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MICROBIOLOGICAL QUALITY OF WHITE SOFT CHEESE AND YOGHURT SOLD IN THE EGYPTIAN MARKETS

By

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ABSTRACT

Background and Objective: Consumption of contaminated dairy products leads to foodborne illnesses, which considered a serious threat to public health on the global scale. Therefore, this study was conducted to examine the microbiological quality of white sift cheese and yoghurt sold in the Egyptian markets.

MATERIALS AND METHODS

Sixty random samples of white soft cheese and baladi yoghurt (30 of each) were collected from dairy shops and supermarkets in Giza governorate, Egypt, for microbiological examination. Results revealed high contamination level of the examined samples, with high numbers of aerobic mesophilic microorganisms, coliforms, staphylococci, yeast, mold, and aerobic spore formers. *E. coli* and *S. aureus* could be isolated from soft cheese and baladi yoghurt samples with percentages of 1, 3 and 19, 15%, respectively. However, *B. cereus* couldn't be detected in all of the examined samples.

CONCLUSION

Unhygienic conditions during production and storage enforce the application of food safety systems and good manufacturing practices (GMP) to produce high quality and safe dairy products.

Key words:

E.coli, *B.cereus*, mold, white soft cheese, baladi yoghurt.

INTRODUCTION

Foodborne pathogens and spoilage microorganisms pose serious public health and financial losses. Every year in the United States, pathogenic microorganisms cause 5000 deaths, 300,000 hospitalizations, and $7 billion economic losses **(Hussain and Dawson, 2013).** Moreover, food deteriorating microorganisms reduce milk production by 20% globally **(Adam *et al*., 2021).** White soft cheese and baladi yoghurt may be a source of undesirable microorganisms like coliforms, aerobic spore formers, staphylococci, yeast, and mold despite having a high nutritional value. Moreover, *Echerichia coli* and *Staphylococcus aureus* have been implicated in different food-borne outbreaks **(Mohamed *et al*., 2020; Hassani *et al*., 2022).** Accordingly, Center of Disease Control **(CDC, 2015)** reported that cheese is considered a main food source for *S. aureus* foodborne outbreaks. Additionally, Six *S. aureus* outbreaks have recently occurred in France as a result of eating soft cheese tainted with *S. aureus* enterotoxin type E **(Halim *et al*., 2022).** The presence of such microorganisms may be attributed to the inadequate thermal treatment, poor handling techniques, using of contaminated equipment, improper storage, and transportation condition **(Halim *et al*., 2022).**

S. aureus is one of the most common causes of bacterial food poisoning outbreaks. In the US, it causes over 241,000 instances of food poisoning each year **(Girmay *et al*., 2020).** Another significant food-borne pathogen that can cause serious diseases is *E. coli*. Its presence in dairy products is considered indication of faecal contamination and increases the likelihood of presence of other pathogenic enteric bacteria. The majority of *E. coli* strains are saprophytic, but some are known to be pathogenic bacteria affecting human health. It causes a variety of intestinal and extra-intestinal illness problems such as hemorrhagic colitis and hemolytic uremic syndrome **(Halim *et al*., 2022).**

Among the spoilage microorganisms, yeast and mold are considered an important cause of fermented products deterioration because their low pH provides a suitable environment for fungal growth **(Adam *et al*., 2022).** Spoilage by fungi (mold and yeast) was regarded as a quality concern rather than a food safety issue however, many toxigenic mold species are able to produce mycotoxins that represent a threat for public health **(Ahmed *et al.* 2014).** On the other hand, aerobic spore-forming bacteria are considered a challenge due to their ability to produce spores under harsh environmental conditions, such as nutrient deficit, osmotic pressure

and temperature deviations; with subsequent production of hydrolytic extracellular enzymes that cause off-flavors in dairy products **(Ahmed *et al*., 2014).**

Therefore, this study was conducted to evaluate the hygienic quality of white soft cheese and baladi yoghurt sold in the Egyptian markets with assessing their agreement with the Egyptian standards and their safety for Egyptian consumers.

MATERIAL AND METHODS

2.1. Sample collection:

A total of 60 random samples of white fresh soft cheese and baladi yoghurt (30 of each) were collected from retail markets in various areas in the Giza governorates, Egypt, between September 2021 and May 2022. Samples were transported to the laboratory as soon as possible in an insulated icebox for direct examination.

