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INCIDENCE OF COLIFORMS IN WHITE SOFT CHEESE WITH SPECIAL REFERENCE TO E. COLI

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ABSTRACT

Coliforms generally are pointer to possible fecal contamination and reflect the hygienic standards of cheese processing. To determine the chemical and microbiological quality of some traditional Egyptian white soft cheese, a total of 200 samples represented by Tallaga, Feta, Baramili and Istamboli (50 samples each) were collected randomly from supermarkets, dairy shops and street-vendors in Giza Governorate, Egypt. Chemically; the averages of pH value and NaCl% in the examined cheese were 6.354, 3.732%; 5.018, 7.0%; 3.441, 6.702% and 4.465, 5.94% for Tallaga, Feta, Baramili and Istamboli cheese, respectively. Microbiologically; *Coliforms, faecal Coliforms and Escherichia coli (E.coli)* bacteria were detected in 86, 76 and 60% of Tallaga cheese, 64, 54 and 32% of Feta cheese, 88, 68 and 50% of Baramili cheese and 78, 64 and 54% of Istamboli cheese, respectively. The average *Coliforms* count was $1.3x10^4$, $1.2x10^3$, $6.5x10^3$ and $3.6x10^3$ cfu/g, while, the *fecal Coliforms* average values were 2.1×10^3, 3.1×10^2, 1.2×10^3 and 6.3×10^2 cfu/g and *E.coli* average was $2.7x10^2$, $1.8x10^2$, $2.9x10^2$ and $1.2x10^2$ cfu/g for the examined samples, respectively. Serological identifications of the isolated *E.coli* revealed that 14, 6, 12 and 8% out of all examined cheese samples were pathogenic. The most relevant detected serotypes in cheese were related to enterohemorrhagic (EHEC) and enterotoxigenic (ETEC) strains. Also, enteroinvasive (EIEC) and enteropathogenic (EPEC) related serotypes have been detected in some examined cheese samples. The *Coliforms* content and incidence of different pathogenic *E.coli* serotypes reflect the poor hygienic conditions of manufacturing and absence of microbial load elimination. It could be concluded from the obtained results that there is a lack of a standardized method for production and keeping quality of white soft cheese. So, it's suggested to apply strict hygienic measures during all stages of white soft cheese production.

***Key Words*:** Cheese; Colifoms; E.coli; fecal Coliforms

INTRODUCTION

Nowadays people mainly eat milk rather than drinking, as well as, white soft cheese the most popular local type of cheese produced and consumed in Egypt. Domiati cheese represents about 75% of pickled cheeses consumed by all socioeconomic classes (El-Baradei *et al.*, 2007), however, it is consumed fresh or pickled. While traditional Feta cheese is a type of white soft cheese manufactured by using UF technique, ripened and kept in brine, originally processed in Greece (Anifantakis, 1991).

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Domiati cheese is manufactured from fresh cow and/or buffalo's milk. The unique step in its processing is adding salt at the first step directly to milk, and then milk is mixed and renneted. For refrigerated cheese known locally as Tallaga cheese, only 5-8% salt was added to the cheese milk, which had a less salty flavour as well as creamy texture. Pickling the Domiati cheese by addition of 10 -14% salt directly to cheese milk, then cheese is held to a salty whey for 3-6 months till reaches a sharp distinct aroma, acid flavour and to some extent hard texture. The cheese is consumed after three months (semi ripened named locally 'Baramili cheese' or after 6 months (well ripened known locally as 'Istamboli cheese' (Abou-Donia, 2008 and Hamad, 2015).

The quality of white soft cheese in different Egyptian varieties is affected by some chemical parameters: different salt concentrations, as Istamboli, Baramili and Feta cheese is ripened in brine solutions (El-Sayed *et al.,* 2011). Also, pH values as the presence of moulds in cheese are affected by a wide range of pH values (Osama *et al.,* 2014). Although these cheese types may undergo a heat treatment during the making process, soft cheeses might represent a health risk for the consumers and are considered a possible vehicle of infection or transmission for well-established pathogens (Lotfy *et al.,* 2018), as well as, reduction in the cheese shelf life.

