

## Determination of Chemical Composition and Microbial contamination of Cow's , Buffalo's Milk and Some Dairy Products from Four Different Regions of Basrah, Iraq

Najla Hussien Al -Garory<sup>1</sup>, Raghad Saad Al Musa<sup>1\*</sup>, Zainab Abd Ali<sup>1</sup>, and Alaa gazi al-hashimi<sup>1</sup>

<sup>1</sup>Department of Food Sciences, College of Agriculture, University of Basrah, Iraq.

\*Correspondence author: e-mail: [raghad.saad@uobasrah.edu.iq](mailto:raghad.saad@uobasrah.edu.iq)

### Abstract

Ninety samples of cow and buffalo's milk besides their products (soft white cheese, yogurt, and cream), which were collected from four different regions in Basrah city (Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair), were investigated. The process of estimating milk components such as moisture, fat, protein, lactose, and ash was conducted. Estimate the total counts of *Staphylococcus aureus* and *E. coli* bacteria. It was observed that the highest total logarithmic number of bacteria was in a cow's milk and manufactured products, such as cheese, yogurt, and local cream, reaching 6.082, 9.049, 9.378, and 6.324 CFU/ml, respectively. At Al-Karma and Al-Zubair, the buffalo milk samples were 5.186, 7.328, 8.895, and 5.616 CFU/ml at Al-Ashar, Abu Al-Khasib, and Al-Zubair. The Al-Zubair region recorded the highest logarithmic number of *Staphylococcus aureus* bacteria in cow's milk (2.681 CFU/ml). The Al-Ashar region recorded the highest logarithmic count of this bacterium in buffalo milk (2.361 CFU/ml). The highest numbers of *Staphylococcus aureus* bacteria in soft white cheese made from cow milk (3.363 CFU/ml) and *E. coli* bacteria (4.146 CFU/ml) were in the areas of Al-Zubair and Al-Ashar, The highest number of both types of bacteria appeared in the Al-Zubair area for yogurt made from cow's milk 3.934CFU/ml the highest number of both types of bacteria, respectively, in cow's milk cream (2.544 CFU/ml) is in Al-Zubair,. Statistical analysis showed that the differences between all different regions were statistically significant ( $P < 0.05$ ) .

**Keywords:** milk products, chemical content, pathogens, Safety.

### Introduction

Humans have used milk and its products for thousands of years as an important nutritional and therapeutic source, in addition to its quality. Which depends mainly on its chemical content and microbial cells. With the beginning of the emergence of agriculture, farmers began to ferment milk and make new products from milk, such as cheese, yogurt, and others. Studies related to healthy and functional foods have developed, and knowledge of the microorganisms found in these products has increased because they produce compounds with biological benefits [1]. Lactic acid bacteria found in

fermented dairy products are beneficial bacteria in milk and milk products. Some species from the *Bacillus*, *Bifidobacterium*, and *Lactobacillus* families are considered probiotics that produce bioactive compounds that are beneficial to human health [2]. Lactic acid bacteria produce enzymes that degrade milk components, as they are the powerhouses of the metabolic system because they stimulate biological and chemical reactions faster than in their absence, which improves the nature of their fermented products [3]. Scientific evidence has proven that milk after milking is contaminated with microorganisms that arise from various causes and are transmitted to milk, such as mastitis or pathogens transmitted from

infected animals, from the surrounding environment, milking equipment, milkers, improper transportation or storage, and failure to adhere to hygiene conditions, which increases the growth rate of pathogenic and spoilage bacteria [4]. [5] stated that the total number of bacteria and the numbers of *Escherichia coli* and *Staphylococcus aureus* bacteria are largely associated with a lack of attention to hygiene in animal shelters, unsanitary practices, and low levels of training for workers and require raising awareness among farmers about the importance of following hygiene conditions. Producing milk of high quality and health safety represents a major challenge in the dairy sector, prompting researchers to take quality control measures to reduce contamination. When contaminated microorganisms reach milk and its products, they grow and produce enzymes that decompose the milk components, leading to a deterioration in its quality [6]. The process of monitoring the quality of milk is carried out by meeting safety requirements by receiving milk of high quality with the lowest number of microbial cells, cooling it upon receipt from farmers, and conducting several tests to confirm the microbial content. To do so, use Resazurin purple dye, which turns pink when exposed to oxidation and reduction reactions caused by microorganisms [7]. The positive development of the dairy products market in the world is the development of innovative technology to manufacture safer, higher-quality, and healthier products. One of these techniques is thermal techniques to treat raw milk before manufacturing it, such as regular pasteurization, as well as non-thermal technologies that reduce time, effort, and costs. Pulsed electrical, high-pressure, ultrasound, pulsed light, and cold plasma treatments inactivate microorganisms that cause spoilage and diseases in milk and its products [8]. This study aims to evaluate the microbial quality and physicochemical

properties of milk and its products, which are soft white cheese, yogurt, and local cream, manufactured from cow and buffalo milk in the local market for several areas in Basra Governorate in Iraq: Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair.

