%
Co.	6.69	35.1%	11%	14%	1.5%	3.3%	2%	45%

Table (2): The microbial content of local Kurdish white soft cheese

No.	Standard plate count (SPC)	Coliforms count cfu/g	<i>E. coli</i> cfu/g	<i>S. aureus</i> cfu/g	<i>Salmonella</i> <i>spp.</i>	Yeast and Moulds cfu/g
1	5.9x10 ⁶	6.0 x10 ⁶	1.7 x 10 ⁵	4.8 x 10 ⁴	-	1.4 x10 ³
2	4.7 x10 ⁶	8.1 x10 ⁶	1.3x10 ⁶	-	+	-
3	8.3 x10 ³	1.0 x10 ³	4.6x10 ⁴	-	-	-
4	2.0 x10 ⁴	8.6 x10 ⁴	7.5x10 ⁴	8.3x10 ³	-	2.9 x10
5	7.1 x10 ⁵	1.0 x10 ⁵	4.1x10 ³	-	-	-
6	4.8 x10 ⁶	4.0 x10 ⁴	1.0x10 ⁴	-	-	1.0 x10 ⁴
7	2.5 x10 ⁶	1.0 x10 ⁶	3.4x10 ⁴	-	-	3.2 x10 ⁵
8	1.2 x10 ⁴	4.0 x10 ³	1.2x10 ⁴	-	-	-
9	3.9 x10 ⁶	1.0 x10 ⁵	8.5x10 ³	-	-	-
10	9.4 x10 ⁴	1.0 x10 ³	5.4x10 ⁴	-	-	-
11	3.0 x10 ⁴	6.0 x10 ⁴	6.1x10 ⁴	-	-	-
12	7.8 x10 ⁵	1.6 x10 ⁵	6.7x10 ³	-	-	-
13	9.8 x10 ⁶	8.0 x10 ⁶	1.0x10 ⁶	6.0x10 ⁴	+	4.6 x10 ²
14	1.2 x10 ⁶	9.9 x10 ⁶	9.1x10 ⁶	-	-	1.5 x10 ²
15	4.3 x10 ⁶	4.5 x10 ⁵	6.5x10 ⁴	-	-	9.6 x10
Co.	1.0 x10 ⁵	5.9 x10 ⁴	1 x10 ³	1 x10 ³	-	1 x10 ²

