



APPLIED STUDY OF MICROBIOLOGICAL HAZARDS IN RAW MILK SOFT WHITE CHEESE IN EGYPT

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ABSTRACT

Raw milk Domiati cheese is the main soft white pickled cheese produced in Egypt. The objective of this study was to assess the microbiological hazards, physiochemical quality and safety of some raw Domiati cheese subtypes including Double Cream, Tallaga, Baramely and Istanbully. The cheese samples were randomly collected from different cheese retailers in Cairo. The results indicated that the protein content and fat content were high in Double cream cheese. The pH value was low in Baramely cheese. Total solid, ash, and EC were high in Istanbully cheese because it is high salt level. Also, results indicated high microbiological hazards. the cheese samples were highly contaminated, having microbial load exceeding the acceptable limits such as: Total bacterial count, Total coliform, Fecal *E. coli* and yeast & molds. Pathogenic bacteria (*Staphylococcus aureus*, *E. coli* O157:H7, *Salmonella* sp., *Shigella* sp., *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter jejuni*) were detected in three cheese samples. Except Istanbully cheese sample were found free of most pathogenic bacteria. A high microbial load of the cheese samples present a public health hazard to the consumers and emphasize the need for improving hygienic standard. Microbiological hazards must be limited to the stander limit of food safety. Raw milk soft white cheese must be manufactured according to hygienic standard.

INTRODUCTION

The cheese is an excellent source of protein, amino acids, calcium, phosphorus, vitamins and many micronutrients (CDIC, 2014). In Egypt, different cheese types are mainly produced from cow and buffalo's raw milk. Domiati cheese is the most popular soft white cheese, which represents about 75% of the cheese produced and consumed in Egypt (El-Baradei et al 2007). It is similar to the soft cheese of east European countries. It is consumed fresh or ripened (pickled for 3 months). Domiati cheese is made from buffalo's and cow's milk, with high salt levels up to 15%.

In modern factories, Domiati cheese is made from pasteurized recombined milk with vegetable fat and less salt levels (1–5%). According to the Egyptian Standards (1008-3/2005), the moisture content should not exceed 60% and the salt contents should not exceed 9% in Domiati cheese. Additionally, the cheese should be free from harmful additives and pathogens such as, *Salmonella* sp, *Staphylococcus aureus*, *Clostridium botulinum*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Sreptococcus faecalis* and indicator hygiene include Coliform group and fungi shouldn't exceed 10 cfu/g and the yeast shroud not exceed 400 cfu/g. according to the manufacturing processes, there are many subtypes of Domiati cheese. Tallaga cheese is made with low salt level and ripened refrigerated. Baramely cheese with additional 48- 72 hours at room temperature. The salt level (15 – 17%) added in Istanbully cheese is the highest among Domiati cheese subtypes. Double cream cheese is the subtype with highest moisture and fat with a

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very soft body and texture. There were many outbreaks of infections associated with the consumption of cheese. The predominant organisms identified were *Salmonella* sp., *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* (De Buyser et al 2001; (Nummer et al (2012). Such sources might be during cheese production, storage or from human contamination, air, milking equipment, feed, soil, feces and grass (Gould et al 2014). There are many researches related to the determination of Physio-chemical quality of cheese but there are only a few researches conducted to detect microorganisms that cause quality problems in raw soft white cheese in Egypt and microbiological hazards (Hegazy et al 2012 and El-Baradei et al 2007). The aim of this study are: identify of the microbiological hazards in raw milk soft white cheese.

MATERIALS AND METHODS

1. Sampling

Different Domiati cheese varieties were sampled from Cairo retails. The cheese varieties (Raw milk) include Double Cream, Tallaga, Baramely and Istanbully raw milk soft white cheeses (seven samples each). The cheeses were kept at 4 °C, transported to the laboratory and analyzed within 24 h samples receiving.

2. Physiochemical Analysis

Fat content, total solids and ash content were determined in collected samples according to (AOAC 2006). Salt content was determined according to APHA (1992). Protein content of cheese was determined by measuring the total nitrogen content of samples by the Kjeldahl method and multiplying by a conversion factor 6.38 APHA (1992). The pH values were determined using bench – top pH meters, HANNA Model HI – 9321. Titrable acidity (as a lactic acid) was determined according to AOAC (2006). Electric Conductivity (EC) was measured at 25 °C as standard temperature using ATC bench EC meter, HANNA Model HI-8820.

3. Microbiological Analysis

3.1. Microbial count

Total bacterial count was determined according to FAD, (2005) using plate count method. MRS

agar was used for the enumeration of Lactic acid bacteria incubated at 37°C for 48 h in sealed jar containing anaerobe sachet.