## Material and Methods

The research was conducted in the laboratories of the Food Sciences Department, College of Agriculture, University of Basrah. Samples of cow and buffalo milk and dairy products manufactured from the two types of milk mentioned were collected, namely soft white cheese, yogurt, and cream, with sterile glass containers from different areas of Basra Governorate, namely Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair. The samples were cooled to 4°C, and tests were conducted on the milk samples within 3 hours of milking. After converting soft white cheese, yogurt, and local cream into an emulsion, 90 ml of sterilized sodium citrate buffer was added to 10 g of the samples and mixed with an electric mixer for 5 minutes.

## Physicochemical tests

The pH was estimated using a German-made Sartorius pH meter, and the acidity was determined by testing with 0.1 N NaOH with a phenolphthalein indicator. Protein percentages were estimated by the Kjeldahl method. The percentage of fat was determined by the Gerber method, moisture by drying, ash by burning, and lactose using the difference method between the components, according to the method followed by [9].

## Microbial tests

The total bacterial count was carried out using nutrient agar medium prepared by Oxoid Company. *Staphylococcus aureus* bacteria were isolated using Mannitol Salt Agar medium prepared by Hi-Media. *Escherichia coli* bacteria were determined in MacConkey Agar medium prepared

from Holland, according to the recommendations of the manufacturer of milk and milk products, and samples were taken from different areas of Basra Governorate by the pouring method. The samples were stained with Gram stain for diagnosis according to the method of [5].

### Statistical analysis

The results were analyzed statistically using CRD, and the analysis table ANOVA and the LSD test were used to calculate the significant differences between the means at a probability level of 0.05 using the Genstat program [10].

### Results and discussion

#### Physicochemical structure

Table 1 shows the pH values and percentages of acidity, moisture, fat, protein, lactose, and ash for cow and buffalo milk and the products manufactured from them, which are soft white cheese, yogurt, and local cream taken from different areas of Basra Governorate, such as Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair. The results of the statistical analysis of cow and buffalo milk parameters indicate that there are significant differences ( $P \leq 0.05$ ) for moisture, fat, protein, lactose, ash, and pH. There were no differences in acidity for samples of soft white cheese made from the two types of milk mentioned. There were no significant differences for the values of moisture, protein, and acidity, and there were differences for fat, lactose, ash, and pH. As for the curd treatments, statistically significant differences were observed for moisture, fat, protein, lactose, and ash and it was noted that statistically significant differences appeared in the local cream parameters for moisture, fat, protein, and ash. There were no differences in the values of lactose, pH, and acidity taken from the Four mentioned areas of Basra governorate. It was observed that the moisture was high in milk compared to its products, and its highest value was 87.41%

in cow's milk taken from the Al-Zubair region, which is higher than in buffalo milk, 85.56% in Al-Ashar. Moisture in milk and milk products is the most important factor affecting the microbial load; products with high moisture content are a suitable environment for the growth of microorganisms, especially pathogenic ones. Lactic acid bacteria work to reduce moisture in dairy products compared to raw milk [9]. Attribute the decrease in moisture in buffalo milk to the high percentage of total solids in it, which depends largely on the genetic factor of the buffalo [11]. High moisture leads to a dilution of the proportions of other components present in the milk; they were fat, protein, lactose, and ash. It was also noted that the percentage of protein in local cream was low. The lowest percentage was 2.05% in cow's milk cream in Al-Zubair. The highest percentage of fat for cow and buffalo milk cream, respectively, was 45.17 and 45.20% in Al-Zubair, while a high percentage of protein and low fat was observed in yogurt and soft white cheese. The highest percentage of protein in yogurt for both products made from cow and buffalo milk, respectively, reached 10.14%, and 11.15% in Abu Al Khasib. It was lower in soft white cheese, 16.80% in Al-Ashar and 17.71% in Abu Al-Khasib. The proportions of the ingredients varied according to the regions from which they were taken. The decrease in the percentage of soluble solids is due to the decomposition of protein and lactose, which leads to an increase in the acidity of the product [12]. Lactic acid bacteria benefit from amino acids, peptides, proteins, and nitrogen sources, as well as carbohydrates and fatty acids as a source of energy. The proportions of components in milk products decrease after fermentation [9]. pH value increased, the acidity of both types of milk, soft white cheese, yogurt, and cream decreased. The acidity of milk and its products

manufactured from buffalo's milk was higher than that of milk and its products manufactured from cow's milk. The highest value of acidity in milk and its products manufactured from the aforementioned cow's milk was 0.19, 0.33, 1.12, and 0.23%, respectively. Buffalo milk samples of 0.23, 0.40, 1.15, and 0.25% were superior to curd with the

highest percentage of acidity. The result of adding starter culture and fermentation, followed by soft white cheese and local cream, which tests showed to be less acidic. The pH and acidity values have a positive relationship with the ripening process. The acidity in dairy products is higher than that of raw milk due to the fermentation and ripening process [13].