2.2. Microbiological examination:

2.2.1. Preparation of decimal dilutions of the examined samples.

11 g of the sample and 99 mL of either 0.1% peptone water (Himedia, RM001) (for yoghurt samples) or sterile 2% sodium citrate solution (Sigma-Aldrich) (for cheese samples) were mixed in a polyethylene bag to make a 1/10 dilution. Next, tenth-fold decimal dilutions were made using 1 mL of the previously prepared homogenate and 9 mL of water with 0.1% peptone **(APHA, 2015).**

2.2.2. Counting the tested microorganisms:

On duplicate plates of Standard plate count agar, Baired Parker media enriched with egg yolk tellurite and Malt extract agar (Hi-media, UK), 0.1 ml of the previously prepared serial dilution was spread. Next, the plates were incubated at 30°C for 72 hours, 35°C for 24-48 hours, and 25°C for 3-5 days to determine the aerobic mesophilic, staphylococcal, yeast, and mold counts, respwctively. However, the coliform count was determined using the Most Probable Number (MPN) method. Additionally, the food homogenate was first heated in a water bath for 12 minutes at 80°C before being immediately chilled in an ice bath to prepare the decimal dilutions. Then, duplicate plates of plate count agar were dispensed with 0.1 ml, and incubated at 32°C for 48 hours for counting total aerobic mesophilic spore formers **(APHA, 2015).**

2.2.3. Biochemical Identification:

The isolated strains of staphylococci species were identified based on the catalase test, mannitol salt agar, tube coagulase, and deoxyribonuclease tests. While, indole, methyl red, voges proskauer, and citrate utilization tests were used to identify the isolated coliform strains. However, aerobic spore formers were identified using egg yolk reaction test, starch hydrolysis, glucose fermentation test, voges proskauer, and growth at different salt concentrations (2 and 7%) and different temperature degrees (50 and 65^{O}C) **(De Vos *et al*., 2009).**

Stastical analysis:

Results were calculated in the form of mean ±standard deviation using the program Statistical Package for Social Science (SPSS), version 26.

RESULTS

Total aerobic mesophilic count (log_{10}cfu/g): Results revealed that all of the examined samples of cheese and yoghurt were contaminated with microorganisms Fig. (1), with a mean count of 9.95±0.3 and 9.22±0.3 log_{10}cfu/g, respectively (Table 1).

Coliform content (MPN/g): Regarding the results presented in (Table 1), it showed that coliforms were detected in all tested cheese and yoghurt samples with a mean value of 7.74±3.23 and 2.76±0.8 log_{10}MPN/g, respectively (Table 1). Moreover, all examined samples were disagreed with the Egyptian standard of soft cheese and yoghurt (1767/2005) and (1000/2005) (Table 2).

Isolated coliforms: *Enterobacter intermedius* was the most frequent one (51%) in cheese followed by *Enterobacter Cloacae* (23%), while *Serratia fonticola* (30%), *Klebsiella oxytoca* (28%) were the most frequent one in yoghurt samples. *E.coli* could be isolated from the examined cheese and yoghurt with percentages of 1 and 3%, respectively Fig. (1). According the incidence of *E. coli* in the examined samples, 96 and 93% of cheese and yoghurt samples, respectively were agreed with the Egyptian standard (1767/2005) & (1000/2005) (Table 2).

Total Staphylococcal count: Results indicated that staphylococci were present in all of the analyzed cheese and yoghurt samples with mean counts of 7.9 ±1.3 and 5.9±1.48, respectively (Table 1).

Confirmed *S. aureus*: The incidence of *S. aureus* in the examined samples of cheese and yoghurt depending on the results of coagulase test were 17.8 and 15%, while depending on the results of TNase test were 36.9 and 26%, whereas depending on both tests, it was 19 and 15%,

respectively Fig. (2). Moreover, about 54% and 64% of the analyzed cheese and yoghurt, respectively were disagreed with the Egyptian standard (1767/2005) & (1000/2005) (Table 2).

Total yeast count: Data depicted in (Table 1) revealed that contaminated yeast was detected in all examined cheese and yoghurt samples with mean values of 7.19±1.34 and 5.88±1.09 $\log_{10}$ cfu/g, respectively. Additionally, according to the Egyptian standards, all examined samples were incompatible with the recommended viable yeast count which should not exceed 400 cfu/g (1767/2005) (Table 2).

Total mold count: Regarding the data recorded in (Table 1), mold was present in 43% and 77% of the examined cheeses and yoghurt samples with mean values of 5.49±1.36 and 3.7±1.23 $\log_{10}$ cfu/g, respectively. Furthermore, 57 and 23% of the tested cheese and yoghurt samples, respectively were agreed with the Egyptian standard (1767/2005) & (1000/2005) (Table 2).

