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MICROBIOLOGICAL EVALUATION OF LOCALLY MANUFACTURED SOFT CHEESE

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Received: 25 May 2022; **Accepted:** 13 June 2022

ABSTRACT

One hundred and fifty random samples of soft cheeses represented by Domiati, Kareish and Tallaga (50 samples each) were collected from different local retails, dairy shops and street vendors in Assiut city, Egypt, for microbiological evaluation. Aerobic bacteria were enumerated with average count values of 4.2×10^4, 2.6×10^5 and 6.1×10^4 cfu/g in Domiati, Kareish and Tallaga cheese, respectively. Coliforms were detected with average count values of $8.5X10^2$, $3.3X10^3$ and $2.0X10^3$ cfu/g in Domiati, Kareish and Tallaga cheese, respectively. Fecal coliforms were detected with average count values of $5.3x10^2$, $9.7x10^2$ and $8.4x10^2$ cfu/g in Domiati, Kareish and Tallaga cheese, respectively. Isolation of *E. coli* in 32, 50 and 42 % samples that were examined with average count values of $3.0X10^2$, $4.0X10^2$ and $4.4X10^2$ cfu/g in Domiati, Kareish and Tallaga cheese, respectively. Isolation of *Staph. aureus* in 74, 72 and 64 % samples that were examined with average count values of $1.8x10^3$, $1.7x10^3$ and $8.7x10^2$ cfu/g in Domiati, Kareish and Tallaga cheese, respectively. Anaerobic bacteria were found in 46, 66 and 54% in Domiati, Kareish and Tallaga cheese samples, respectively. Yeasts and molds were present with average count values of $2.3x10^2$, $5.4x10^2$ and $8.2x10^2$ cfu/g and $3.8x10^2$, $4.1x10^2$ and $3.9x10^2$ cfu/g in Domiati, Kareish and Tallaga cheese, respectively. The results reflect the poor general hygiene conditions during production and storage which call for more restriction and preventive measures in milk herds, milk production and dairy factories in respect to quality control, sanitation and health care.

Keywords: Domiati cheese, Kareish cheese, Tallaga cheese, Aerobic bacteria, Coliforms, Fecal coliforms, *E. coli*, *Staph. Aureus*, Anaerobic bacteria, Yeasts, Molds.

INTRODUCTION

Kareish cheese is made from non-pasteurized milk with basic equipment. coagulation of fresh milk causes the survival of various pathogens during its manufacture and ripening (Deeb *et al.*, 2004). Domiati cheese is most popular type in Egypt. Tallaga cheese is white and unpickled. The three kinds of cheese are called soft cheese.

The sources of microbial contamination of cheese may during cheese production as: curd cutting knife, packaging material, brine,

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cheese vat, cold room, floor, cheese cloth and air of production room (Temelli *et al.*, 2006). Moreover, the number of bacteria in milk directly influences the quality and safety of cheese (Arcuri *et al.*, 2006).

Cheese must be pathogens free as, *Staphylococcus aureus* while Coliform and mold must be not more than 10 cfu/g, while the yeast should be not more than 400 cfu/g Egyptian Standards (2005). The presence of coliforms indicates environmental or fecal contamination. Amongst the coliforms, *E. coli* indicated faecal contamination of milk and dairy products, which cause health hazards (Todar, 2008).

Coliforms are responsible for diarrheal disease of 30% in industrial countries and hundreds of millions in developing countries (El-Kosi, 2001). *Staph. aureus* can adapt to grow in various foods and causes many diseases. The presence of yeasts does not commonly cause a defect in milk and dairy products unless they started to ferment lactose, so they grow faster and produce obvious gas and fruity flavour (Davis and Wilbey, 1990). Molds may influence the organoleptic characteristics of the dairy products, and they represent a potential health risk due to they produce mycotoxins (Wouters *et al.*, 2002).

Anaerobic spore formers detection is of great concern to indicate the faecal pollution since, they are a normal inhabitant of the lower part of the intestinal tract of warm-blooded animals and about 25% of the human populations excrete the bacteria in their feces (Smith and Holdeman, 1981).

The most efficient way of reducing the initial milk contamination and, consequently, producing a safer cheese is by improving the hygienic conditions during milking and cheese production (Poli *et al.*, 2007). However, these practices have not been enough to ensure cheese quality and safety because many studies have associated cheese with food poisoning (Magenis *et al.*, 2014).