**Table (1):** Physicochemical composition of cow and buffalo milk and products manufactured from it taken from local markets in Basra Governorate.

Products	Regions	Chemical composition						
		Moisture %	Lipid%	Protein%	Lactose %	Ash%	pH	Acidity %
Milk								
Cow's milk	Al-Ashar	87.31	4.34	3.97	3.79	0.59	6.80	0.15
	Karma Ali	87.20	3.72	3.56	4.92	0.60	6.78	0.16
	Abu Al-Khasib	86.96	3.96	3.61	4.82	0.65	6.70	0.18
	Al-Zubair	87.41	3.95	3.65	4.62	0.57	6.65	0.19
Buffalo's milk	Al-Ashar	85.56	4.45	4.42	4.85	0.72	6.65	0.19
	Karma Ali	83.40	6.71	3.86	5.27	0.76	6.62	0.20
	Abu Al-Khasib	83.15	6.66	4.22	5.18	0.79	6.56	0.22
	Al-Zubair	82.76	6.96	4.56	5.72	0.80	6.45	0.23
L.S.D		0.0173	0.0173	0.0173	0.0081	0.0173	0.0916	0.0149
Soft white cheese								
Cow's cheese	Al-Ashar	62.00	16.22	16.80	3.42	1.56	6.20	0.30
	Karma Ali	62.41	16.24	16.75	3.06	1.54	6.40	0.25
	Abu Al-Khasib	62.21	16.20	16.76	3.28	1.55	6.34	0.27
	Al-	61.77	16.38	16.55	3.56	1.74	6.23	0.33

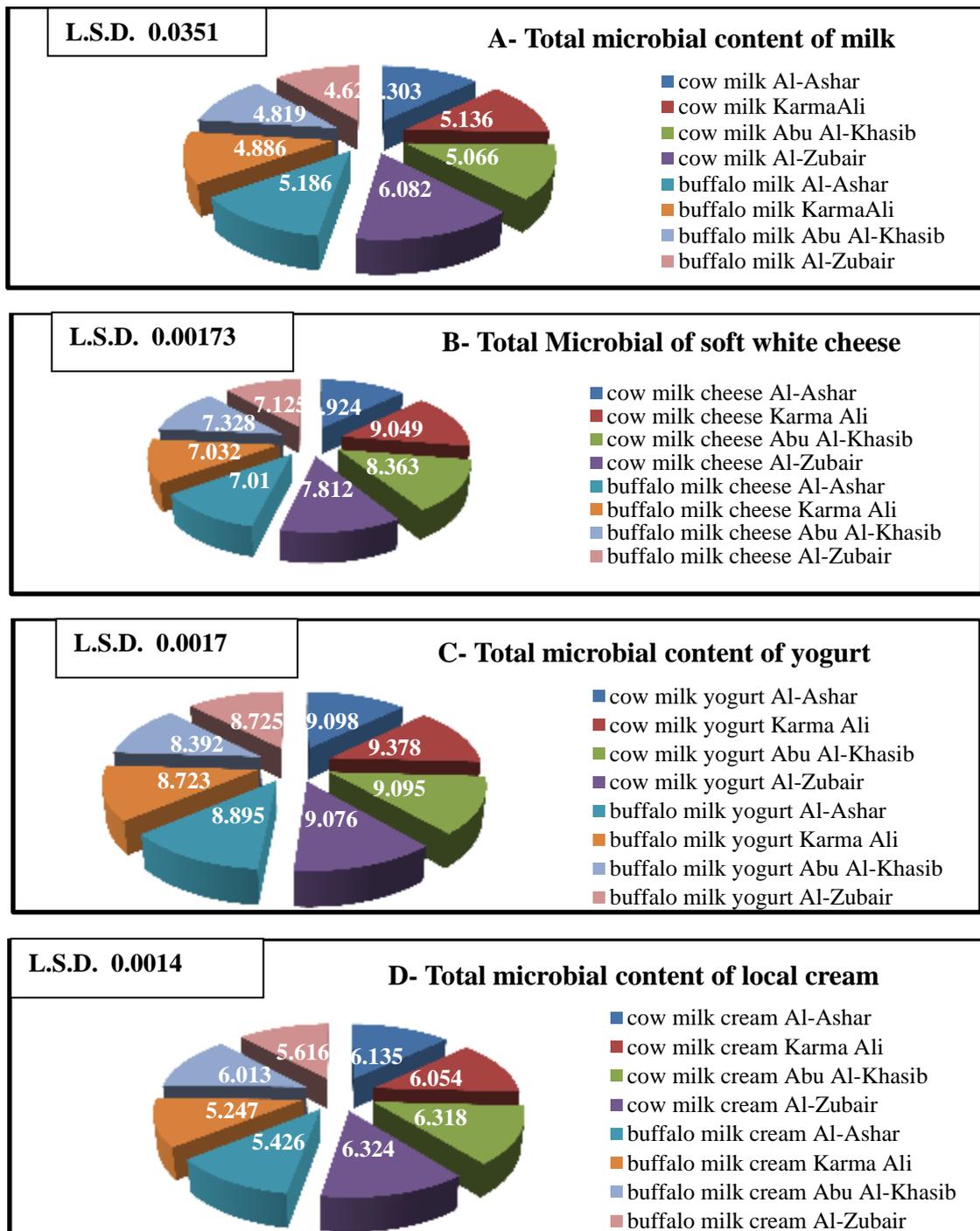
	Zubair							
Buffalo's cheese	Al-Ashar	57.27	19.17	17.50	4.24	1.82	6.22	0.32
	Karma Ali	57.30	18.90	17.55	4.45	1.80	6.12	0.40
	Abu Al- Khasib	57.69	18.80	17.71	4.50	1.30	6.20	0.35
	Al- Zubair	57.65	18.78	17.70	4.55	1.32	6.30	0.29
L.S.D		0.6122	0.0173	0.7121	0.0158	0.0158	0.0149	0.0158
Yogurt								
Cows yogurt	Al-Ashar	79.89	4.70	10.01	4.49	0.91	4.45	1.00
	Karma Ali	79.90	4.65	10.10	4.45	0.90	4.40	1.10
	Abu Al- Khasib	79.89	4.63	10.14	4.43	0.91	4.38	1.12
	Al- Zubair	79.85	4.62	10.12	4.48	0.93	4.43	1.05
Buffalo's yogurt	Al-Ashar	78.19	5.31	11.05	4.50	0.95	4.40	1.10
	Karma Ali	78.15	5.25	11.10	4.54	0.96	4.38	1.12
	Abu Al- Khasib	78.12	5.29	11.15	4.46	0.98	4.36	1.15
	Al- Zubair	78.15	5.25	11.11	4.53	0.96	4.39	1.11
L.S.D		0.0165	0.0158	0.0158	0.0165	0.0165	0.348	0.0136
Local cream								
Cow's cream	Al-Ashar	48.19	45.14	2.10	3.00	1.57	5.91	0.20
	Karma Ali	48.17	45.15	2.09	3.01	1.58	5.93	0.23
	Abu Al- Khasib	48.20	45.10	2.10	3.05	1.55	5.91	0.20
	Al- Zubair	48.25	45.17	2.05	3.00	1.53	5.92	0.21
Buffalo's	Al-Ashar	47.82	45.19	2.15	3.22	1.62	6.94	0.23

