



BACTERIOLOGICAL HAZARD OF WHITE CHEESE PROCESSED IN SOME SMALL PRIMITIVE PLANTS (DAIRY SHOPS) IN TANTA CITY

Gamal I. Heikal¹; Dalia F. khater¹; and Safaa A. Al-wakeel²

¹Food Hygiene Dept., Tanta Lab., Animal Health Research Institute. ² Animal Reproductive Research Institute

ABSTRACT

This study has been conducted to assess the bacteriological quality and safety of traditional white cheese made from raw milk, as well as, to investigate possible contamination sources and their microbial loads during production of the cheese in some dairy shops, Tanta, Egypt. A total of 120 samples and swabs were obtained from 3 different local dairy shops (40 samples/shop); five samples or swabs of raw milk, rennet, whey, cheese, cheese vat, cheese cloth, curd cutting knife and polyethylene bags were collected and analyzed for enumeration of total aerobic bacteria, staphylococci, and coliforms, with isolation of *Escherichia coli*, *Salmonella* spp. *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*. Generally, cheeses showed high microbial counts reached to 6.80, 4.41 and 4.37 Log cfu/g for total aerobe, coliforms and staphylococci respectively. *Bacillus cereus* and *Clostridium perfringens* were not detected in any cheese sample, while *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* was found in 26.7%, 6.7% and 6.7% of examined cheese samples respectively. The raw milk was also of unsatisfactory quality and contaminated with similar pathogenic flora; but the effects of rennet and whey on the bacterial profile of cheese was found to be marginal. In conclusion, it was observed that the hygienic quality of white cheeses sold in dairy shops in Tanta city was low and does not have enough assurance in terms of public health. The results emphasized that the raw milk and equipment has been shown to be a possible contamination sources during processing of soft cheese.

Keywords: cheese; microbiological quality; pathogens; food safety.

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1. INTRODUCTION

Fresh soft cheeses are made by using only traditional methods in the different geographical locations in Egypt. The traditional method of manufacture involves renneting, curd formation and preparation for markets. This cheese is made from raw milk (usually heat-treated) without starter cultures. Microbial contamination of cheese may originate from various sources. Such sources might be during cheese production (Temelli et al., 2006), storage (Brito et al., 2008) or from humans contamination (Callon et al., 2008). However, in this study an attempt is made

to study the main sources of cheese contamination with pathogenic bacteria, i.e. the raw milk and routes of contamination at cheese processing levels. The safety of raw milk cheese has been questioned, however, as several large outbreaks of foodborne disease due to consumption of raw milk cheeses have been reported in the past 10-20 years (Ryser, 2001; West, 2008). In addition, several studies in which milk was intentionally inoculated with some pathogenic bacteria have shown that some pathogens can survive the manufacturing process (D'Amico et al., 2008a; D'Amico

et al., 2010; Schwartzman et al., 2011). There are many researches related to the determination of chemical and microbiological quality of cheese (Gulmez et al., 2001; Kalkan et al., 1991; Nizamlioglu et al., 1989) but there are only a few researches conducted to detect microorganisms that cause quality problems and to determine the contamination sources during cheese production (Kasimoglu, 1998; Evrensel et al., 2003). Moreover, analyses of the microbiological quality of raw milk and cheese made from raw milk have revealed varying levels of quality and safety, ranging from raw milk samples that met pasteurized milk standards to those that tested positive for several pathogens (D'Amico et al., 2008a, b; D'Amico and Donnelly, 2010; Giannino et al., 2009; Little et al., 2008). However, there have been few nation-wide surveys of raw milk cheese that have specifically screened for the presence of pathogenic bacteria. Therefore, the objective of this study was to assess the microbiological quality and safety of Egyptian white soft cheese made from raw milk, as well as, to investigate possible microbiological contamination sources and their microbial loads during production of such cheese type in some dairy shops, located in Tanta, Egypt. Identifying these points helped us to find several solutions to eliminate or minimize the microbial contamination that might occur during cheese production.

