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Safety and Public Health Hazards Associated with Egyptian Soft Cheese Consumption

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ABSTRACT

White soft cheese is widely consumed by the Egyptian population and could be contaminated during manufacturing, handling and distribution. So, to assess safety and detect the public health hazards of traditional white soft cheese, one hundred samples represented as (40 Kareish cheese, 30 Damietta cheese and 30 Feta cheese) were collected randomly from supermarkets, and street-vendors at Alexandria Governorate. The results revealed that the incidence of *Coliforms* group were 75 and 20 % in the examined Kareish and Damietta cheeses, respectively with respective logarithmic (log) mean values were 5.49 ± 0.62 and 4.10 ± 0.15 cfu/g, on the other hand coliforms failed to be detected in Feta cheese samples. The incidence of *Staphylococcus aureus* were 55, 83.33 and 26.66% in Kareish, Damietta and Feta cheeses samples, respectively, with a log mean values 4.42 ± 0.89; 4.94 ± 0.61 and 2.70 ± 0.62 cfu/g. Staphylococcal enterotoxin A could be detected qualitatively by using ELISA in the examined Kareish and Damietta cheeses and Enterotoxin C could be detected only in Damietta cheese while, Enterotoxin D could be detected only in Feta cheese. Salmonella was failed to be detected in all examined samples. While, the incidence of mould were 35 , 46.67 and 10 % in Kareish, Damietta and Feta cheeses samples, respectively with the log mean values 4.49 ± 0.31, 4.26 ± 0.26 and 1.20 ±0.17cfu/g, respectively. The Aflatoxin M1 was detected in 40 and 53.33% of Kareish and Damietta cheeses samples, respectively. While, the Aflatoxin M_2 was detected only in 20% 0f examined Kareish samples but failed to be detected in Damietta cheese samples. Neither M1 nor M_2 were detected in Feta cheese. In conclusion, it was observed that the hygienic quality of white cheeses sold in dairy shops in Alexandria was low and does not have enough assurance in terms of public health. These results emphasize the need for applying more strict hygienic practices, efficient heat treatment, and applying HACCP system.

1. INTRODUCTION

Cheese is known to be of a great nutritional value for human consumption as its fat and protein have a high biological value and contains all essential fatty and amino acids. Also it is a source of vitamins and minerals. However, cheese is very susceptible to mould growth and is normally kept under refrigeration (Awad et al., 2012).

Coliform bacteria are the main contaminants of raw milk and dairy products, including fresh cheeses. *Coliforms* are easily destroyed by heat treatments usually employed for milk, being an indicator of process failures or post-processing contamination in pasteurized foods (Okura et al., 2010).

E.coli is considered as a harmless bacteria that are most often used as indicator organisms for fecal contamination and breaches in hygiene. However, several E.coli clones have acquired virulence factors that have allowed them to adapt to new niches and in some cases to cause serious disease. There are six categories of pathogenic E.coli that affect the intestines of humans: Shiga-toxin-producing E.coli (STEC; also called verocytotoxin-producing E. coli or VTEC) (STECs are associated disease symptoms. STECs are so named because they produce one or more cytotoxins, called Shiga

toxin 1 (stx1) and Shiga toxin 2 (stx2), of which enterohaemorrhagic E. coli (EHEC) are a pathogenic sub-group; enteropathogenic E. coli (EPEC); enterotoxigenic E. coli (ETEC); enteroaggregative E. coli (EAEC); enteroinvasive E. coli (EIEC); and diffusely adherent E. coli (DAEC).

The presence of *Staphylococcus aureus* in cheese constitutes a potential public health hazard since many strains of *Staphylococcus aureus* produce enterotoxins that cause food poisoning if ingested. Neither the absence of *Staphylococcus aureus* nor the presence of small numbers is complete assurance that a food is safe. Conditions inimical to the survival of *Staphylococcus aureus* may result in diminishing operation or death of viable microbial cells, while sufficient toxins remain to elicit symptoms of staphylococcal food poisoning. The most common symptoms are nausea, vomiting and diarrhea. However, in severe cases they may be accompanied by acute prostration and abdominal cramps. Symptoms usually occurring 2 to 6 hrs after ingestion of the contaminated food (AOAC, 1984) and (Lancette and Tatini, 1992).

Salmonella organisms are the major pathogenic bacteria in humans and animals and their species causing acute gastroenteritis in several countries (Dalal et al., 2009).