Coliforms count is a well-known reflection to possible contamination with manure and inferior microbial quality of cheese manufacturing. *Coliforms* including *fecal Coliforms* and *E. coli* have gained more concern among most other groups of bacteria owing to their powerful indication in the routine evaluation of cheese quality (Synge, 2000). The contamination of white soft cheese with these microorganisms might be an alarm for low-quality ingredients and bad hygienic measures during handling and distribution. As well, their presence is a true sign of fecal pollution and the possibility of other enteric pathogens' existence (Sayed *et al.,* 2011). As *E.coli* constitutes a part of the intestinal normal flora of humans and some animals, the microbiological criteria involving *E. coli* are commonly related to fecal contamination (Abo El-Makarem *et al.,* 2017). Generally, its presence in cheese might be non-pathogenic, but if virulence genes were carried in *E. coli* cells, they mostly are dangerous to consume (Ombaraka *et al.,* 2016). The most common *E. coli* strains associated with cheese contamination are enterohemorrhagic E. coli (EHEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC) and enteropathogenic E. coli (EPEC). However, O26, O91, O103, O111, O121, O113, O145 and O157 are the most somatic serotypes in developing countries responsible for serious chronic diseases in human (Pizarro *et al.,* 2014).

Therefore, it should bear in mind the *E. coli* priority as food-borne pathogens, and the vital role of cheese as a human disease vehicle, especially in Egypt. As consumption of such cheese has been associated with safety concerns (Abd El-Tawab *et al.,* 2020). For this reason, it's required to use pasteurized milk for white soft cheese production, implementation of good manufacturing and hygienic practices over production stages from farm to table.

MATERIALS AND METHODS

1. Collection of samples:

A total of 200 random samples of white soft cheese represented by Tallaga, Feta, Baramili and Istamboli (50 samples of each) were collected randomly from supermarkets, dairy shops and street vendors in Giza Governorate, Egypt. The samples were preserved in an ice box and transferred directly to the laboratory without undue delay to be chemically and Microbiologically evaluated.

2. Chemical evaluation of examined cheese samples:

Sodium chloride % was determined according to AOAC (2016) and the determination of pH was carried out by an Electrical pH meter (Bye model 6020, USA) (Pearson, 2006).

3. Microbiological evaluation of examined cheese samples:

Preparation of cheese samples was carried out according to ISO 4833-1 (2013).

Total *Coliforms* count was determined using the "most probable number method MPN/g" and *fecal Coliforms* were counted using Brilliant Green Lactose Bile Broth (BGLBB) tubes by MPN/g, as reported by FDA (2013). *Escherichia coli* (*E. coli*) was isolated by MPN/g on Eosin Methylene Blue Agar (E.M.B.) at 37°C for 24h (FDA, 2013), and identified morphologically and biochemically (MacFaddin, 2000).

According to (Kok *et al.,* 1996), using (Denka Seiken Co., Japan) as rapid diagnostic *E. coli* antisera sets for the identification of the Enteropathogenic types, the biochemically suspected isolates were serologically identified in *Animal Health Research Institute*, Doki, Egypt.

The diagnostic *E. coli* antisera sets used for identification include the following sets:

Set 1: O- antisera:

Polyvalent antisera 1: O1, O4, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O113, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O76, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O2, O6, O7, O27, O78, O148, O159 and O168.

Polyvalent antisera 5: O20, O25, O63, O91, O153, O163 and O167.

Polyvalent antisera 6: O8, O15, O17, O102, O115, O141, O169 and O171.

Polyvalent antisera 7: O28ac, O112ac, O117, O124, O136 and O144.

Polyvalent antisera 8: O29, O45, O121, O143, O152 and O164.

Set 2: H- sera: H2, H4, H6, H7, H11, H18 and H21.

1. Statistical Analysis:

The obtained results were statistically evaluated by using SPSS (2007) for Windows (SPSS, version 16, Inc., Chicago, IL). Significant differences were at values of $p < 0.05$.