2. MATERIALS AND METHODS

2.1. Sample collection

120 samples and swabs of raw materials, cheese, equipment and packaging material used during production of traditional white cheese were obtained from 3 dairy shops (40 samples /shop) in Tanta/Egypt. From each dairy shop, 5 samples were collected from raw milk, rennet, whey and cheese; while 5 swabs were taken from cheese vat, cheese cloth, curd cutting knife and

packaging material (polyethylene bags) in different periods. All samples were stored at 4°C till examined within 24 hours of collection.

2.2. Preparation of samples

For microbiological analyses minimum of 500 g from cheese was taken into sterile polyethylene bags while 200 ml from each of raw milk, rennet and whey were collected into sterile laboratory bottles with screw caps. Surface swab samples were taken from the cheese vat, cheese cloth, curd cutting knife and packaging material, where a 10 cm² area has been swabbed by a prewetted swab in 0.1% sterile peptone water, placed and transported in the same diluent in a cooler within an hour of sampling. All samples were diluted up to 10⁻⁸ in 0.1% sterile peptone water and microbiological analyses were performed.

2.3. Bacteriological examination

All samples were analyzed for total aerobic count; coliform count; staphylococci count; isolation and identification of *Escherichia coli*, *Salmonella* spp. *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens* according to standard procedures (Wehr and Frank, 2004).

2.4. Statistical analysis

Statistical analyses were carried out using SPSS program (SPSS 2009) using Completely Randomized Design and General linear models. Duncan's multiple range test was used for mean separation at $p < 0.05$.

3. RESULTS

3.1. Total Aerobic Plate Counts

The mean total aerobic counts of the examined samples are presented in Table 1. The total aerobic plate counts in all samples were in the range of 2.73 to 7.30 log cfu/ml. Generally, the raw milk and cheese samples showed insignificant

differences in the 3 shops ($p>0.05$), and recorded the highest (4.42 – 7.30 log cfu/ml) numbers of bacterial growth, as shown in Table 1. However, significant increase ($P>0.05$) was obtained when comparing means for raw milk or cheese samples with other samples. The total aerobic counts for equipment (cheese vat, cheese cloth and cutting knife) were in the interval 3.21 – 5.21 log cfu/cm²; while rennet and whey had lower counts (2.73 – 3.57 log cfu/ml) as shown in Table 1.

3.2. Coliform Counts

The mean total coliform count in all samples ranged from 2.00 to 7.66 log cfu/ml, with raw milk samples having the highest count especially in shop I (Table 2). Significant differences ($P>0.05$) were found between raw milk and other samples. Moreover, the coliform count was not detected in equipment samples or packaging material.

3.3. Staphylococci Counts

The mean range of staphylococci count was 2.15 to 6.36 log cfu/ml in all examined samples, with raw milk samples in shop I recording the highest followed by cheese samples of the same shop (Table 3). Comparison of the different samples in shop I or II, raw milk samples showed significant increase ($P>0.05$); while insignificant decrease were found between the raw milk and cheese samples in shop III (Table 3).

3.4. Prevalence of foodborne pathogens

Importantly, the entire samples tested were negative for the two pathogens: *Bacillus cereus*, and *Clostridium perfringens*; furthermore, the analysis of rennet, whey and equipment were negative for all tested foodborne pathogens. *E. coli* was detected in 46.7% of raw milk and 26.7% of cheese; *Salmonella* spp. was detected in 20% of raw milk and 6.7% of cheese; while *Staphylococcus aureus* was detected in only 6.7% of both raw milk and cheese samples (Table 4).

4. DISCUSSION

Egyptian white cheese falls into the family of soft cheese of east European countries, the east Mediterranean region and North Africa (Abdalla, 1992). Soft white 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).