Fungal growth on cheese is a common problem for the cheese manufacture during ripening and curing as well as for the retailer and consumer during refrigeration storage. Species of Penicillium and Aspergillus are the most common contaminants of cheese (Gandomi, et al., 2004). By the searching in the medical references, it was observed that, most of this fungi had the ability to human and animal pathogenicity or produced toxins (Ghibaudo and Peano, 2010).

Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic compounds produced mainly by *Aspergillus falvus* and *Aspergillus parasiticus*. The main target organ for their toxicity and carcinogenicity is the liver. Milk and milk products are major nutrient for humans, especially children. For this reason, AFM1 in milk and dairy products should be controlled systematically (Akkaya et al., 2006) & (Azizollahi et al., 2012).

Therefore, the objective of this study was to assess safety and detect the public health hazards of Egyptian white soft cheese sold at Alexandria city, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 100 samples of white soft cheeses (40 Kareish cheese, 30 Damietta cheese and 30 Feta) were collected randomly from supermarkets, and street-vendors at Alexandria Governorate. The samples were obtained as sold to the public and transferred directly in an icebox at 4 ± 1 ^{o}C to the laboratory with a minimum of delay to be examined. Preparation of the samples was carried out according to (APHA, 2004).

2.2. Microbiological evaluation of examined white soft cheese

Representative 10 g of each sample was aseptically homogenized in 90 ml of a sterile 2% sodium citrate solution, in a stomacher for 1 minute. Decimal dilutions were prepared in 0.1% sterile peptone water and appropriate dilutions were used to enumerate the following as descried by Roberts and Greenwood (2003).

2.2.1. *Coliforms* count was conducted according to the method described by (APHA, 2001). Typical colonies were picked up and identified as Escherichia coli by standard biochemical reactions (characteristic greenish metallic color on EMB), and confirmed with the indole, methyl-red, Voges-Proskauer, and citrate utilization (IMVIC) tests (Collee et al., 1996).

2.2.2. *Staphylococcus* aureus was counted using Baird Parker RPF medium after incubation at 35℃ for 24- 48 hours (Oxoid, 2006), for identification of *S. aureus*, typical and atypical presumptive Staphylococci species colonies were examined by Gram stain, coagulase, catalase, and latex agglutination.

Qualitative Detection of Staphylococcal enterotoxins (ELISA test) (Tasci et al., 2011)

2.2.3. Isolation and identification of Salmonella organisms (APHA, 2004).

2.2.4. Mould count according to (APHA, 2004).

The identification of isolated mould genera were carried out based on their micro morphological properties (Pitt and Hocking,

2009). Qualitative and quantitative estimation of aflatoxins (Shundo and Sabino, 2006), and ("EC" (2006).

3. RESULTS AND DISCUSSION

Table (1): Statistical analytical results of Log *Coliforms* count (cfu/g) of examined soft cheese samples.

Product	No. of Examined samples	Positive samples		Mean±SD	ES (2005)	Samples comply with ES	
		No.	%			No.	%
Kareish cheese	40	30	75	5.49 ± 0.62	Not more than 10 cell/gm	10	25
Damietta cheese	30	6	20	4.10 ± 0.15	Not more than 10 cell/gm	24	80
Feta cheese	30	0	0	0	Not more than 10 cell/gm	30	100

Table (2): Frequency distribution of *Coliforms* isolated from the examined soft cheese samples.

Isolates	Kareish cheese		Damietta cheese	
	No.	%	No.	%
Citrobacter diversus	5	12.5	1	5.56
Citrobacter freundii	8	20	4	22.22
Enterobacter aerogenes	5	12.5	2	11.11
Enterobacter cloacae	4	10	3	16.67
Escherichia coli	18	45	8	44.44
Total	40	100	18	100

Table (3): Statistical analytical results of Log *Staphylococcus aureus* count (cfu/g) of examined soft cheese samples.

Product	No. of Examined samples	Positive samples		Mean±SD	ES (2005)	Samples comply with ES	
		No.	%			No.	%
Kareish cheese	40	22	55	4.42 ± 0.89	Free	18	45
Damietta cheese	30	25	83.3	4.94 ± 0.61	Free	5	16.7
Feta cheese	30	8	26.7	2.70 ± 0.62	Free	22	73.3

Table (4): Incidence of enterotoxins of *Staphylococcus aureus* in the examined soft cheese samples.