cream	Karma Ali	47.73	45.20	2.21	3.23	1.63	5.92	0.25
	Abu Al-Khasib	47.88	45.17	2.14	3.21	1.60	5.95	0.22
	Al-Zubair	47.80	45.20	2.16	3.22	1.62	5.93	0.24
L.S.D		0.0193	0.0165	0.0141	0.8660	0.0158	0.0173	0.0149

### Total microbial content

Figure 1 indicates the total logarithmic numbers of bacteria present in cow and buffalo milk and products manufactured from it, which are soft white cheese, yogurt, and local cream taken from local markets at Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair in Basra Governorate. The results of the statistical analysis showed that there were significant differences ( $P \leq 0.05$ ) in the total bacterial counts for cow's and buffalo's milk and the local yogurt and cream produced from them; there were no significant differences in the number of bacteria for soft white cheese. Figure 1-A shows that the highest total logarithmic number of bacteria in cow's milk was in the Al-Zubair area (6.082 CFU/ml). In buffalo milk, the highest total number of bacteria (5.186 CFU/ml) is in the Al-Ashar area, and the lowest (4.628 CFU/ml) is in Al-Zubair. The reason for the higher bacterial numbers in cow's milk than in buffalo milk may be attributed to the nature of buffalo milk and its components, especially the high percentage of fat, which may affect the growth of some microorganisms [14]. High bacterial counts are also related to moisture content; the numbers increase with high moisture content in the milk or a lack of attention to hygiene. Figure 1-B shows an increase in the total logarithmic numbers of bacteria in soft white cheese due to the addition of lactic acid bacteria when manufacturing cheese to the total numbers of bacteria. The numbers in