The bacteriological examination of the examined Egyptian white cheese commercialized in Tanta city was not good and the mean total aerobic plate counts were > 6.2 Log cfu/g. in all shops (Table 1); also, the mean count of total coliform and staphylococci reached to 4.41 and 4.37 Log cfu/g in examined cheese samples of shop I (Table 2 and Table 3). These results might be due to the poor sanitary conditions during cheese processing. Several studies have detected high total bacterial counts in raw milk-white cheese (Elsanhoty *et al.*, 2009; Kheir *et al.*, 2011; Alper and Nesrin, 2013). Similarly, high levels of faecal contamination and staphylococci were previously recorded in soft cheese (Araújo, *et al.*, 2002; Pešić Mikulec, *et al.*, 2012; Jaber, 2011). On contrary, previous study in Egypt (Ghada *et al.*, 2002) showed that total aerobic bacterial count did not exceed 1.4×10^5 cells/gm, which is close to what allowed by the Standard Egyptian Guidelines; and 47.5 % of the tested cheese were free from coliform bacteria and *Escherichia coli*. The 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. Truzyan (2003) stated 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. Generally, the milk used in

Table (1) Mean of total aerobic counts (n = 5) of the examined soft cheese samples, raw materials and equipment.

| Sample | Shop | I | | II | | III | |
|-------------------------|------|-------------------|------|-------------------|------|-------------------|------|
| | | Mean | SD | Mean | SD | Mean | SD |
| Raw milk ^α | | 7.30 ^a | 0.68 | 4.42 ^b | 0.22 | 6.13 ^a | 0.25 |
| Rennet | | 3.57 ^c | 0.42 | 3.21 ^c | 0.20 | 2.77 ^c | 0.15 |
| whey | | 3.12 ^c | 0.56 | 3.25 ^c | 0.24 | 2.73 ^c | 0.41 |
| Cheese ^β | | 6.80 ^a | 0.55 | 6.47 ^a | 0.37 | 6.22 ^a | 0.18 |
| Cheese vat ^γ | | 4.53 ^b | 0.20 | 3.31 ^c | 0.19 | 4.25 ^b | 0.32 |
| Cheese cloth | | 5.21 ^b | 0.51 | 3.79 ^c | 0.39 | 5.05 ^b | 0.28 |
| Cutting knife | | 4.27 ^b | 0.20 | 3.21 ^c | 0.19 | 4.12 ^b | 0.17 |
| Packaging material | | <1.0 | 0 | <1.0 | 0 | <1.0 | 0 |

^α Log cfu/ml.

^β Log cfu/g.

^γ Log cfu/ cm².

a–c: Differences between the samples demonstrated with different small letters in the same column are significant ($P < 0.05$).

Table (2) Mean of total coliform counts (n = 5) of the examined soft cheese samples, raw materials and equipment.

| Sample | Shop | I | | II | | III | |
|-------------------------|------|-------------------|------|-------------------|------|-------------------|------|
| | | Mean | SD | Mean | SD | Mean | SD |
| Raw milk ^α | | 7.66 ^a | 0.39 | 5.56 ^a | 0.88 | 5.33 ^a | 1.01 |
| Rennet | | 2.15 ^d | 0.22 | 2.15 ^c | 0.22 | 2.00 ^c | 0.10 |
| whey | | 3.09 ^c | 0.64 | 2.43 ^c | 0.12 | 2.26 ^c | 0.28 |
| Cheese ^β | | 4.41 ^b | 0.27 | 3.75 ^b | 0.54 | 3.39 ^b | 1.35 |
| Cheese vat ^γ | | <1.0 | 0 | <1.0 | 0 | <1.0 | 0 |
| Cheese cloth | | <1.0 | 0 | <1.0 | 0 | <1.0 | 0 |
| Cutting knife | | <1.0 | 0 | <1.0 | 0 | <1.0 | 0 |
| Packaging material | | <1.0 | 0 | <1.0 | 0 | <1.0 | 0 |

^α Log cfu/ml.

^β Log cfu/g.

^γ Log cfu/ cm².

a–d: Differences between the samples demonstrated with different small letters in the same column are significant ($P < 0.05$).