Product	No. of samples	Enterotoxins of *Staphylococcus aureus*									
		A		B		C		D		E	
		No	%	No	%	No	%	No	%	No	%
Kareish cheese	**40**	**8**	**20**	0	0	0	0	0	0	0	o
Damietta cheese	**30**	**10**	**25**	0	0	**5**	**16.67**	0	0	0	0
Feta cheese	**30**	0	0	0	0	0	0	**6**	**20**	0	0

Table (5): Statistical analytical results of Log mold count (cfu|g) of examined soft cheese samples.

Product	No. of Examined samples	Positive samples		Mean±SD	ES (2005)	Samples comply with ES	
		No.	%			No.	%
Kareish cheese	40	14	35	4.49 ± 0.31	Not more than 10 cell/gm	26	65
Damietta cheese	30	14	46.7	4.26 ± 0.26	Not more than 10 cell/gm	16	53.3
Feta cheese	30	3	10	1.20 ±0.17	Not more than	28	93.3

10 cell/gm

Table (6): Frequency distribution of moulds isolated from the examined soft cheese samples.

Mould Isolates	Kareish cheese		Damietta cheese		Feta cheese	
	No.	%	No.	%	No.	%
Aspergillus. Flavus	5	25	8	25	0	0
Aspergillus. Fumigatus	2	10	2	6.25	0	0
Aspergillus niger	8	40	12	37.5	3	60
Cladosporium spp	0	0	2	6.25	0	0
Mucor spp	2	10	2	6.25	0	0
Penicillium spp	1	5	4	12.5	2	40
Rhizopus spp	2	10	2	6.25	0	0
Total	**20**	**100**	**32**	**100**	**5**	**100**

Table (7): incidence of Aflatoxins M1 and M2 in the examined soft cheese samples

Product	No. of samples	Aflatoxins (ppm)					
		M1			M2		
		No	%	Range	No	%	Range
Kareish cheese	**40**	16	40	0.893-2.184	8	20	0.156- 0.159
Damietta cheese	**30**	16	53.33	1.629-0.510	0	0	0
Feta cheese	**30**	0	0	0	0	0	0

White soft cheese is widely consumed by the Egyptian population. However, raw milk and cheese are frequently implicated as vehicles of transmission of pathogenic bacteria and with outbreaks reported all over the world (Flowers et al., 1992). Moreover, the pathogenic bacteria in cheeses pose a threat to human health due to the increased number of cases and the severity of symptoms (Heikal et al., 2014).

Data in Table (1) showed that the incidence of *Coliforms* group were 75 and 20% in the examined Kareish and Damietta cheeses samples, respectively. Coliforms log mean were higher in Kareish than Damietta, while *Coliforms* was failed to be detected in Feta cheese samples. The Standard Egyptian Guidelines allow maximum possible of *Coliforms* bacterial count in cheese which up to 1.0 Log cfu/ g. according to these standard 25, 80, 100% of examined Kareish, Damietta and Feta cheeses samples were compatible.

High contamination of Kareish cheese may be due to the fact that Kareish cheese is sold uncovered and without container which made it good medium for growth and multiplication of different types of spoilage and pathogenic microorganisms (Ibrahim et al., 2015). The variation between Kareish, Damietta and Feta cheeses in results may be due to the difference in salt concentrations, acidity, and the method of manufacture. Additionally, ripening in brine solution, quality and heat treatment of milk used in the manufacture, handling method, hygienic practices, transportation condition, storage condition and distribution play an important role in its microbial quality.

Data in Table (2) revealed that the most common isolated *Coliforms* from examined Kareish and Damietta cheeses samples were *E.coli* by 45 and 44.44 %, followed by *Citrobacter freundii* by 20, 22.22 %, respectively. Presence of E. coli and high Coliform counts per gram of cheese in this study gives indication of bad hygienic conditions during production, handling and distribution, also used as indicator for fecal contamination in food (Heikal et al., 2014). In addition, there illness. Currently four categories of pathogenic E.coli have been associated with food borne illness: Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC) and Enterohaemorrhagic (EHEC) E.coli (Singh and parakash, 2008).

Truzyan (2003) reported that improper milking hygiene without subsequent pasteurization of milk and the lack of general food–hygiene-related knowledge and infrastructure of marketing could be the sources and causes of such contamination.

Data presented in Table (3) showed that the incidence of *Staph. aureus* were 55, 83.33 and 26.66% in Kareish, Damietta and Feta cheese

samples, respectively. Egyptian Standards stated that white soft cheese must be free from *Staph. aureus*. According to this standard 45, 16.67 and 73.33 % of examined Kareish, Damietta and Feta cheeses samples were compatible.