cheese made from cow's milk were higher than in buffalo milk; the highest numbers for both types reached 9.049 and 7.328 CFU/ml, respectively, in the areas of Karma Ali and Abu Al-Khasib. Figure 1-C indicates the total logarithmic numbers of bacteria in yogurt; their increase was also observed due to the activity of lactic acid bacteria added as a starter. It was noted that the areas of Karma Ali and Al-Ashar recorded the highest total number of bacteria for samples made from cow and buffalo milk (9.378 and 8.895 CFU/ml), respectively. The total logarithmic numbers decreased in local cream, as shown in Figure 1-D, compared to cheese and yogurt. Due to the high percentage of fat, which negatively affects the microbial content, it reduces the water content that encourages bacterial growth, as the highest numbers for both cow milk samples (6,324 CFU/ml) in the areas of Al-Zubair and Abu Al-Khasib, respectively, are due to the high microbial contamination and total numbers of bacteria in dairy products, due to the primitive processes in production and the lack of awareness of the producer, as well as the presence of part of the milk with the product, considered a suitable environment for the growth of various microorganisms. In addition to the inefficiency of heat treatments on milk during manufacturing or marketing, which lack sanitary conditions, exposing cheese, cream, and other products to the air, consequently, the number of bacteria increases [14].



**Figure (1):** The total microbial content of cow and buffalo milk and products manufactured from it taken from local markets in Basra Governorate.

**Count of *Staphylococcus aureus* and *Escherichia coli* bacteria**

Figure 2 shows the numbers of *Staphylococcus aureus* and *E. coli* bacteria in the milk of cows and buffaloes, taken from local markets in the areas of Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-

Zubair in Basra Governorate. The results of the statistical analysis showed ( $P \leq 0.05$ ) that there were statistically significant differences in the numbers of *Staphylococcus aureus* bacteria in the milk of cows and buffalo, soft white cheese, yogurt, and local cream manufactured from

the two types of milk in the aforementioned areas of Basra Governorate. There were significant differences in the numbers of *E. coli* bacteria in milk, white soft cheese, and yogurt made from cow and buffalo milk. There are no differences in the numbers of these bacteria in local cream made from cow and buffalo milk. The Al-Zubair region recorded the highest logarithmic number of *Staphylococcus aureus* bacteria in cow's milk (2.681 CFU/ml). The Al-Ashar region recorded the highest logarithmic count of this bacterium in buffalo milk (2.361 CFU/ml). The samples showed different logarithmic numbers of *E. coli* bacteria, with the highest numbers in the milk of cows and buffaloes, respectively (3.633 and 2.707 CFU/ml), in the areas of Al-Zubair and Al-Ashar. The reason for these numbers may be due to coliform bacteria and *Staphylococcus aureus* contaminating the milk, to the primitive methods of milking and the lack of attention to washing the cleanliness of the animal, the hands of the milkers, and the utensils for collecting milk, in addition to the process of selling milk, which is done using plastic water bottles or plastic soft drink bottles measuring 1.5-2 liters after washing them with tap water, which reinforces the presence of these neighborhoods and the lack of awareness and cultural and educational level among livestock breeders, which damaged the samples, which negatively affected the quality of the milk and its products [4].

Figure 3 shows the numbers of *Staphylococcus aureus* and *E. coli* bacteria in soft white cheese made from cow and buffalo milk in the areas of Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair. The results showed higher logarithmic numbers for both types of bacteria mentioned compared to the numbers in cow and buffalo milk. The highest numbers of *Staphylococcus aureus* bacteria in soft white cheese made from cow and