Table (3) Mean of total staphylococci counts (n = 5) of the examined soft cheese samples, raw materials and equipment.

| Sample | Shop | I | | II | | III | |
|-------------------------|------|-------------------|------|-------------------|------|-------------------|------|
| | | Mean | SD | Mean | SD | Mean | SD |
| Raw milk ^α | | 6.36 ^a | 0.80 | 3.46 ^a | 0.12 | 2.31 ^a | 0.24 |
| Rennet | | <2.0 | 0 | 2.15 ^b | 0.22 | <2.0 | 0 |
| whey | | 2.15 ^c | 0.22 | <2.0 | 0 | <2.0 | 0 |
| Cheese ^β | | 4.37 ^b | 0.72 | 2.26 ^b | 0.24 | 2.51 ^a | 0.13 |
| Cheese vat ^γ | | <2.0 | 0 | <2.0 | 0 | <2.0 | 0 |
| Cheese cloth | | <2.0 | 0 | <2.0 | 0 | <2.0 | 0 |
| Cutting knife | | <2.0 | 0 | <2.0 | 0 | <2.0 | 0 |
| Packaging material | | <2.0 | 0 | <2.0 | 0 | <2.0 | 0 |

^α Log cfu/ml.

^β Log cfu/g.

^γ Log cfu/ cm².

a–c: Differences between the samples demonstrated with different small letters in the same column are significant ($P < 0.05$).

Table (4) Prevalence of some foodborne pathogens in examined raw milk and cheese samples.

| Shop Sample | Pathogen | I | | II | | III | | Total | |
|-------------|----------------|---|--------------|----|--------------|-----|--------------|-------|--------------|
| | | N | Prevalence % | N | Prevalence % | N | Prevalence % | N | Prevalence % |
| Raw milk | <i>E. coli</i> | 3 | 60% | 2 | 40% | 2 | 40% | 7 | 46.7% |
| | <i>Salm.</i> | 2 | 40% | 0 | 0% | 1 | 20% | 3 | 20% |
| | <i>Staph.</i> | 1 | 20% | 0 | 0% | 0 | 0% | 1 | 6.7% |
| | <i>E. coli</i> | 2 | 40% | 1 | 20% | 1 | 20% | 4 | 26.7% |
| Cheese | <i>Salm.</i> | 1 | 20% | 0 | 0% | 0 | 0% | 1 | 6.7% |
| | <i>Staph.</i> | 1 | 20% | 0 | 0% | 0 | 0% | 1 | 6.7% |

making cheese in this study shows high total aerobic, coliforms and staphylococci counts; but the effects of rennet and whey on the bacterial profile of cheese was found to be marginal. This result is parallel to the researches proving that the raw milk is a contamination source in cheese production (Pesic-Mikulec and Jovanović, 2005; Temelli et al., 2006). Average total aerobic counts of cheese vat in the three dairy shops were determined as 4.53, 2.31 and 4.25 log cfu/cm², respectively (Table 1). In addition, cheese cloth was determined to affect the total aerobic count significantly (Table 1). The effect of cheese vat and cheese cloth came out to be insubstantial for the other bacteria analyzed. The curd cutting knife's had relatively high (>4 Log cfu/cm²) total aerobic bacterial count particularly in dairy shop I & III; (Table 1). This result complies with previous studies indicating that the curd-cutting knife is a contamination source in cheese production (Mavropoulos and Arvanitoyannis, 1999). The low contamination of the equipment used in dairy shop II may attributed to the efficient cleaning and sanitization. These results indicate that final product quality is affected by the disinfection of equipment used in production (Legnoni et al., 2004; Temelli et al., 2006). Bacteriological examination of packaging material (polyethylene bags) used in the production indicated absence of aerobic bacteria (Table 1). No coliforms (Table 2) or