Staphylococci food poisoning resulting from contaminated milk and dairy products, especially cheeses produced from raw milk in unclean conditions, causes staphylococcal intoxication (Can and Celik, 2012). Differences between the results may be based on the differences in the cheese production techniques, storage conditions, type of cheese and whether the milk used was raw or pasteurized. It could be also related to the unclean conditions where the cheese is produced and the personnel involved in production.

Staph.aureus is capable of producing several enterotoxins that when ingested through contaminated food could cause food poisoning in human with varying intensity (Brightwell et al., 2006) So, presence of *Staph.aureus* in milk and dairy products even in low numbers must be regarded as public health hazard, because it has been established that *Staph.aureus* may lose its viability in food but its enterotoxin still exist. The viability of *Staph.aureus* during the manufacture of dairy products as in cheese depends on the addition of starter culture, salt concentration and storage time (Erkman, 1995).

Data recorded in Table (4) showed that the incidence of Enterotoxin A was detected only in 20 and 25% of Kareish and Damietta cheeses samples, respectively. Enterotoxin C were detected only in 16.67 % of Damietta cheeses samples and failed to be detected in other samples. The incidence of Enterotoxin D were detected

Table (5) showed that only in 20% of Feta cheese samples but failed to be detected in other samples.

The enterotoxins of *Staphylococcus aureus* are antigenically different types include (A, B, Cl, C2, D, E and TST "toxic shock toxin"). All these types of enterotoxins except TST were involved in food borne diseases (Awad et al., 2005).

Salmonella was failed to be detected in all examined cheeses samples. But that is not mean white soft cheese in Alexandria free from salmonella.

The incidence of mould were 35, 46.67 and 10 % in Kareish, Damietta and Feta cheeses samples, respectively. The Standard Egyptian Guidelines allow maximum possible of mould count in cheese which up to 1.0 Log cfu/ g. according to these standard 65, 53.33, 93.33% of examined Kareish, Damietta and Feta cheeses samples were compatible.

Table (6) showed that the most common mould isolated were *Aspergillus niger* by 40, 37.5 and 60 of examined Kareish, Damietta and Feta cheeses samples, respectively.

Moulds are the most common cheese spoilage organisms which can lead to economic loss as well as raising public health hazards such as *Penicillium solitum, Aspergillus versicolor, Cladosporium herbarum, Mucor circinelloides* and *Geotrichum candidum* (Cheong et al., 2014). Mould counts in cheese are used as an index of the proper sanitation and quality defects in soft cheese as rancidity, softness and color defects which arise mainly from contamination by mould. In addition to the potential ability of some mould to produce mycotoxins during their growth thus, their presence poses potential hazards to food safety and human illness (Besancon et al., 1992).

Table (7) showed that the incidence of Aflatoxin M1 were detected in 40 and 53.33% of Kareish and Damietta cheeses samples, respectively while, the incidence of Aflatoxin M2 were detected only in 20% 0f examined Kareish samples but failed to be detected in Damietta cheese samples. Neither M1 nor M2 were detected in fete cheese samples.

Aflatoxins are relatively stable compounds, not destroyed by processing and may even be concentrated. Their thermal stability disqualifies pasteurization and ultra-pasteurization as methods of control (Carvajal et al., 2003 and Honikel, 2003).

The presence of AFM1 concentration in cheese has been explained by the affinity of AFM1 for casein (Brackett and Marth, 1982). Generally, presence of aflatoxins in animal or human bodies cause a disease named aflatoxicosis, so the presence of AFM1 may be specified as aflatoxicosis M1. It can be considered that AFM1 is an etiological factor for a foodborne zoonosis terming aflatoxicosis M1 (El-Tras et al., 2011).

AFM1 is known to be hepatotoxic and carcinogenic. The World Health Organization changed its classification from group 2 to group 1 (IARC 2008). Aflatoxicosis causes anemia, reduction of immune function, hepatotoxicosis, hemorrhage, teratogenesis, carcinogenesis and mutagenesis. The most prevalent symptoms of aflatoxicosis in animals are reduced growth rate and poor intellectual and behavioral performance. The liver is considered a target organ for the toxic and carcinogenic effects of AF (Buldu et al., 2011).

In conclusion, it was observed that the hygienic quality of white cheeses sold in dairy shops in Alexandria was low and does not have enough assurance in terms of public health. These results emphasize the need for applying more strict hygienic practices, efficient heat treatment, and applying HACCP system.

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