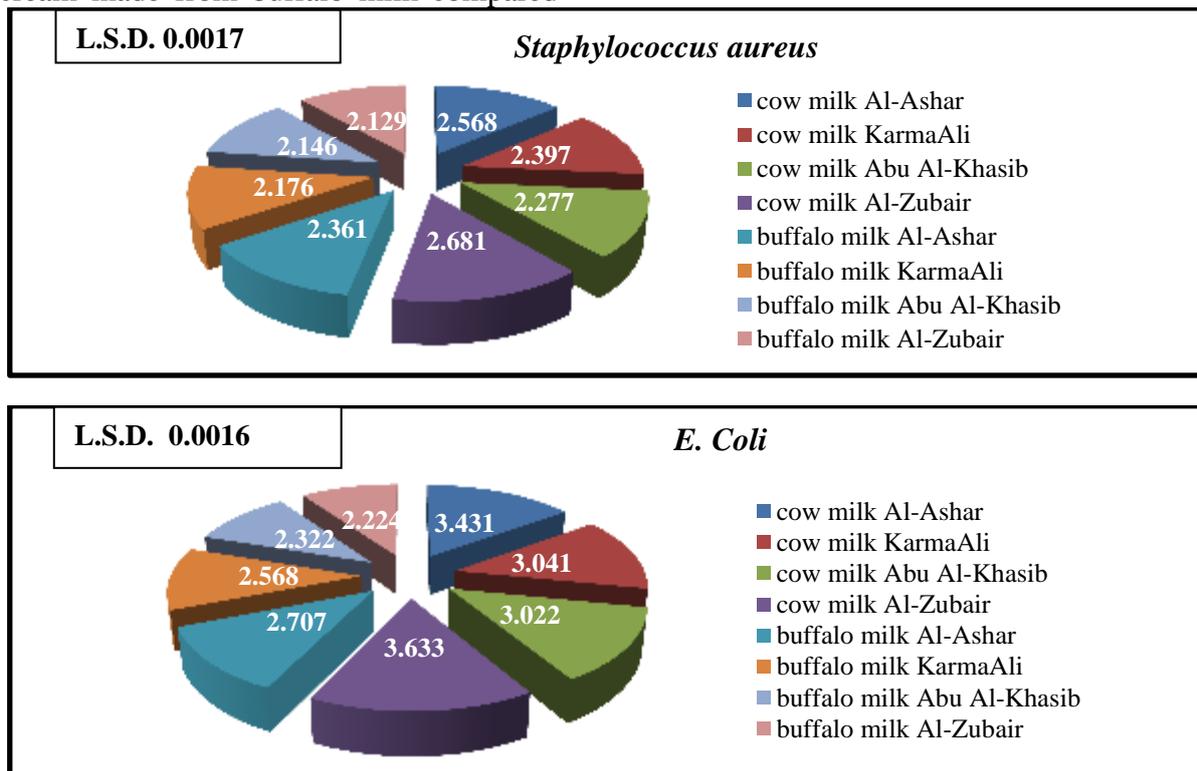
buffalo milk (3.363 and 3.053 CFU/ml) and *E. coli* bacteria (4.146 and 3.892 CFU/ml) were in the areas of Al-Zubair and Al-Ashar, respectively, compared to the rest of the areas of Basra Governorate. Bacteria associated with the spoilage of cheese, such as *Staphylococcus aureus* and coliform bacteria, produce gas and butyric acid as they grow, leading to the appearance of gaps in the cheese and thus negatively affecting the sensory acceptability of the cheese. In addition to producing toxins, these bacteria are transmitted to the cheese from the milk through the udder by a sick or injured animal, or they are transmitted from animal skins, from the surrounding environment, equipment, or from the hands of milkers. This requires health intervention at the farm level or when treating the milk upon receipt to reduce the transmission of bacteria and pathogens into milk and cheese [15].

Figure 4 shows the numbers of *Staphylococcus aureus* and *E. coli* bacteria in yogurt made from cow and buffalo milk in the areas of Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair. Variation in bacterial numbers was observed due to the presence of starter and probiotic bacteria as well as contaminating bacteria; it was observed that the numbers of bacteria varied due to the presence of starter and probiotic bacteria as well as contaminating bacteria. The highest number of both types of bacteria appeared in the Al-Zubair area for yogurt made from cow's milk, and the numbers (3.934, and 4.695 CFU/ml) for samples manufactured from buffalo milk (3.695 and 4.067 CFU/ml) in the Al-Ashar region, respectively, although these numbers are slightly higher than the acceptable limits for the presence of coliform bacteria in yogurt ( $< 2 \log_{10}$  CFU/ml); However, it is considered more polluted in the rest of the areas from which the samples were taken in Basra Governorate. The presence of numbers of

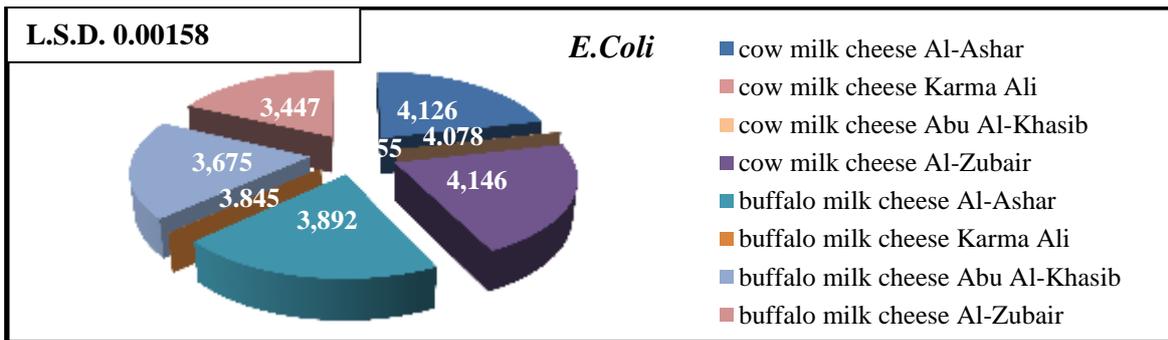
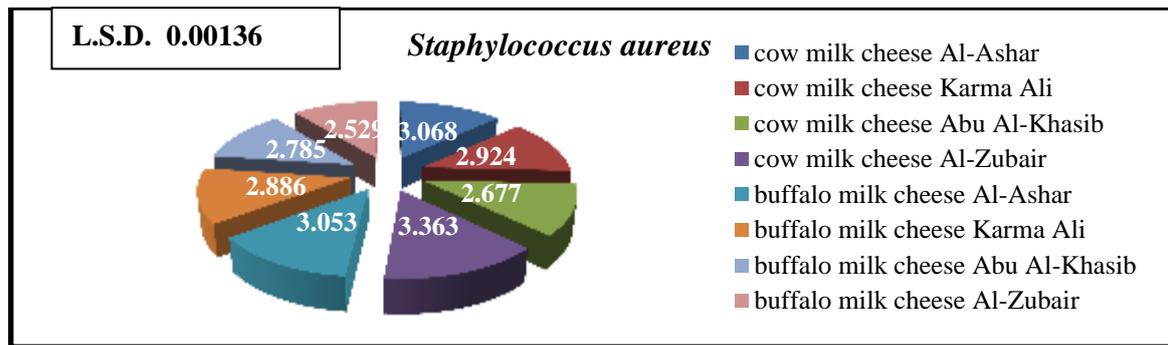
coliform bacteria and *Staphylococcus aureus* indicates contamination of the milk from which the yogurt is made with fecal materials and a lack of attention to the cleanliness of the animal’s udder, vessels, and surrounding environment, in addition to poor storage and transportation in an unrefrigerated manner, which increases the risk of consuming the products to human health [16].