RESULTS

Results of the examined samples are illustrated in Tables (1-5).

Table 1: Statistical analytical results of salt% and pH value in the examined samples.

Cheese samples N=50	NaCl%			pH		
	Min.	Max.	Average	Min.	Max.	Average
Tallaga	3.40	4.20	3.7	5.97	9.47	6.35
Feta	5.50	8.40	7.0	4.74	5.27	5.01
Baramili	5.30	8.10	6.7	2.96	4.39	3.44
Istamboli	4.60	7.90	5.9	3.81	5.27	4.46

Table 2: Statistical analytical results of total *Coliforms* count in the examined samples.

Cheese samples	Positive samples		Count/ g		
	No. =50	%	Min.	Max.	Average
Tallaga	43	86%	5.0×10^{2}	7.6×10^{4}	1.3×10^{4}
Feta	32	64%	1.0×10^{2}	3.6×10^{3}	1.2×10^{3}
Baramili	44	88%	4.0×10^{2}	2.6×10^{4}	6.5×10^{3}
Istamboli	39	78%	2.0×10^{2}	9.5×10^{3}	3.6×10^{3}

Table 3: Statistical analytical results of *faecal Coliforms* count in the examined samples.

Cheese samples	Positive samples		Count/ g		
	No. =50	%	Min.	Max.	Average
Tallaga	38	76%	3.0×10^{2}	6.0×10^{3}	2.1×10^{3}
Feta	27	54%	5.0×10	9.0×10^{2}	3.1×10^{2}
Baramili	34	68%	1.0×10^{2}	3.7×10^{3}	1.2×10^{3}
Istamboli	32	64%	1.0×10^{2}	2.3×10^{3}	6.3×10^{2}

Table 4: Statistical analytical results of *E.coli* count in the examined samples.

Cheese samples	Positive samples		Count/ g		
	No. =50	%	Min.	Max.	Average
Tallaga	30	60%	5.0×10	9.0×10^{2}	2.7×10^{2}
Feta	16	32%	1.0×10	6.0×10^{2}	1.8×10^{2}
Baramili	25	50%	2.0×10	7.0×10^{2}	2.9×10^{2}
Istamboli	27	54%	1.0×10	5.0×10^{2}	1.2×10^{2}

Table 5: Incidence of different *E.coli* serotypes in the examined cheese samples.

E. coli serotypes	Serotypes characteristics (strains)	Cheese							
		Tallaga		Feta		Baramili		Istamboli	
		No.=50	%	No.=50	%	No.=50	%	No.=50	%
O26:H2	EHEC							1	2%
O26:H11	EHEC					1	2%		
O91: H21	EHEC	2	4%					1	2%
O103:H2	EHEC	1	2%						
O111:H2	EHEC			1	2%	2	4%		
O121: H7	EHEC							1	2%
O125:H21	ETEC	1	2%						
O128: H2	ETEC			1	2%	1	2%		
O17:H18	ETEC							1	2%
O124	EIEC			1	2%				
O159	EIEC					1	2%		
O119:H6	EPEC	2	4%			1	2%		
O44:H18	EPEC	1	2%						
Total		7	14%	3	6%	6	12%	4	8%

DISCUSSION

Results of chemical evaluation in Table 1 revealed that the average values of salt% of examined Tallaga, Feta, Baramili and Istamboli cheese samples were 3.7, 7.0, 6.7 and 5.9 with a range of 3.4 to 4.2, 5.5 to 8.4, 5.3 to 8.1 and 4.6 to 7.9 %, respectively. According to Egyptian Standard (ES, 1008-3/2005) which stipulated that salt content in Domiati cheese should not be more than 9%, all examined Domiati cheese-related samples (Tallaga, Bramili and Istamboli) were within the permissible limit. The average pH values of examined Tallaga, Feta, Baramili and Istamboli cheeses samples were 6.35, 5.01, 3.44 and 4.46, respectively, with a range of 5.97 to 9.47, 4.74 to 5.27, 2.96 to 4.39 and 3.81 to 5.27, respectively. Relative variations in pH of the examined cheese samples may be attributed to variations in manufacturing methods, ripening time and/or cheese samples' age. The delayed manufacturing process, the prolonged period of ripening, warm storage temperature and cheese aging increase the acidity of such product (Mohamed, 2016). That clarifies the reason for Baramili and Istamboli lower pH values, while Tallaga and Feta were much higher.