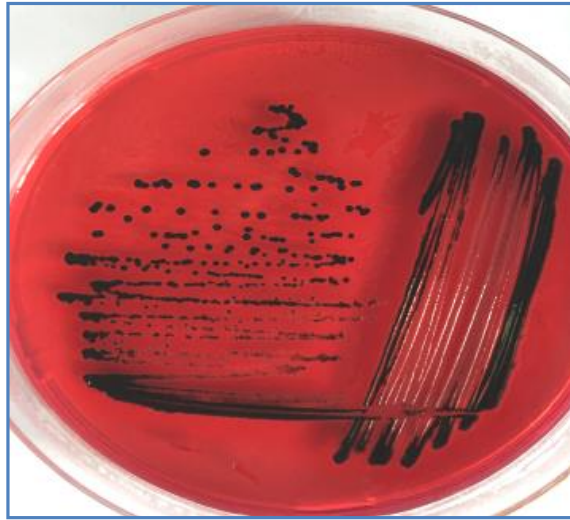


Figure (1): *Salmonella* Typhimurium on Xylose Lysine Deoxycholate (XLD) agar

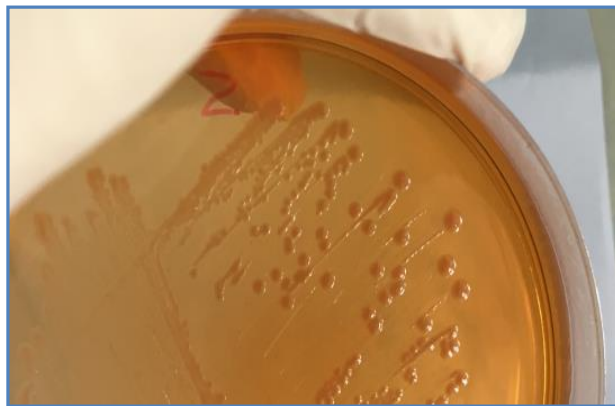


Figure (2): *Salmonella* Typhimurium on MacConkey agar

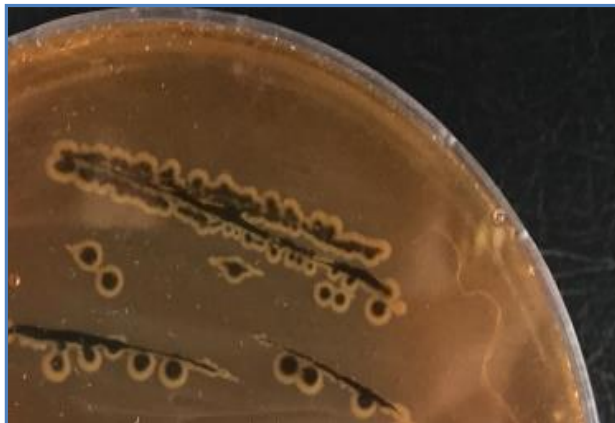


Figure (3): *Salmonella* Typhimurium on Salmonella and Shigella Agar (SSA)

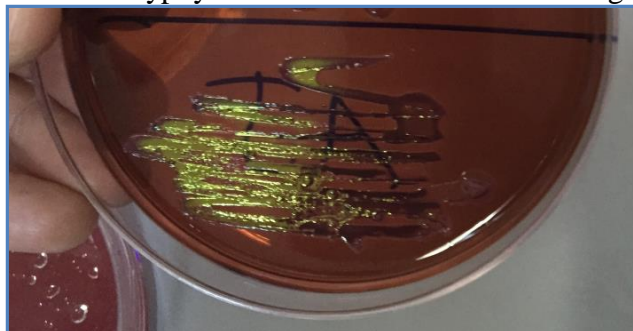


Figure (4): *E. coli* on Eosin Methylene Blue agar (EMB).



Figure (5): *Staphylococcus aureus* on Mannitol Salt Agar

DISCUSSION:

Our result show that the average of pH, acidity, moisture, salt, ash, protein and fat were 6.53, 1.70%, 45.04%, 2.89%, 2.20%, 19.55%, and 2.36% respectively. Haddad and Yamani (2017) they reported that the average of pH, acidity, moisture, salt, and ash were 6.0, 0.53%, 56.5%, 9.4%, and 9.5% respectively. Al-Manhal, (2013) reported that the average of pH (4.886), moisture (63.05%), salt (2.555%), ash (2.852%), protein (17.58%), and fat (13.31%).

The high standard plate count of the cheese samples reflected the general unhygienic conditions used during production and storage (Tannous, 1991; Haddad and Yamani, 2017).

Coliforms counts were generally high and were in most samples unacceptable. Presence of coliforms in food samples generally indicates direct or indirect fecal contamination of the milk (Quinto and Cepeda, 1997) or the product during processing, handling, and distribution, and thus the possibility of having pathogenic bacteria, virus, or protozoa of fecal origin in the food (Tannous, 1991; Haddad and Yamani, 2017).

Some spoilage microorganism 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 consumer's acceptance (Ledenbach and Marshall, 2009). Yeast and molds count in cheese are used as an index of the proper sanitation quality. Moreover, some species constitute a public health hazard due to production of mycotoxin (Rippon, 1982).

The *S. aureus* contamination presumably coming from the hands of the cheese-sellers. However, cows may excrete *S. aureus* from the udder, often without clinical evidence of mastitis (Vaishnavi *et al*, 2001). *S. aureus* may be main cause of several food intoxication outbreaks for their production of heat stable enterotoxin (ICMSF,1996). The hazards associated with the presence of *S. aureus* in food are essentially related to ingestion of enterotoxin previously released by this organism (Adams and Moss, 1995).