Staphylococcus aureus enumeration on Baird Parker agar supplemented with egg yolk tellurite medium incubated at 37°C for 48 h. Yeast glucose chloramphenicol agar medium was used for yeast and molds enumeration were incubated at 25°C for 5 days. MacConkey Agar medium was used for the enumeration of coliform bacteria and fecal coliform were incubated at 37°C for 24 h for coliform bacteria and 44.5°C for 24 h for fecal coliform Mucchetti, et al (2008).

3.2. Detection of *Salmonella* sp. and *Shigella* sp.

The sample was mixed with sterile 1% peptone water in a ratio of 1:10 and incubated at 37°C for 24 hr. One ml of the pre-enriched broth was transferred to a sterile test tube containing 9 ml Gram-negative broth (selenite broth) as an enrichment medium. The tubes were incubated for 16-18 h at 37°C after which a loopful from each enriched broth was streaked on the surface of both XLD and S.S. agar media to detect *Shigella* sp. The plates were incubated at 37°C for 24 hr. On XLD agar, *Shigella* sp colonies are red, on S.S. agar, *Shigella* sp. gives translucent small colonies. While detection of *Salmonella* sp. a loopful from each enriched broth was streaked on the surface of both S.S agar, Brilliant green agar and Bismuth Sulfite Agar media. The plates were incubated inverted at 37°C for 24 hr. On S.S agar, *Salmonella* sp colony is small translucent with or without a black center, but on Brilliant green agar *Salmonella* sp colony is red purple surrounded by a red halo and tan to brown or black colonies on Bismuth Sulfite Agar medium.

3.3. Detection of *Campylobacter jejuni*

Two grams of each sample were transferred to 10 ml of thioglycolate medium with Skirrow supplement (SR 69, Oxoid) and incubated at 37°C for 24 hours under microaerophilic conditions (6% O₂ + 10% CO₂).

A loopful from each enriched broth was streaked on the surface of both *Campylobacter* blood agar base media (10% sheep blood) and *Campylobacter* blood free selective agar base. The plates were incubated inverted at 43°C for 48 hours under microaerophilic conditions using gas generation kits specific for *Campylobacter* sp

3.4. Detection of *Bacillus cereus*

The sample was mixed with sterile 1% peptone water in a ratio 1:10 and incubated at 37°C for 24 h to enrichment. After which a loopfull from each enriched broth was streaked on the surface of *Bacillus cereus* Agar Base medium **FAD (2005)**. The plates were incubated inverted at 37°C for 24 hr. Colonies appear turquoise to peacock blue colonies developed on PPEMPa and surrounded by a precipitate.

3.5. Detection of *Escherichia coli* O157: H7

Samples dilutions were spread onto plates of Sorbitol MacConkey agar medium (Oxoid, England). After 18-24h at 35°C incubation, sorbitol negative colonies (pale - colored, typical *E. coli* O157: H7) were serologically tested, as outlined by **FAD (2005)**.

3.6. Detection of *Listeria monocytogenes*

Detection of *Listeria monocytogenes* was carried out according to the methods of **FDA (2005)**. Twenty five grams from each sample were added to 225 ml of *Listeria* enrichment broth medium. Enrichment broth incubated at 30°C for 7 days. After that, 0.1 ml of the inoculated enrichment broth culture was streaked on Palcam agar media incubated at 35°C for 24-48 hours **Hitchins (2003)** and loopful from *Listeria* enrichment broth culture was streaked on Oxford agar incubated at 37°C for 24-48 hours. Up to five typical colonies (dew-drop-like, dark brown or black colonies with brown or black halo).

The pathogenic detected were subjected to preliminary identification tests including Gram staining, catalase, MR/VP, gas production and coagulation....etc. and second identification by serological test at species level with Vitek 2 system Version: 07.01 (BioMérieux) in Air Force Specialized Hospital.

RESULTS AND DISCUSSION

Microbial counts in Domaiti raw milk soft white cheeses

Data in **Table (1)** show that the microbial count (cfu/g) in Tallaga soft white cheese, Istanbul, Double cream and Baramely soft white cheese from traditional dairy factories in Egypt.

Lactic Acid Bacteria (LAB) was 8.4×10^2 , 8.1×10^2 , 12.9×10^3 and 3.1×10^3 cfu/g in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were lower than results as reported by **Beuvoir and Buchin (2004)** who found that lactic acid bacteria count was high 10^8 - 10^9 cfu/g, and regularly decreased during storage. LAB are biopreservatives, it produce a variety of inhibitory substances including hydrogen peroxide, diacetyl, carbon dioxide, ethanol and bacteriocins against the growth of pathogenic and spoilage microorganisms. LAB improve the safety and increase the quality of dairy products **D'Amico et al (2010)**.