Aerobic mesophilic spore former: They were present in 87% of the examined yoghurt samples with a mean count of 3.14±0.63 $\log_{10}$cfu/g. The biochemical identification of aerobic spore former revealed that, the most frequent organism was *B. pumilus* (30%), followed by *B. megaterium* (23%), *B. mycoides* (16%) and *B. gelatini* (16%). On the other hand, *B.cereus* couldn't be detected in all of the examined samples Fig. (3).

DISCUSSION

Hygienic milk production is very essential to provide safe and high-quality products. During different handling procedures, dairy products may be contaminated with numerous bacteria, either directly or indirectly from exogenous or endogenous origins.Contaminating microorganisms impair their utility and render the products unfit for human consumption, in addition to the public health significance of the pathogenic one **(Mohamed *et al*. 2020).**

The source of contaminating microorganisms varies depending on the type of product, how it was made and processed, the quality of raw milk, and the inadequate conditions of processing, handling, and distribution **(Halim *et al*. 2022).**

Standard plate count: Is one of the techniques that is mostly used worldwide for determining the general level of quality and safety of the dairy products during production, collecting, and processing **(Hasan *et al*., 2016).** The examined cheese and yoghurt samples were highly contaminated with aerobic mesophilic microorganisms, which are considered a mirror of the poor microbial quality of raw milk used in processing, inadequate sanitary conditions applied

during production, bad storage conditions, the resistance of spore forming organisms to the heat treatment, post heat treatment contamination, and cross contamination **(Adam *et al*., 2022).** The obtained results were higher than that recorded by **Mohamed *et al.* (2020)** and lower than those reported by **Karima (2012).**

Coliform Count: The examined samples were highly contaminated with coliform bacteria, which indicated the bad sanitary measures, poor quality raw milk, inadequate thermal treatment and/or post pasteurization contamination **(Atef *et al*., 2017).** The examined samples were disagreed with the Egyptian Standards (1767/2005) & (1000/2005), which recommend that the coliform count should be less than 10 cells/g. Coliforms pose a risk to the public's health in addition to reducing the quality of dairy products and causing financial losses. A comparable result of yoghurt reported by **Ahmed *et al*. (2014), while Adam *et al*. (2021) and Mohamed *et al*. (2020)** reported lower results in cheeses.

Among the isolated coliforms, *E. coli* could be detected in the examined sample of cheese and yoghurt and this were disagreed with the Egyptian Standards. *E.coli* presence is an indicator for the fecal contamination and the unhygienic condition applied during the manufacturing process. To overcome this problem, milk pasteurization is recommended during cheese or yoghurt production as recommended by the Egyptian Organization for Standardization and Quality Control in addition to applying the good hygienic practice and safety systems while processing, transportation and storage **(Atef *et al*., 2017).**

Total Staphylococci: All examined samples were highly contaminated with staphylococcal spp. indicating poor sanitation and mishandling, as well as presence of high incidence of pathogenic staphylococcus carriers among producers **(Garbaj *et al*. 2004).** Additionally. The high frequency of *S. aureus* in the examined samples may be attributed to the genus' dominance on human body parts such as the nose, hands, and skin (Mohamed and Mazyed, 2015). Therefore, the poor hygienic practices during processing, transportation, storage, as well as the poor personal hygiene might be the cause of this high prevalence and counts **(Halim *et al*., 2022).** Similar results were obtained by **Karima *et al*. (2012). While Mohamed *et al.* (2020) and Adam *et al*. (2021)** recorded a lower mean count of 3.4×10^6 cfu/g in cheese. **Where Ahmed *et al*. (2014) and Adam *et al*. (2021)** recorded lower results in yoghurt. *S. aureus* can produce heat-resistant enterotoxins when their count exceeds 5 log cfu/g. these enterotoxins are frequently linked to food poisoning outbreaks which usually appear

quickly (within 1-6 hours) and characterized by presence of nausea, vomiting, diarrhea, and severe abdominal discomfort **(Zeinhom and Abed 2020).**

Total yeast count: Yeast plays diverse roles in the quality and safety of dairy products. It has a proteolytic and lipolytic activity, which cause off-flavors, discolorations and changes in texture of the products. Moreover, some species of yeast cause gastrointestinal diseases, endocarditis, and occasionally fatal systemic diseases **(Ahmed *et al*., 2016).**

