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Study of Microbial Contaminants in White Cheese in Basra Markets, Iraq

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Abstract
This study was conducted to detect and identify the types of microbial contaminants in the homemade soft white cheese in Basra city markets and from different regions to assess their validity and their compliance with the approved Iraqi health standards. Fifty samples of soft white cheese samples were collected from the local markets of the city of Basra, represented by the regions (Al-Ashar, Old Basra, Abi Al-Khateeb, Al-Qarma, and five miles) during the period from February 2020 to April 2020 by using 250 g for each sample of soft white cheese. total bacterial count and the total coliform count was investigated, as well as the *Staphylococcus aureus* preparation and the presence of *Pseudomonas spp* was also investigate compared with specific control rates, biochemical identification was done by using the Enterosystem18R system. The results of the current study showed the high content of soft white cheese from the microorganisms that included many pathogens in humans and food poisoning. Total bacterial count ranges from 5.6×10^5 to 6.8×10^9 cfu /g, coliform bacteria ranged from 4.6×10^4 to 9.99×10^7 cfu/g and it was found that the bacterial count of *E. coli* was between 2.1×10^2 to 3.9×10^9 cfu/g,. *Pseudomonas* spp. was found between 2.4×10^4 to 3.1×10^7 cfu/g and *Staphylococcus aureus* bacteria was 1.8×10^7 to 5.3×10^7 cfu/g .It was concluded through this study that soft white cheese samples contain high bacterial contamination that may threaten consumers' general health, exceeding health standards.
Keywords: Soft white cheese, *S. aureus, Pseudomonas* spp., Basra city market

Introduction

In particular, for expectant mothers and growing infants, Milk is a distributional factor of typical diet (1). For many microbes, milk is an excellent growth medium because it has a neutral pH, high water content and a complex biochemical composition (2). Microorganisms can be infected from a wide range of sources that indicate a very massive growth of cheese, a food that is very rich in protein and calcium (3). Milk and milk products are important in human nutrition, specifically cheese that is very rich in protein and calcium (4).

Now, a substantial amount of milk production in our country is generated by dairy farmers, who are deprived of technical expertise and advanced technology, and by women from the village. While classical methods are widely used to transform milk into cheese, there are some variations in the submission. Significant variations are pasteurization, coagulation (yeast addition) and maturation. Among the most important reasons influencing TAMB (total aerobic mesophilic bacteria) count are not pasteurization of milk, noncompliance with hygienic regulations and the development of fresh cheese for consumption without

maturation (5). In milk and cheese, the types of species may be increased either by degradation or by the growth of emerging microorganisms. Production, handling and manufacturing methods should be planned to eliminate both. Mentioned milk contact surfaces and milk works' hands are the most effective sources of contamination, but starter culture, rennet, calcium chloride and brine solution have some effect on the value of cheese (6, 7).
Alternatively, the total viable count (VC) of bacteria (TBC) tests the amount of bacterial contamination in milk. Generally, the regulatory cap is as long as the milk colony TBC consists of less than 100,000 units (cfu)/ml. However, a TBC of < 10,000 cfu / ml is present in highly quality milk. Most of these bacteria are damaged by pasteurization (8). The aim of this study was to diagnose certain forms of bacterial contamination of cheese, to measure the total bacterial count of samples and to compare them with relevant control rates.

MATERIALS AND METHODS

Sample collection

Collecting samples: Fifty samples of soft white cheese were collected from the local markets of the city of Basra, represented by the regions (Al-Ashar, Old Basra, Abi Al-Khateeb, AL-Karma, and five miles) during the period from February 2020 to April 2020 by using 250 g for each sample of soft white cheese. All samples were placed in a new bag from Polyethylene tightly closed, kept in an icebox, and transferred immediately to the laboratory where they were prepared and examined for the presence of bacteria.

Isolation bacteria

Ten gram of each sample was weighed under sterile conditions and diluted to 90 ml of sterile phosphate-buffer and mixed well. This mixture was considered 1: 100 dilution and prepared series dilutions until 10^9 dilutions and then 0.1 ml of specimens, collected from dilutions, were cultured with spread method in the Plate Count Agar (Alper and Nesrin, 2013). The samples were then streaked on the on selective media as mentioned above to differentiate different types of bacteria based on its morphology and Gram's staining. Nutrient agar was applied to estimate the total number of bacteria and incubated at 37°C for 24 hours. Eosin methylene blue (EMB) Agar to estimate the number of coliform bacteria and incubated at 37°C for 24 hours. Mannitol Salt Agar (MSA) Agar to estimate the number of *Staphylococcus aureus* bacteria and incubated at 37°C for 24 hours, and *Pseudomonas* Selective Agar to estimate the number of *Pseudomonas* spp. at 35-37°C for 48 hours (3).

Biochemical identification

Biochemical identification for genus and species of bacteria was then carried out by using the system (Enterosystem 18R). All the culture media were prepared according to the manufacturer's instruction and sterilized by autoclaving at 121°C.

Statistical analysis

The t-test in the GraphPad Prism Software was applied to detect significant differences between study values at a level of $P<0.05$ (9).