The higher salt content of white soft cheese was obtained by Haddad and Yamani (2017) in Jordan, Mohamed and El Zubeir (2018) in unpickled white soft (Tallaga) cheese in Sudan, in pickled white soft cheese by Moawad and Khalil (2021), and Egyptian Istamboli cheese as postulated by Mohamed (2017). While the results were nearly similar in soft cheese noted by Abdulghani and Kareem (2018) in Iraq, however lower percentage in Baramili cheese was reported by Mohamed (2020). On the other hand, a lower pH value was demonstrated by Mohamed (2017) in Baramili cheese, and a similar pH value was stated by Moawad and Khalil (2021) in pickled white soft cheese, also in white soft cheese by Haddad and Yamani (2017), in Jordan, Abdulghani and Kareem (2018) and Hussein and Isa (2021) in Iraq.

Data in Table 2 showed that total *Coliforms* group average values were $1.3x10^4$, $1.2x10^3$, $6.5x10^3$ and $3.6x10^3$ cfu/g in the examined Tallaga, Feta, Baramili and Istamboli cheeses samples. According to the Egyptian Standard (ES, 1008-1/2005), that allows the maximum possible count of *Coliforms* in cheese up to 10 cells/g, there were 86, 64, 88 and 78% of the examined samples, respectively not accepted due to the high counts of Coliforms. The variation in total *Coliforms* between Tallaga, Feta, Baramili and Istamboli cheeses may be due to salt concentration differences, the method of manufacture, brine solution ripening, milk quality and heat treatment, storage and distribution condition. High *Coliforms* incidence in this study indicates how these white soft cheese varieties were of inferior quality and risky hazardous as food, which might be a foodborne illness etiology.

Close results in soft cheese samples were recorded by Meshref and Hassan (2009) in Tallaga cheese and Ahmed (2021) in Baramili cheese. Higher *Coliforms* count was recorded by Abdulghani and Kareem (2018) in white soft cheese, and higher incidence by Hassan and Gomaa (2016) in Istamboli cheese. Inferior average counts were reported by Lotfy *et al.* (2018), Hassan *et al.* (2019) and Eid *et al.* (2022) in Tallaga cheese, and El-Shaheer (2013) in Feta. Also, the lower incidence was indicated by Sayed *et al.* (2011) and Abo El-Makarem *et al.* (2017) in Tallaga cheese, Sayed *et al.* (2011) in Baramili and Istamboli cheese, and Moawad and Khalil (2021) in Istamboli cheese. While, *Coliforms* were detected neither by Al Jedah and Robinson (2001) and Mohamed (2017) in Feta, nor by Mohamed (2020) in Baramili cheese.

The data presented in Table 3, revealed that 76, 54, 68 and 64% of Tallaga, Feta, Baramili and Istamboli, respectively were contaminated with *faecal Coliforms*. The minimum counts/g were 3.0×10^2, 5.0×10, 1.0×10^2 and 1.0×10^2 cfu/g, while the maximum counts/g were 6.0×10^3, 9.0×10^2, 3.7×10^3 and 2.3×10^3 cfu/g with average

values 2.1×10^3, 3.1×10^2, 1.2×10^3 and 6.3×10^2 cfu/g, respectively. The higher incidence in Tallaga cheese samples than in Feta, Baramili and Istamboli cheese samples could be due to the Tallaga cheese production method, in addition to the high salt content and ripening in brine in Feta, Baramili and Istamboli. The occurrence of *fecal Coliforms* in white soft cheese is an indication of fecal pollution and possible other enteric pathogens' existence (Brooks *et al.*, 2010). Virtually comparable results were noted by Meshref and Hassan (2009) and Eid et al. (2022) in Tallaga cheese samples, whereas lesser results were recorded by Sayed *et al.* (2011) in the examined Tallaga and Barmili cheese. Also, El-Tayeb (2019) showed that all Feta cheese samples had *fecal Coliforms* count below –3 cfu/g.