MATERIALS AND METHODS

1. Collection of samples:

A total of 150 random samples of soft cheeses represented by Domiati, Kareish and Tallaga (50 samples each) were collected from different local retails, dairy shops and street vendors in Assiut city, Egypt. All samples were transferred directly to the laboratory in an insulated icebox and examined rapidly. The microbiological examination was carried out for determination of the quality of such examined cheese samples as well as their acceptability for human consumption. The samples were prepared according to ISO (2013).

2. Microbiological examination

Total Aerobic Count according to ISO (2013) by using standard plate count technique.

Coliform's, *Fecal coliforms* and *E. coli* count according to FDA (2013), which the obtained results confirmed by MPN table.

Isolation and Enumeration of *Staphylococcus aureus* according to ISO (2013) by using Baird Parker agar plate. Characteristic colonies surrounded by a yellow zone were picked up for confirmatory tests.

Detection of anaerobes according to Rhodehamel and Harmon (2001) which was performed by a stormy fermentation test.

Mold and yeast count according to ISO (2008) using DRBC agar, which "star-shape" mold growth enumerated and recorded as mold count/g. In the case of yeast, the colonies were separately counted by the naked eye and recorded as yeast count/g.

3. Statistical analysis:

By using SPSS (2007) for Windows (SPSS, version 16, Inc., Chicago, IL). Significant differences at values of $p < 0.05$.

RESULTS

Table 1: Statistical analytical results of total aerobic count in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	50	100	4.5×10^{3}	2.7×10^{5}	4.2×10^{4}
Kareish cheese	50	100	9.8×10^{3}	1.5×10^{6}	2.6×10^{5}
Tallaga cheese	50	100	6.1×10^{3}	4.2×10^{5}	6.1×10^{4}

Table 2: Statistical analytical results of coliforms count in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	35	70	1.5×10^{2}	4.6×10^{3}	8.5×10^{2}
Kareish cheese	43	86	2×10^{2}	1.2×10^{4}	3.3×10^{3}
Tallaga cheese	38	76	1.5×10^{2}	7.3×10^{3}	2.0×10^{3}

Table 3: Statistical analytical results of fecal coliforms count in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	33	66	1×10^{2}	1.9×10^{3}	5.3×10^{2}
Kareish cheese	37	74	2×10^{2}	4.6×10^{3}	9.7×10^{2}
Tallaga cheese	35	70	1.5×10^{2}	7.3×10^{3}	8.4×10^{2}

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

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	16	32	1.0 X 10	9.0×10^{2}	3.0×10^{2}
Kareish cheese	25	50	1.0 X 10	2×10^{3}	4.0×10^{2}
Tallaga cheese	21	42	5.0 X 10	1.5×10^{3}	4.4×10^{2}

Table 5: Frequency distribution of the positive samples based on their *E. coli* count.

Count / g	Cheese					
	Domiati		Kareish		Tallaga	
	No./16	%	No./25	%	No./21	%
$10 - <10^2$	5	31.25	10	40	3	14.29
$10^2 - <10^3$	11	68.75	12	48	16	76.19
$10^3 - <10^4$	-	-	3	12	2	9.52
Total	16	100	25	100	21	100

Table 6: Statistical analytical results of *Staph. aureus* counts in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	37	74	4×10^2	8×10^3	1.8×10^3
Kareish cheese	36	72	2×10^2	7×10^3	1.7×10^3
Tallaga cheese	32	64	2×10^2	3.6×10^3	8.7×10^2

Table 7: Incidence of anaerobic bacteria in the examined samples.

Soft cheese Samples	No. of examined samples	Positive samples	
		No.	%
Domiati	**50**	**23**	**46**
Kareish	**50**	**33**	**66**
Tallaga	**50**	**27**	**54**

Table 8: Statistical analytical results of yeasts count in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	16	32	1.0×10^2	6.0×10^2	2.3×10^2
Kareish cheese	30	60	1.0×10^2	2.1×10^3	5.4×10^2
Tallaga cheese	28	56	1.0×10^2	2.2×10^3	8.2×10^2

Table 9: Statistical analytical results of mold count in the examined samples.