Total bacterial count (TBC) of different cheese samples were 9.2×10^5 , 5.7×10^4 , 7.4×10^5 and 8.2×10^5 cfu/g in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were higher than results as reported by **Anhar (1989)** who found that TPC in Domiati white soft cheese aged for 1-3 months ranged from 5.3 to 9.6×10^5 with a mean of 8.2×10^5 . **Abdel Moneim et al (2016)** found TBC in soft white cheese from camel and cow raw milk was 5.0×10^6 (cfu/g). The high TBC level in this study may be due to different factors such as: contaminated raw milk, unhygienic processing conditions and practices during milk collection.

Coliform bacteria may exist in cheese in large numbers; they have been used as indicators of unhygienic manufacturing practices. Total coliforms in cheese samples were 4.9×10^2 , 9.5×10^3 , 7.4×10^3 and 4.1×10^3 cfu/g in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were above the limits required by the **Egyptian standards (1008-3/2005)**. In Egyptian standard requirements not exceed 10 cfu/g.

Also, data in **Table (1)** show that fecal coliforms in cheese samples were 2.3×10^2 , 3.2×10^2 , 4.1×10^2 and 3.1×10^2 cfu/g in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were higher than results as reported by **Ahmed et al (1988)**, who suggested that coliforms may lose their viability in Domiati cheese due to the inhibitory effect of salt, low pH value and competition with other microorganisms during ripening period. **Trmcic et al (2016)** found fecal *E. coli* in raw milk soft white cheese. The contamination with coliform and fecal *E. coli* indicates a need to improve efficient cleaning and sanitation program in cheese chain processing. Coliforms in soft white cheese. Coliform caused some diseases may include gastrointesti-

nal illnesses such as severe diarrhea, nausea, and possibly jaundice as well as associated headaches and fatigue (CDFA, 2016).

Total yeast and molds in different cheese samples were 13.0×10^4 , 22.0×10^2 , 7.5×10^3 and 48.0×10^3 cfu/g in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were above the limits required by the **Egyptian standards (1008-3/2005)** which should not exceed 10 cfu/g for fungi and 400 cfu/g for yeast. These results agree with the results reported by **Abou Dawood et al., (2005)** how found that many of Domiati cheese samples collected from Giza Government, contained higher counts of yeast and molds than the standard specifications. The presence of molds and yeasts in dairy products is undesirable hazard. It could be comes from the environmental factors such as: walls and shelves of ripening rooms, air, equipment, water, milk, brine, etc.

Enumeration of *Staphylococcus* sp in four kinds soft white cheese from traditional dairy factories in Egypt presented in **Table (2)**. Cheese is a good substrate for growth of *S. aureus* and other pathogens. Such product is involved in foodborne diseases due to: the occurrence of coagulase-positive staphylococci in raw milk cheese; cross-contamination during the process; the possible cross-contamination thereafter (**Addis and Kyule, 2011**). *Staphylococcus aureus* in different cheese samples ranged from 1.6×10^2 cfu/g (Istanbul cheese) to 9.0×10^3 cfu/g (Baramely cheese). These results were higher than the limits required by the Egyptian standards (1008-3/2005). These are results lower than that found by **El Kholy et al (2008)** who found similar results for the incidence and counts of *staphylococcus aureus* in Tallaga soft white cheese samples collected from Cairo & Giza areas during 2004 to 2005 reported that *Staphylococcus aureus* was detected in Domiati soft white cheese samples collected from different sites in Egypt. Nearly similar results obtained by **Eid and Eltalawy (2014)**. Staphylococcal counts should reach approximately 10^6 cfu/g¹ to be associated with enterotoxin production (**Necidova et al 2009**).

(**Makita et al 2012**) also isolated *Staphylococcus aureus* from soft white cheese. *Staphylococcus aureus* is often present on human skin and in nasal passages and is potentially a post processing contaminant.

Pathogenic bacteria in Domaiti soft white cheeses

Data in **Table (2)** shows pathogenic bacteria (cfu/g) in Tallaga, Istanbul, Double cream and Baramely soft white cheese from traditional dairy Plants in Egypt. The absence of pathogens in Istanbul soft white cheese is a result of a salting level up to 16 %, which is an efficient control measure, regardless the environmental and health burdens. The contamination of other cheese types with the microbial pathogens (*E. coli* O157:H7, *Salmonella* sp., *Shigella* sp., *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter jejuni*) was observed in **Table (2)**. These results were above the limits required by the **Egyptian standards (1008-3/2005)**.