The CHROM agar Salmonella detected Salmonella as mauve colonies at 18 to 24 h of incubation, which other members of the family Enterobacteriaceae appearing as blue or uncolored colonies (Maddocks *et al*, 2002). This technique based on production substrate material for specific microorganism enzyme, according to the produced color the microorganism can be identified easily (Manafi *et al*, 2005). An important Irish soft unpasteurized cow's milk cheese was the reason for an outbreak of *Salmonella Dublin* infection that occurred in England and Wales (Maguire *et al*, 1992). Also an outbreak of *Salmonella enterica* serotype Typhimurium infection occurred in France due to presence of *Salmonella typhimurium* in unpasteurized soft cheese, and they considered this soft cheese an effective vehicle of *S. typhimurium* transmission (De Valk *et al*, 2000). Al-Manhal, 2013, reported that the prevalence of *S. typhimurium* was 40 %.

Escherichia coli in milk and milk products is an indication of direct or indirect fecal contamination (Morgan, 1978). The highest number of *E. coli* in this study could be related to low quality of unpasteurized milk used, unhygienic conditions during production, manufacturing, and storage (Moraes *et al.*, 2009). The primitive methods used for manufacturing cheese from unpasteurized raw milk with the absence of standard hygienic measures afford the pathway for its contamination with different food borne pathogen and subsequently hazard to consumers (Brooks *et al.*, 2012).

CONCLUSION:

The high microbial content of the soft cheese samples reflects the poor general hygiene conditions during production and storage of milk and cheese, lack of refrigeration and absence of steps such as heat treatment to eliminate microorganisms. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving should be taken. The results obtained in the research demonstrated that the hygienic quality of fresh white cheese sold in Erbil is low and does not have enough assurance in terms of public health. Measures to control the quality of the raw material, environmental and hygienic conditions during preparation and serving should be taken, markets and processing should be periodically inspected by specialists. In addition to that, pasteurized milk should be used for the manufacturing of local soft white cheese with its preservation inside the brine.

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الجودة الميكروبية والفيزيوكيميائية للجبين الطري الكردي المتداول في أسواق أربيل

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المستخلص

تعتبر منتجات الألبان المواد الغذائية الأكثر اكتمالا التي تزود الإنسان بمعظم احتياجاته الحيوية. تهدف هذه الدراسة إلى تقييم بعض العوامل الكيميائية والجودة البكتريولوجية للجبين الطري الأبيض التقليدي. تشير النتائج إلى أن قيم الرقم الهيدروجيني تراوحت بين 5.72-7.25 بمعدل 6.53 ، تراوحت نسبة الرطوبة بين 37.76 % - 54.95 % بمعدل 45.03 % ، ونسبة الدهون بين 1.2 % - 4.3 بمعدل 2.36 % ، بروتين تراوحت النسبة بين 16.3% - 23.16 % بمعدل 19.55 % . تراوحت نسبة الرماد بين 1.7 % - 2.8 % بمعدل 2.20 . تراوحت النسبة المئوية للمواد الصلبة بين 25 % - 37.7 % بمعدل 31.26 % . تراوحت الحموضة بين 1.3 % - 2.5 % بمعدل 1.70 % . تراوحت نسبة الملح بين 1.4 - 4.3 بمعدل 2.89 % . تم الكشف عن العدد الكلي للبكتريا الهوائية للجبين الطري ليكون المدى من (1.2×10^4) - (4.7×10^6) وحدة تكوين المستعمرة/غم في جميع عينات الجبن ، في حين تراوح عدد بكتريا القولون بين $(10^3 \times 4.0)$ و $(10^5 \times 8.6)$ وحدة تكوين المستعمرة /غم أما تم اكتشاف اعداد الخمائر والاعفان في المدى بين (10×9.6) - (10×3.2) وحدة تكوين المستعمرة/غم. أظهرت النتائج ان 20 % من العينات كانت ملوثة بالمكورات العنقودية الذهبية، 13.33 % من العينات اظهرت تواجد بكتريا السالمونيلا، وعليه فان الجبن الطري المنتج في أربيل غير مطابق مع المواصفة القياسية العراقية.

الكلمات المفتاحية: الجبن الطري الكردي، التلوث المايكروبي، والمحتوى الكيميائي.