RESULTS

The current study revealed that there were a high percentage of microorganisms in soft white cheese. We found that total bacterial count in samples of soft white cheese ranges from 5.6×10^5 to 6.8×10^9 cfu /g, coliform bacteria count ranged from 4.6×10^4 to 9.99×10^7 cfu/g. The

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bacterial count *of E. coli* was between 2.1 $\times 10^2$ to 3.9 $\times$ 10^9 cfu/g. *Pseudomonas* spp. was found between 2.4 $\times 10^4$ to 3.1 $\times$ 10^7 cfu/g, and *S. aureus* bacteria was 1.8 $\times 10^7$ to 5.3 $\times 10^7$cfu/g. Biochemical identification for the genus and species of bacteria were carried out by the method (Enterosystem18R). Various types of Gram-negative bacteria have been diagnosed (*Enterobacter aerogenes*, *Klebsiella pneumonia*, *Shigella* spp, and *proteus* (Tables 1, 2; Figure 1).

Table (1): Number and percentage of isolated bacteria from cheese samples that showed growth on different media

Region	**Total No.**	**No. (%)**	**No. (%)**	**No. (%)**
Al-Ashar	13	7(53.8)	9(69.2)	7(53.8)
Old Basra	7	5(71.4)	6(85.7)	5(71.3)
AL-Karma	11	6(54.5)	9(81.8)	8(72.7)
five miles	10	4(40)	8(80)	6(60)
Abi- Elkhasib	9	5(55.5)	4(44.4)	5(55.5)
Total	50	27(54)	36(72)	31(62)

Table (2): Diagnosis some types of bacteria in cheese

Type of bacteria		
	No. of positive	**Percentage**
Klebsiella pneumonia	11	30.5
Proteus spp	9	25
Shigella spp	5	13.8
Enterobacter aerogenes	6	16.6

Figure (1): Biochemical identification by using Enterosystem18R system showing various types of Gram-negative bacteria (*Enterobacter aerogenes, Klebsiella pneumonia, Shigella* spp, and *proteus*) have been diagnosed

Discussion

White soft cheese is one of the most delicious cheeses used in many countries of the world because of its high moisture content, white cheese is sensitive to proteolytic and microbiological alteration. Furthermore, raw milk and cheese or pasteurized milk are often involved as vehicles for disease transmission, and outbreaks are recorded worldwide (10, 22). The present study collected 50 soft white cheese samples from local markets in the city of Basra. The result was high levels of bacterial contamination and the total amount of bacterial samples in fresh white cheeses sold at the markets was found to be 5.6 x 10^3 to 6.8 x 10^9 cfu /g and mean TBC was 5.2 x 10^4 x 5.78 x 10^{11} cfu /g. This result is higher than the previous analysis, and the total coliform count was also detected at the time interval. Also, 4.6 x 10^4 to 9.99 x 10^7 cfu/g cfu/g were revealed higher than the previous study 1.0 x 10^3 – 9.58 x 10^8 (3, 6). The total bacteria count in foods is influenced by several factors. The consistency of microbiological products is compromised by not pasteurizing the milk that is used to manufacture cheese, not to the standards of hygiene and new cheese without ripening. Indeed, the general bacterial count has been demonstrated to be lower in pasteurized milk cheeses than in raw milk cheeses (3).

The higher fecal coliform counts contributed to being found *E. coli*, samples from cheese are isolated. We know that cheese can be caused by many enteropathogens as a vehicle of foodborne disease (11). Pasteurized milk cheese should be free of coliforms and *staphylococci.* Cheese regulations in Canada require that coliforms do not exceed 10^2 CFU / g, and *staphylococci* in pasteurized cheese do not exceed 102 CFU / g and require coliforms / g under 5.0 × 10^2 CFU/ g and *stapylococci* less than 10^3 cfu in raw milk cheese (8, 22). So, in this study showed higher than the previous study 1.8 × 10^7 to 5.3 ×10^7 cfu/g cfu/g that's mean High level of bacterial contamination (3). These enzymes can survive pasteurization and UHT-treatment by their predominance gram-negative bacteria that can spoil the milk products due to the production of thermal stable extracellular enzymes (12, 13, 18, 21). The results of this study revealed high *Pseudomonas* spp. count number 2.4 × 10^4 to 3.1 × 10^7 cfu/g perhaps because of the enzymes produced by the bacteria or because of the poor pasteurization level of the milk from which the cheese is produced (11, 17).

Cheese production is subject to physical and chemical conditions, including pH increases as the yeast grows, the availability of oxygene, the water activity (Aw), proteolysis and salt content, as the result of wash processes favoring growth of halophilic species like *Halomonas*, is likely to be influenced by the distribution in and on the surface of enterobaceae within the cheese nucleus. Furthermore, the products in the maturation environment can be contaminated with bacteria. Some Gram-negative bacteria were used as a hygiene marker because coliforms show fecal contamination and are considered undesirable contaminants of the cheese (12, 14). Some bacteria can use proteins as carbon and energy sources, especially pathogenic ones. Amino acids transported and catabolized into the cells with protease enzymes hydrolytizing proteins and polypeptides (15, 20). We conclude from the current study the poor quality of cheese traded in the Basra market for exceeding indicators microbial limits accepted in Iraqi standard.

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