Addis et al (2012) obtained results that is in agreement with results in the presented study, they detected *E. coli* O157: H7 in feta soft white and Egyptian Domaiti soft white cheese. **El Kholy et al (2008) and El Sayed et al (2011)** found *E. coli* O157:H7 from white soft cheese samples. **CDIC, 2014** found *E. coli* O157: H7 in raw milk soft white cheese. Many other researchers obtained similar results such as: detected *Salmonella* spp. in Domaiti soft white cheese. **El Kholy et al (2008)** found *Salmonella* spp in Domaiti soft white cheese, *Listeria monocytogenes* in Tallaga soft white cheese samples collected from Cairo & Giza areas during 2004 to 2005. It is most likely due to post process contamination from environmental sources and cross-contamination in the dairy plant and or retail shops stores or processing chain. **CDIC, (2013)** found *Listeria monocytogenes* in refrigerators samples from retail stores at USA. *Listeria monocytogenes* can survive a number of cheese-making processes and can remain viable in the final product for a considerable length period of time (**Griffiths, 1989**). A combination of control measures was suggested to prevent, eliminate and reduce the contamination with microbial hazards to the safe limits.

Physio-chemical composition in Domaiti soft white cheeses

Data in **Table (3)** show that Physio-chemical composition in Tallaga, Istanbul, Double cream and Baramely soft white cheese from traditional dairy Plants in Egypt. Total protein (TP) content in soft white cheese samples were 11.30, 12.80 and 11.21 % in Istanbul, Double cream and Baramely soft white cheese, respectively. These results were

Table 1. Microbial count (cfu/g) in Domaiti raw milk soft white cheeses.

Microbial Group (cfu/g) ± *SE	Cheese types			
	Tallaga	Istanbully	Double cream	Baramely
Lactic acid bacteria	8.4 x 10 ² ± 0.21	8.1x10 ² ± 0.53	12.9x 10 ³ ± 1.3	3.1x10 ³ ± 0.66
Total bacterial count	9.2 x10 ⁵ ±1.2	5.7x10 ⁴ ± 2.81	7.4x10 ⁵ ±1.23	8.2x10 ⁵ ± 1.31
Total coliform	4.9 x10 ² ±1.98	9.5x10 ³ ±51.0	7.4x10 ³ ±4.3	4.1x10 ³ ± 0.8
Fecal <i>E. coli</i>	2.3 x10 ² ± 6.34	3.2x10 ² ± 1. 6	4.1 x10 ² ± 0.83	3.1x10 ² ± 0. 13
Yeast & Molds	13.0 x10 ⁴ ± 0.08	22.0 x 10 ² ± 0. 12	7.5x 10 ³ ±0.23	48.0x 10 ³ ± 0.09

*SE = Stander error

Table 2. Pathogenic bacteria (cfu/g) in Domaiti raw milk soft white cheeses

Pathogenic bacteria ± *SE	Cheese types			
	Tallaga	Istanbully	Double cream	Baramely
<i>Staphylococcus aureus</i>	1.9 x10 ² ±0.164	1.6x10 ² ± 0.32	7.0x10 ³ ± 0.98	9.0x10 ³ ± 0.72
<i>E. coli</i> O157:H7	ND	ND	+	ND
<i>Salmonella typhi</i>	+	ND	+	+
<i>Shigella</i> sp.	+	ND	ND	+
<i>Listeria monocytogenes</i>	+	ND	ND	+
<i>Bacillus cereus</i>	ND	ND	ND	ND
<i>Campylobacter jejuni</i>	+	ND	ND	ND

*ND = Not detected *SE = Stander error

Table 3. Physio-chemical composition in Domaiti soft white cheeses

Composition± *SE	Cheese types			
	Tallaga	Istanbully	Double cream	Baramely
Protein %	9.60 ± 0.57	11.30 ±0.37	12.80 ± 0.19	11.21 ± 0.62
Fat %	16.80 ± 0.21	20.70 ± 0.24	19.50 ±0.81	21.00 ± 0.32
Moisture %	65.00 ± 0.15	60.50 ± 0.12	62.80 ± 0.03	61.00 ± 0.07
Total solid%	35.00 ±0.02	40.00 ± 0.04	37.20 ± 0.18	39.00 ± 0.38
Ash %	12.40 ± 0.10	13.70 ± 0.14	12.60± 0.25	11.80 ± 0.01
NaCl %	10.60 ± 0. 04	13.20 ± 0.03	11.80 ± 0.16	12.60 ± 0.35
pH	4.30 ± 0.08	3.60 ± 0.01	3.80 ± 0.03	3.20 ± 0.19
Acidity %	0.47 ± 0.05	0.98 ± 0. 14	0.68 ± 0.07	1.30 ± 0.02
EC (µS)	16.20 ± 0.04	34.4 ± 0.02	23.40 ± 0.06	26.20 ± 0.05