Soft cheese Samples	Positive samples		Count / g		
	No./50	%	Min.	Max.	Average
Domiati cheese	**23**	**46**	**1.0×10^2**	**8.0×10^2**	**3.8×10^2**
Kareish cheese	**27**	**54**	**8.0 X 10**	**1.3×10^3**	**4.1×10^2**
Tallaga cheese	**23**	**46**	**1.0×10^2**	**1.3×10^3**	**3.9×10^2**

DISCUSSION

1. Total aerobic count:

Table 1, showed that the maximum total aerobic counts /g of Domiati, Kareish and Tallaga cheese samples were 2.7×10^5, 1.5×10^6 and 4.2×10^5, the minimum was 4.5×10^3, 9.8×10^3 and 6.1×10^3 with average values of 4.2×10^4, 2.6×10^5 and 6.1×10^4, respectively. The limits proposed by Egyptian Standards (ES, 2005), must be not more than 10^5 CFU/g. In this respect, 10, 42 and 16 % of the Domiati, Kareish and Tallaga examined cheese samples, respectively, were not matching with the acceptable limits.

Nearly similar results of total aerobic count were recorded by El-Leboudy *et al.* (2017) in samples of Domiati cheese. Lower scores were recorded by Soliman *et al.* (2004), and higher scores were obtained by Abdelall *et al.* (2006) and El-Bessery (2006). The obtained results in samples of Kareish cheese were like that recorded by Hussien *et al.* (2013) and Hassan and Gomaa (2016). Lower results were recorded by Abo El-Makarem *et al.* (2017) and higher than that recorded by Abd-El-Moneim (2004) and Baraheem *et al.* (2007). The obtained results in samples of Tallaga cheese were like that recorded by Abo El-Makarem *et al.* (2017), while lower than that recorded by Soliman *et al.* (2004) and higher than that detected by Meshref and Hassan (2009) and Hassan *et al.* (2019).

2. Coliforms and fecal coliforms count:

From the summarized results presented in Table 2, it is clear that 70, 86 and 76% of the examined samples of Domiati, Kareish and Tallaga cheese, respectively, were contaminated with coliform bacteria. The maximum coliform counts /g in Domiati, Kareish and Tallaga cheese samples were $4.6X10^3$, $1.2X10^4$ and $7.3X10^3$, the minimum was $1.5X10^2$, $2X10^2$ and $1.5X10^2$ with average values of $8.5X10^2$, $3.3X10^3$ and $2.0X10^3$, respectively. The higher incidence in Kareish cheese samples than in Domiati and Tallaga cheese samples could be attributed to the production of Kareish cheese from unpasteurized raw milk in addition to the high salt content and lowered pH of Domiati cheese. Nearly similar results of coliform count in samples of Domiati cheese were noted by El-Kholy *et al.* (2014) and Lotfy *et al.* (2018). Lesser results were documented by Soliman *et al.* (2004) and Sharaf *et al.* (2014) and higher than that recorded by Amer and Ewina (2003) and El-Leboudy *et al.* (2017). Close results in samples of Kareish cheese were noted by Abd Allah (2017) and Abo El-Makarem *et al.* (2017). The results were lower than that noted by Soliman *et al.* (2004) and Salem *et al.* (2016), while higher than that documented by Meshref and Hassan (2009) and Hassan *et al.* (2019). Virtually comparable results were noted by Abo El-Makarem *et al.* (2017) in samples of Tallaga cheese, whereas lesser results were recorded by Soliman *et al.* (2004) and Hassan *et al.* (2019), meanwhile higher results were recorded by Meshref and Hassan (2009).

The data presented in Table 3, revealed that 66, 74 and 70% of the examined samples of Domiati, Kareish and Tallaga cheese were contaminated with fecal coliform bacteria. The maximum fecal coliform counts /g in Domiati, Kareish and Tallaga cheese samples were $1.9X10^3$, $4.6X10^3$ and $7.3X10^3$, the minimum was $1X10^2$, $2X10^2$ and $1.5X10^2$ with average values of $5.3x10^2$, $9.7x10^2$ and $8.4x10^2$ cfu/g, respectively. Nearly similar results of coliform count in samples of Domiati cheese were verified by El-Kholy *et al.* (2014). The results were lower than that recorded by Sharaf *et al.* (2014), while higher than that noted by Amer and Ewina (2003). Near parallel results in samples of Kareish cheese were recorded by Baraheem *et al.* (2007), while the lower results were recorded by Salem *et al.* (2016), moreover, higher results were recorded by Meshref and Hassan (2009) and Hussien *et al.* (2013). Higher results in samples of Tallaga cheese were verified by Meshref and Hassan (2009) and the lower results were recorded by Lotfy *et al.* (2018).

According to the limits proposed by Egyptian Standards (ES, 2010) count of coliforms in cheese mustn't exceed 10 cfu/g. In this respect, 70, 86 and 76% of the Domiati, Kareish and Tallaga examined cheese samples respectively, were not in agreement with the acceptable limits of coliforms count.