Figure 5 indicates the numbers of *Staphylococcus aureus* and *E. coli* bacteria in local cream made from cow and buffalo milk, taken from local markets in the areas of Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair in Basra Governorate. The total number of *Staphylococcus aureus* and coliform bacteria was lower compared to other dairy products. The number of mentioned bacteria was lower in the local cream made from buffalo milk compared

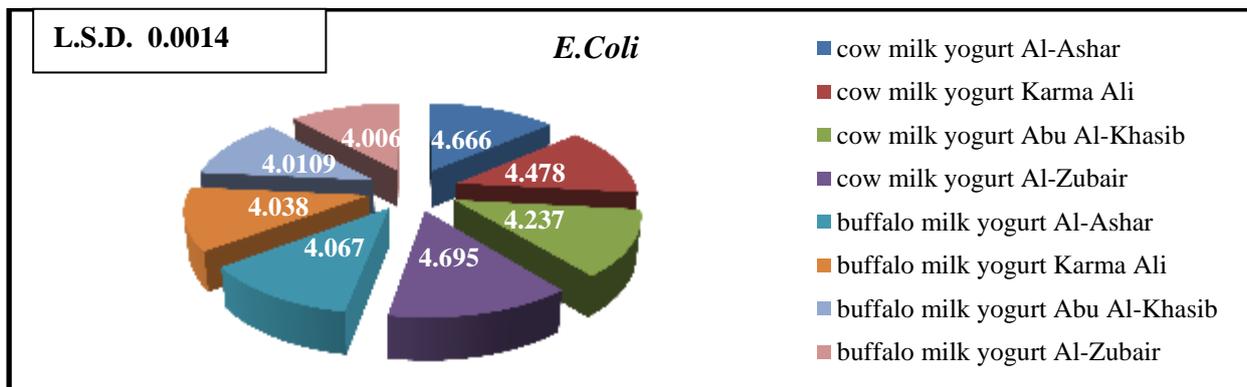
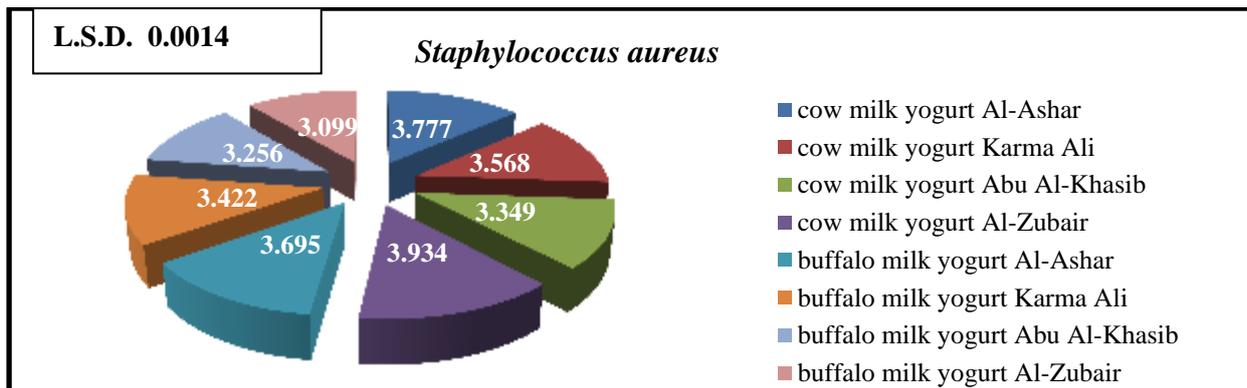
to cow’s milk samples. Due to the high percentage of fat, which reduces the percentage of water in the cream and thus decreases its numbers, the highest number of both types of bacteria, respectively, in cow’s milk cream (2.544 and 2.240 CFU/ml) is in Al-Zubair, while the highest numbers in buffalo milk cream (2.510 and 2.048 CFU/ml) are in Al-Zubair. The reason for the appearance of the two types of bacteria mentioned in dairy products is a lack of attention to cleanliness, insufficient pasteurization, contamination after pasteurization, especially with feces, or the use of milk contaminated with these bacteria, which affects the health of the consumer, especially in the case of the presence of *Staphylococcus aureus*, which produces a chemical enterotoxin [14].