*SE = Stander error

above limit of protein content required by **Egyptian standards (3-1008/2005)** which should be 10% at least. Total protein in Tallaga cheese was 9.6%, these results were below limit **Egyptian standards (1008-3/2005)**. The results reported by **Abou-Dawood et al (2005)** emphasized that a mean of total protein of Domiati cheese was 7.89%. The results were lower than that reported by **Dabiza et al (1999)** and **Ojedapo et al (2014)** who reported that TP of soft white cheese ranged from 17.7 to 20.5%. Concerning the fat content, in soft white cheese samples were 16.80, 20.70, 19.50 and 21.00 % in Tallaga, Istanbully, Double cream and Baramely soft white cheese, respectively. These results were less than limit of fat content required by **Egyptian standards (1008-3/2005)** which should be in rang 45 – 60 % fat and in agreement with the results reported by **Idris (1989)** and **Ojedapo et al (2014)** whose found that fat content of Domatii soft white cheese ranged from 14.00 to 15.31 with a mean of 14.46%. **Hofi et al (2010)** found that fat content of Domiati soft white cheese ranged from 38.70 to 51.50 with a mean of 45.50%. Low of fat percentage in cheese samples may be due to distributor of milk is remove and segregate fat from milk to made butter for high prices.

The total solids (TS) of soft white cheese samples ranged from 35.00, 37.00% for 39.00 % in Tallaga cheese, Double cream, and Baramely cheese, respectively. These results were lower than results as reported by **El-Kenany et al (2001)** who found that total solids of fresh Domiati cheese was 43.27%. While the total solid in Istanbully cheese was 40.0 % this result was agreement with the limits required by **Egyptian standards (1008-3/2005)**, This may be due to high salt content in Istanbul soft white cheese or add additives such as powder milk.

The pH values in soft white cheese samples were 4.30, 3.80, 3.60, and 3.20 in Tallaga, Double cream, Istanbully and Baramely soft white cheese, respectively. These results were lower than results reported by **Anhar (1989)** who found that pH values of Domiati soft white cheese ranged from 5.6 to 5.8 with a mean of 5.72. Also, **Hofi et al (1975)** found that pH values of Domiati soft white cheese ranged from 4.5 to 4.8 with a mean of 4.6. The pH of soft white cheese reflected the change in acidity. The relatively decreased pH of soft white cheese samples in this study may be attributed to the fact that they were made from raw milk with high level of natural microflora.

The acidity developed in Tallaga cheese (0.47%) and Double Cream cheese (0.68%) are lower than measured in Baramely soft white and Istanbul soft white cheese (1.30% and 0.98%, respectively). These results identified Istanbul soft white as a cheese with salt and acidity levels controlling the microbial hazards (**Abou-Dawood et al 2005**).

In this type of cheese, sodium chloride (NaCl) is normally added to milk before renneting (**Abd El-Salam et al 1976**). Salt content in cheese samples were 10.60, 13.20, 11.80 and 12.60 % in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were above the limits required by the **Egyptian standards (1008-3/2005)** which should not exceeded 9%. These results were higher than the results reported by **Abou-Dawood et al (2005)** who found that salt content of Domiati cheese ranged from 3.0 to 6.0 with a mean of 4.2. The relation between salt content and cheese quality could be explained on the basis that the high salt content would interfere with normal ripening processes. Besides it would overcome the sensory effect of the flavor constituents (**Hofi et al 1975**). The ash content is reflecting the salt content of cheese samples which were 12.40, 13.70, 12.60 and 11.80 % in Tallaga, Istanbul, Double cream and Baramely soft white cheese, respectively. These results were higher than the results reported by **Idris et al (1989)** who found that ash content of Domiati soft white cheese ranged from 8.10 to 10.07 with a mean of 9.13%.

CONCLUSION

In conclusion, it can be said that the raw milk cheese in the study area was satisfactory. It is confirmed by the high values of total bacterial count, Coliform, *Staph. aureus* and yeasts & molds counts. Soft cheeses might represent a health risk for the consumers and considered as a possible vehicle of infection or vehicle of transmission for well established pathogens so the cheese made in street, farmers' home or in unlicensed factories is not safe for human consumption. More and more of proper inspection must be done on street markets, supermarkets, stores, factories, unlicensed factories of dairy products. The development and application of microbiological hazard must follow established basic principles to control presence or growth of pathogenic microorganisms.

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