3. *Escherichia coli* (*E. coli*) count:

The results obtained in Table 4, verify the enumeration of *E. coli* in 32, 50 and 42 % of the Domiati, Kareish and Tallaga examined cheese samples. The maximum *E. coli* counts /g in Domiati, Kareish and Tallaga cheese samples were $9.0X10^2$, $2.0X10^3$ and $1.5X10^3$, the minimum was 1.0X10, 1.0X10 and 5X10 with average values of $3.0X10^2$, $4.0X10^2$ and $4.4X10^2$, respectively. The highest frequency distribution of *E. coli* in the positive soft cheese samples lied within the range of 10^2 - $<10^3$ (68.75, 48 and 76.19%) in Domiati, Kareish and Tallaga cheese, respectively (Table 5). Approximately parallel results were documented by Ibrahim *et al.* (2015) and Abo El-Makarem *et al.* (2017) in samples of Domiati cheese, while, the lower results were recorded by El-Bessery (2006). The results were higher than that noted by Abd Allah (2017). Near results were recorded by Baraheem (2007) and Abd Allah (2017) in samples of Kareish cheese, while the lower results were recorded by Hussien *et al.* (2013) and Hassan and Gomaa (2016). The results were higher than that verified by Abo El-Makarem *et al.* (2017) and Abd El-Halem (2019). In Tallaga cheese samples lower results were recorded by El Bagoury *et al.* (2019), while greater than that verified by Soliman *et al.* (2004). Egyptian Standards (ES, 2010) have stated that *E. coli* should be absent, accordingly, 32, 50 and 42 % of the Domiati, Kareish and Tallaga examined cheese samples respectively, didn't comply to the standards.

4. *Staphylococcus aureus*:

Data in Table 6, revealed the detection of *Staph. aureus* in 74, 72 and 64% of the Domiati, Kareish and Tallaga examined cheese samples. The maximum *Staph. aureus* counts /g in Domiati, Kareish and Tallaga cheese samples were $8x10^3$, $7x10^3$ and $3.6x10^3$, the minimum was $4x10^2$, $2x10^2$ and $2x10^2$ with average values of $1.8x10^3$, $1.7x10^3$ and $8.7x10^2$, respectively. Results that were nearly similar in samples of Domiati cheese were recorded by El-Bessery (2006), whereas, the lower results were recorded by Eid and Eltalawy (2014) and Elshall (2019), and higher results were recorded by Ahmad (2012). Results that nearly similar in samples of Kareish cheese were recorded by Hassan (2008), while the lower results were recorded by El-Bessery (2006) and Eid and Eltalawy (2014) and higher results were recorded by Hussien *et al.* (2013) and Elshall (2019). In Tallaga cheese samples lower results were detected by Soliman *et al.* (2004) and Lotfy *et al.* (2018), while higher results were detected by Sayed *et al.* (2011). The Egyptian Standards (ES, 2005) indicate that *Staph. aureus* (coagulase-positive) should be absent in soft cheese, hence 74, 72 and 64% of Domiati, Kareish and Tallaga cheese samples were not compatible with that standard.

5. Anaerobic bacteria:

Results given in Table 7, showed that anaerobic bacteria in soft cheese, were found in 46, 66 and 54% in Domiati, Kareish and Tallaga cheese samples, respectively. lower Results in samples of Domiati cheese were recorded by Hassan (2008), while greater than that listed by Abd El-Raheem (2009) and El-Prince *et al.* (2018). Results that were nearly similar in samples of Kareish cheese were recorded by Mostafa (2008), while lower results were recorded by El-Bessery (2006) and Hassan (2008), meanwhile the results that higher were recorded by Abd El-Raheem (2009) and El-Prince *et al.* (2018). lower results in samples of Tallaga cheese were documented by Sayed *et al.* (2011).

6. Yeasts and molds count:

In Table 8, the detection of yeasts was in 32, 60 and 56% of the Domiati, Kareish and Tallaga examined cheese samples. The maximum yeast counts /g in Domiati,