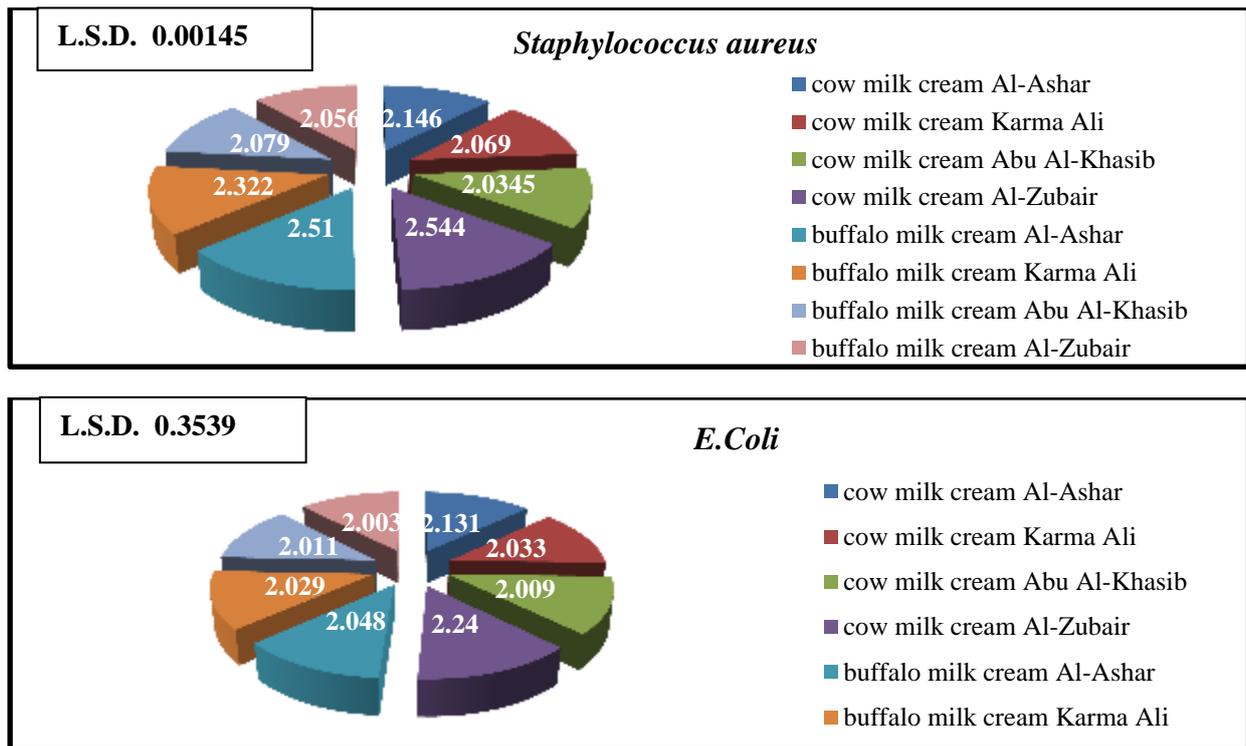
**Figure (2):** Number of *Staphylococcus aureus* and *E. coli* bacteria present in cow and buffalo milk taken from local markets in Basra Governorate.



**Figure (3):** Number of *Staphylococcus aureus* and *E. coli* bacteria in soft white cheese made from cow and buffalo milk taken from local markets in Basra Governorate.



**Figure (4):** Number of *Staphylococcus aureus* and *E. coli* bacteria in yogurt made from cow and buffalo milk taken from local markets in Basra Governorate.



**Figure (5):** Number of *Staphylococcus aureus* and *E. coli* bacteria in cow and buffalo milk cream taken from local markets in Basra Governorate.

### Conclusions

This study was conducted as a result of microbial indicators exceeding internationally accepted limits. The results of the microbiological examination of milk and milk products manufactured from cow and buffalo milk from local markets showed that, for different areas of Basra Governorate, they are Al-Ashar, Karma Ali, Abu Al-Khasib, and Al-Zubair. There was contamination in these products due to

lack of attention to hygiene and neglect of the health aspect, whether from milkers or during manufacturing, storage, and marketing, or inefficiency of heat treatments. We recommend that farmers and manufacturers should be educated, observing safety conditions and procedures, and officials in the health control sector should follow and implement quality and safety standards.

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