As shown in Table 4, the ranges of *Escherichia coli (E. coli)* count from minimum to maximum were from 5.0×10 to 9.0×10^2, from 1.0×10 to 6.0×10^2, from 2.0×10 to 7.0×10^2 and from 1.0×10 to 5.0×10^2 with average values $2.7x10^2$, $1.8x10^2$, $2.9x10^2$ and $1.2x10^2$ in the examined samples, respectively. Egyptian Standard (ES, 1008-1/2005) stated that cheese samples must be free from *E. coli*, according to these standards 60, 32, 50 and 54% of the examined samples were not complying with their limits. The presence of *E.coli* with high incidence and count in the examined samples reveals improper sanitation. It's a good sign of faecal contamination, as *E. coli* is a part of the normal intestinal flora of humans and animals (WHO 1995 and Sayed *et al.*, 2011).

Approximately parallel results of *E. coli* incidence in soft cheeses samples were obtained by Mohamed (2017) in Tallaga and Baramili cheese samples, also, Lotfy *et al.* (2018) and Eid *et al.* (2022) in Tallaga cheese, and Moawad and Khalil (2021) in Istamboli cheese. The results of *E. coli* incidence were higher than that noted by Meshref and Hassan, 2009, Eid *et al.* (2022) in Tallaga cheese, and Sayed *et al.* (2011) in both Baramili and Tallaga cheese samples. Also, *E. coli* was absent in Feta and Istamboli cheese samples examined by Mohamed (2017). However, a greater average value was reported by Abo El-Makarem *et al.* (2017) and incidence was noted by Badawy (2021) in Tallaga cheese.

It is evident from Table 5 that, 14, 6, 12 and 8% out of all examined samples were contaminated by *E. coli*, which is serologically identified as pathogenic *E.coli*. Furthermore, different serotypes were identified in Tallaga cheese: O91:H21 (4%) and O103:H2 (1%) as EHEC, O125:H21 (2%) as ETEC, O119:H6 (4%) and O144:H18 (2%) both as EPEC. In Feta cheese O111:H2 (EHEC), O128:H2 (ETEC) and O124 (EIEC) each was identified in 2% of samples, while in Baramili O26:H11 (2%) and O111:H2 (4%) as EHEC, O128:H2 (2%) as ETEC, O159 (2%) as EIEC and O119:H6 (2%) as EPEC serotypes were identified. Moreover, 2% of the examined Istamboli cheese samples were found to have one serotype of each O26:H2, O91:H21, O121:H7 (EHEC), and O17:H18 (ETEC).

In addition, the identified serotypes were related to four different strains of pathogenic *E. coli* namely, EHEC, ETEC, ETEC and EPEC. The most relevant detected strain was EHEC, which had been noticed in all examined cheese types as well as, ETEC in all sample varieties. While, EIEC-related serotypes were identified in some of Feta and Baramili cheese samples, also, serotypes related to EPEC strain were found in some of the Tallaga and Baramili cheese samples. Such *E. coli* strains act as a risky worldwide health problem, and could cause severe GIT illness in humans, such as watery or bloody diarrhea and might develop into hemorrhagic colitis (Pennington, 2010**)**.

The identified serotypes were nearly similar to that found by Abd El-Tawab *et al.* (2020) in white soft cheese, while higher incidence of different pathogenic *E.coli* were obtained by El-Badry and Raslan (2016) and Ombaraka *et al.* (2016). Contamination of cheese with pathogenic *E.coli* is attributed to post-processing contamination as a result to minimum personal hygiene and unhygienic

equipment, utensils, kitchen and packaging (Darwish *et al.,* 2015).