Kareish and Tallaga cheese samples were $6x10^2$, $2.1x10^3$ and $2.2x10^3$, the minimum was $1x10^2$ for the three types of cheese with average values of $2.3x10^2$, $5.4x10^2$ and $8.2x10^2$, respectively. In Table 9, the detection of molds was in 46, 54 and 46% of the Domiati, Kareish and Tallaga examined cheese samples. The maximum mold counts /g in Domiati, Kareish and Tallaga cheese samples were $8x10^2$, $1.3x10^3$ and $1.3x10^3$, the minimum was $1x10^2$, 8x10 and $1x10^2$ with average values of $3.8x10^2$, $4.1x10^2$ and $3.9x10^2$, respectively. The lower results in samples of Domiati cheese were recorded by Nasser (2001) and Sharaf *et al.* (2014), meanwhile the results that higher were recorded by El-Shazly (2007). Results that were nearly similar in samples of Kareish cheese were detected by ELbagory *et al.* (2014). Inferior results were recorded by Nasser (2001) and the higher results were recorded by El-Bessery (2006) and El-Refaay (2016). Results that were nearly similar in samples of Tallaga cheese were recorded by Hassan *et al.* (2019), although, minor results were verified by Nasser (2001). The higher results were recorded by Abo El-Makarem *et al.* (2017) and Lotfy *et al.* (2018). Moreover, results indicated that 40 (80%), 22 (44%) and 25 (50%) of the Domiati, Kareish and Tallaga examined cheese samples were within the permissible limit of yeast counts stipulated by the Egyptian Standards (ES, 2005) which is <400 /g. Furthermore, 27 (54%), 23 (46%) and 27 (54%) of the examined Domiati, Kareish and Tallaga cheese samples were within the permissible limit of mold counts <10 /g.

CONCLUSION

The bacterial load in milk products depends upon the overall hygienic condition from the production till consumption. Information given by the obtained results allows concluding that the overall hygienic condition was not satisfactory. Unhygienic practices adopted during production, handling, processing, storage and distribution are responsible for the poor sanitary and bacteriological quality of the examined soft cheese samples exposed for consumption in Assiut city.

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التقييم الميكروبيولوجي للجبن الطري المصنع محلياً

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تم جمع مائة وخمسون عينة من الجبن الطري (خمسون عينة من كل من الجبن الدمياطي والجبن القريش والجبن الثلاجة) عشوائيا من مختلف الباعة الجائلين ومحلات الألبان والسوبر ماركت بمدينة أسيوط. وقد تم فحص الحالة الميكروبيولوجية لتلك العينات. دلت النتائج على ان متوسط العدد الكلي للبكتيريا في عينات الجبن الدمياطي و الجبن القريش والجبن الثلاجة بمتوسط ٤,٢×١٠^٤ و ٢,٦×١٠^٥ و ٦,١ ×١٠^٤/جرام على التوالي. تواجدت الميكروبات القولونية بنسبة ٧٠% في عينات الجبن الدمياطي و٨٦% في عينات الجبن القريش و٧٦% في عينات الجبن الثلاجة بمتوسط ٨,٥×١٠^٢ و٣,٣×١٠^٣ و٢×١٠^٣ / جرام على التوالي. تواجدت الميكروبات القولونية البرازية بنسبة ٦٦% في عينات الجبن الدمياطي و٧٤% في عينات الجبن القريش و٧٠% في عينات الجبن الثلاجة بمتوسط ٥,٣×١٠^٢ و٩,٧×١٠^٢ و٨,٤×١٠^٢ / جرام على التوالي. تواجد ميكروب الايشيريشيا كولاي بنسبة ٣٢% في عينات الجبن الدمياطي و٥٠% في عينات الجبن القريش و٤٢% في عينات الجبن الثلاجة بمتوسط ٣×١٠^٢ و٤×١٠^٢ و٤,٤×١٠^٢ / جرام على التوالي. تواجد ميكروب المكور العنقودي الذهبي بنسبة ٧٤% في عينات الجبن الدمياطي و٧٢% في عينات الجبن القريش و٦٤% في عينات الجبن الثلاجة بمتوسط ١,٨×١٠^٣ و١,٧×١٠^٣ و٨,٧×١٠^٢ / جرام على التوالي. تواجدت الميكروبات اللاهوائية بنسبة ٤٦% في عينات الجبن الدمياطي و٦٦% في عينات الجبن القريش و٥٤% في عينات الجبن الثلاجة. تواجدت الخمائر بنسبة ٣٢% في عينات الجبن الدمياطي و٦٠% في عينات الجبن القريش و٥٦% في عينات الجبن الثلاجة بمتوسط ٢,٣×١٠^٢ و٥,٤×١٠^٢ و٨,٢×١٠^٢ / جرام على التوالي. كما تواجد العفن بنسبة ٤٦% في عينات الجبن الدمياطي و٥٤% في عينات الجبن القريش و٤٦% في عينات الجبن الثلاجة بمتوسط ٣,٨×١٠^٢ و٤,١×١٠^٢ و٣,٩×١٠^٢ / جرام على التوالي.