Nearly similar results in yoghurt were obtained by **Ahmed *et al.* (2016)** while, Adam *et al*. (2022) reported a lower result in soft cheese. Using poor quality raw materials or insufficient hygienic precautions during production might be responsible for the high level of yeast contamination in the examined samples **(Mohamed *et al*., 2020).**

Total mold count: Yoghurt was more contaminated with mold (77%) compared to soft cheese (43%) due to its acidic condition that favors the growth of fungi. In addition to the quality problems caused by mold contamination safety concern was also recorded because some toxigenic mold species can produce mycotoxins that have carcinogenic effect on the public health **(Ahmed *et al*., 2016).** Lower mold count in soft cheese was reported by **Mohamed *et al.* (2020) while Karima *et al.* (2012)** assessed similar results in cheese and yogurt.

Aerobic mesophilic spore formers: The high incidence of aerobic spore formers in yoghurt samples reflected the unhygienic conditions applied during production and storage. **Moreover, Ahmed *et al.* (2014)** reported higher results with mean count of $1.2 \times 10^4 \pm 38 \times 10^2$.

CONCLUSION

The current study assessed the microbiological quality of some Egyptian dairy products (White soft cheese and baladi yoghurt). The results revealed that the examined samples were contaminated with high numbers of aerobic mesophilic bacteria, coliforms, Staphylococci, aerobic spore formers, yeast, and mold. In addition to presence of some biological hazards such as *E. coli, S. aureus.* Consequently, it is important to raise the public awareness targeting factories and households should be encouraged to follow the strict hygienic control measures with application of Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Point (HACCP) system and Food Safety Management Systems eg. ISO 22000: 2005 to produce safe dairy food and to save a lot of dairy products from being spoiled.

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Table (1): Descriptive statistical analytical results of the determined microbial parameters in the examined samples

Parameter		Prevalence		Min	Max	Mean± SEM
		No.	%			
Total aerobic mesophilic count (log $_{10}$cfu/g)	Cheese (n=30)	30	100	5.39	12.30	9.95±0.3
	Yoghurt (n=30)	30	100	5	11.2	9.22±0.3
Coliform count (log_{10}MPN/g)	Cheese (n=30)	30	100	1.56	12.87	7.74±3.23
	Yoghurt (n=30)	30	100	0.96	4.66	2.76±0.8
Total staphylococci count (log 10cfu/g)	Cheese (n=30)	30	100	5.78	10.92	7.9 ±1.3
	Yoghurt (n=30)	30	100	4.23	9.36	5.9±1.48
Total yeast count (log $_{10}$cfu/g)	Cheese (n=30)	30	100	4.85	9.23	7.19±1.34
	Yoghurt (n=30)	30	100	3.15	7.30	5.88±1.09
Total mold count (log $_{10}$cfu/g)	Cheese (n=30)	13	43	4	8	5.49±1.36
	Yoghurt (n=30)	23	77	2	6.85	3.7±1.23
Aerobic mesophilicspore former (log $_{10}$cfu/g)	Yoghurt (n=30)	26	87	2	4.02	3.14± 0.63

No.: number of positive samples

Table (2): Compatibility of the examined samples with the Egyptian Standards.

Parameter	Products	Permissible limits	compatibility	
			No.	%
Coliform count	Cheese	10MPN/g	0	0
	yoghurt	10MPN/g	0	0
E.coli	Cheese	Nil	29	96
	yoghurt	Nil	28	93
S. aureus	Cheese	Nil	16	54
	yoghurt	Nil	19	64
Yeast count	Cheese	400cfu/g	0	0
	yoghurt	-	-	-
Mold count	Cheese	10cell/g	17	57
	yoghurt	10cell/g	7	23
B. cereus	Cheese	Nil	30	100
	yoghurt	Nil	30	100
Over all acceptability	Cheese		0	0
	Yoghurt		0	0

No: number of samples that were agree with the Egyptian Standards.