CONCLUSION

It's emphasized that the higher microbial contamination of food, the higher risk of foodborne illness, due to consumption of such hazardous food. The current study data informed that some white soft cheese samples, particularly Tallaga cheese, were of inferior quality, and overall unsatisfactory hygienic condition. Probably, it's due to the production, handling and distribution of the products under neglected sanitary measures. Therefore, it's suggested to rise hygienic knowledge awareness, where cheese is made, handled and served to the public and apply good manufacturing practices as well as HACCP system to ensure white soft cheese varieties' safety.

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مدى تواجد الميكروبات القولونية في الجبن الأبيض الطري مع إشارة خاصة لميكروب الإيشيرشيا كولاي

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تعد المكروبات القولونية مؤشـــر علي التلوث بالبراز وتعكس معايير الرقابة في تصـــنيع الجبن. ولتعيين الجودة الكيمائية والمكروبيولوجية لبعض أنواع الأجبان المصـــرية المحلية، تم تجميع ٢٠٠ عينة من الجبن الأبيض الطري (خمســون عينة من كل من الثلاجة، الفيتا، البراميلي والإسطنبولي) عشوائيا من المتاجر، ومحلات الألبان والباعة الجائلين بمحافظه الجيزة. كيميائيا: كـان متوســـط الرقم الهيدروجيني ونســـبه الملح ٦,٣٥٤، ٣,٧٣٢٪ و ٥,٠١٨، ٧,٠٠٪ و ٣,٤٤١، ٦,٧٠٢٪ و ٤,٤٦٥، ٥,٩٤٪ في الأجبان المختبرة، علي التوالي. مكروبيولوجيا: قد تواجدت الميكروبات القولونية والقولونية البرازية وميكروب الإيشيرشيا كولاي بنسبه ٨٦ و ٧٦ و٦٠ ٪ في عينات الجبن الثلاجة، ٦٤ و ٥٤ و ٣٢٪ في عينات الجبن الفيتا، ٨٨ و ٦٨ و٥٠٪ في عينـات الجبن البراميلي و٧٨ و٦٤ و ٥٤٪ في عينـات الجبن الإســـطنبولي. وتواجدت الميكروبات القولونية بمتوســـط ١,٣×١٠^٤، ١,٢×١٠^٣، ٦,٥×١٠^٣ و٣,٦× ١٠^٣/جرام، والميكروبـات القولونيـة البرازيـة بمتوســـط ٢,١×١٠^٣، ٣,١×١٠^٢، ١,٢×١٠^٣ و ٦,٣×١٠^٢/جرام وميكروب الإيشـيرشـيا كولاي بمتوسـط ٢,٧×١٠^٢، ١,٨×١٠^٢، ٢,٩×١٠^٢ و١,٢×١٠^٢ في الأجبان المفحوصـة. وبالتعريف السـرولوجي لميكروب الإيشـيرشـيا كولاي وجد ممرضـا في ١٤٪، ٦٪، ١٢٪ و٨٪ من عينات الجبن المختبرة. وكانت اكثر الفصـــائل المتواجدة في الجبن تابعه لعترات الايشـــيرشـــيا كولاي النزفية المعوية و الايشـيرشـيا كولاي المسـممة المعوية كما تواجدت ايضـا فصـائل تابعه لعترات الايشـيرشـيا كولاي المخترقة المعوية الايشيرشيا كولاي الممرضة المعوية في بعض عينات الجبنة المختبرة. ويعكس العد للميكروبات القولونية ومدي تواجد الفصـــائل الممرضـــة المختلفة للايشـــيرشـــيا كولاي ظروف الرقابة السـيئة للتصـــنيع وغياب تخفيف الحمل الميكروبي. ويتلخص من النتائج المعروضـة ان هناك عجز في قياسيه طرق التصنيع وحفظ الجودة للجبن الابيض الطري. لذلك من المقترح تطبيق رقابه حازمه خلال كل مراحل تصنيع الجبن الابيض الطري.