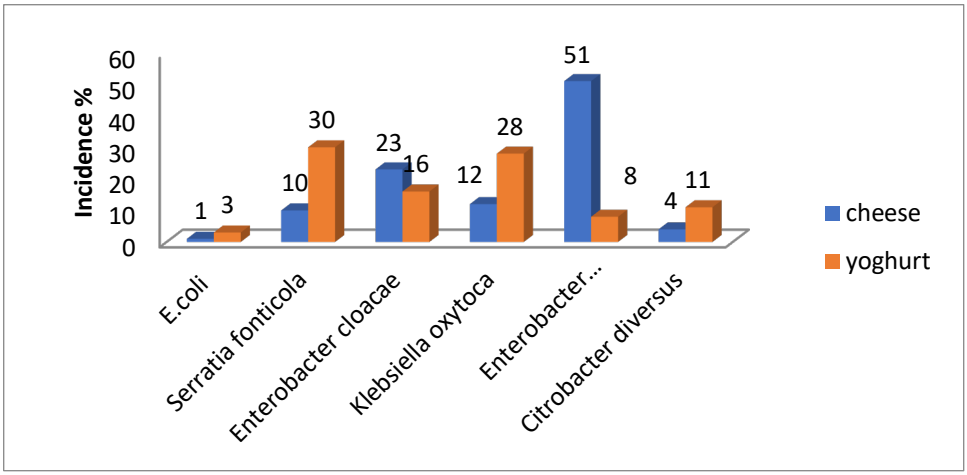

Fig. (1): Incidence of the isolated coliforms from the examined samples.

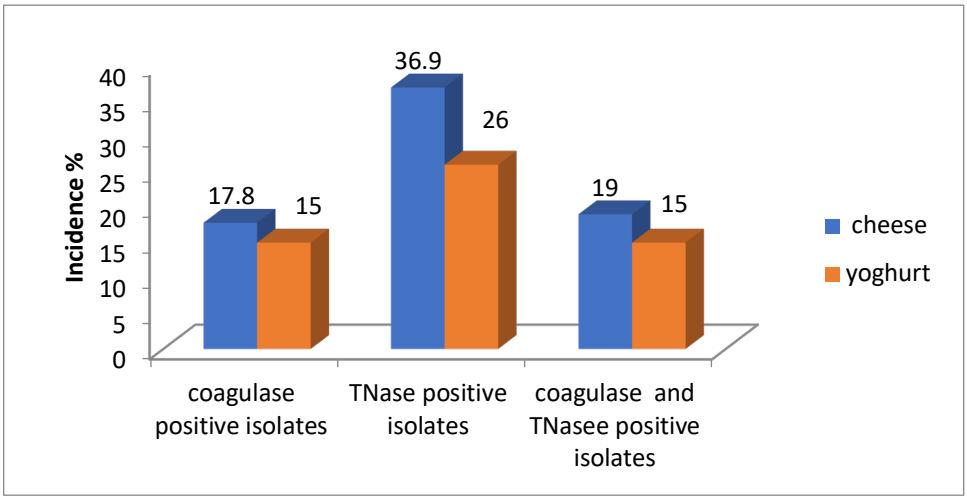

Fig. (2): Incidence of the isolated staphylococcal spp. in the examined samples.

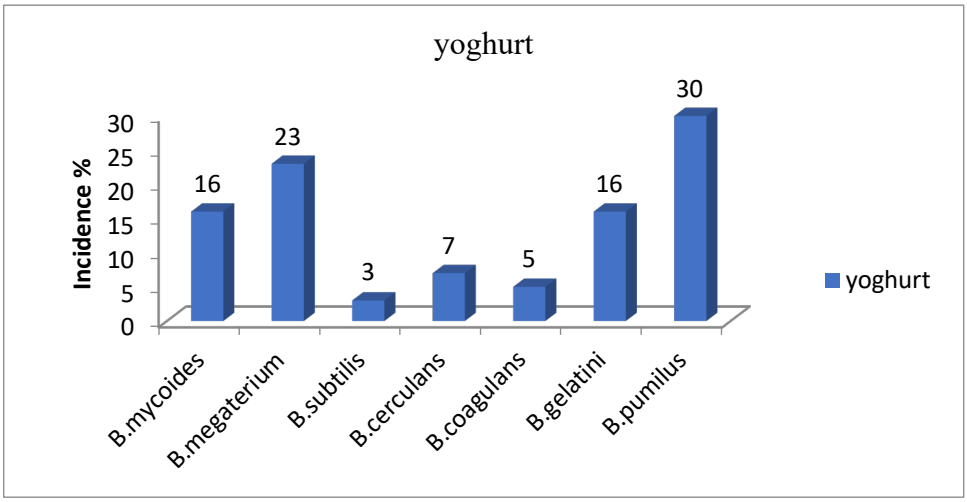

Fig. (3): Incidence of the isolated aerobic mesophilic spore former in the examined yoghurt samples