staphylococci (Table 3) were detected from the packaging material samples analyzed. These results show that the packaging material did not pose a microbiological risk for the examined cheese. In this study an attempt was made to detect main sources of cheese contamination. The bacteriological analysis of the raw materials and equipment used in the manufacture of soft cheese indicated that raw milk, most probably, is the main contamination source of total aerobic bacteria, coliforms and staphylococci. It has been shown that raw milk is a potential source of cheeses contamination (André et al., 2008; Tondo, et al., 2000). Additionally, the present results determined equipment as probable contamination sources of total aerobic bacteria. The important role of equipment in cheese contamination was previously reported (Temelli et al., 2006; Kousta et al., 2010). The foodborne pathogens that were examined in the present study were: *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens*. These pathogens have been selected because it is well documented that foodborne outbreaks caused by the consumption of various types of cheeses were contaminated with that pathogens (Araújo et al., 2002; Haeghebaert et al., 2003; Gaulin et al., 2012). Moreover, the presence of these pathogenic bacteria in raw milk or cheeses pose a threat to human health due to the

increased number of cases and the severity of symptoms. Unexpectedly, *Bacillus cereus*, and *Clostridium perfringens* were not detected in any cheese sample in this study. This is contrary to results reported by Tudor et al. (2009), who detected *Bacillus cereus* and *Clostridium perfringens* in 1.47% and 1.68% respectively, out of 954 traditional Romanian cheeses. However, problems with *Bacillus cereus* and *Clostridium perfringens* do exist as evidenced by outbreaks of gastrointestinal food poisoning (Fricker et al., 2007; Sanz et al., 2002).

The examined Egyptian white cheese samples were produced under unmechanised conditions. Various types of microorganisms may enter the cheese during manufacture and subsequent handling (Turantas et al., 1989). In the 15 cheese specimens examined in this study, the coliform bacterial count was detected to be between 3.39 and 4.41 Log cfu/g. (Table 2) and the *E. coli* was found in 4 (26.7%) samples. Furthermore, one sample (6.7%) of cheese in shop I contained *Staph. aureus* and another sample revealed *Salmonella* spp. (Table 4). These results are not in accordance with the Standard Egyptian Guidelines which allow maximum possible coliform bacterial count in cheese up to 1.0 Log cfu/ g. In addition, there should be no *E. coli*, *Staph. aureus* or *Salmonella* spp. in cheese (EOSQ, 2005). Several surveys have shown the presence of foodborne pathogens in various types of cheeses (De Reu et al., 2004; Kongo et al., 2008; Rosengren et al., 2010; Aydin et al., 2011; Brooks et al., 2012). Most of the published studies refer to the prevalence of foodborne pathogens in cheese at retail level and there is little information on the prevalence of pathogenic bacteria in cheese at processing level. Subsequently, however, several challenge studies suggested that pathogenic bacteria, if present in milk prior to cheese making,

could survive the manufacture and aging process (Bachmann and Spahr, 1995; Reitsma and Henning, 1996; Mohammadi et al., 2009). There were rare studies about detection of *Salmonella* spp. in cheese sold at markets. In the present study, one cheese sample (6.7%) was positive for *Salmonella* spp. (Table 4). *Salmonella* spp. was previously detected in only 6 (2.4%) and 3 (2.5%) samples of local cheeses sold in Istanbul and Sulaimani (Colak et al., 2007; Arif et al., 2012). On the other hand, the present study detected that out of 15 raw milk samples, 7, 1 and 3 samples were found to be contaminated with *E. coli*, *Staph. aureus* or *Salmonella* spp. respectively (Table 4). These data showed that the quality of incoming raw milk was poor. In previous study (Tondo et al., 2000), raw milk was established as the main source of contamination with *S. aureus* for four contaminated final products. Contamination of milk and cheese with *S. aureus* by food handlers was also reported (Callon et al., 2008; Roberson et al., 1994). In this study, the presence of *E. coli*, *Salmonella* spp. and *staph. aureus* in the examined cheeses seems to be related with the use of raw milk and non-hygienic production processes. Furthermore, it was reported by various researchers that low microbiological quality of white cheese is referred to the poor hygienic conditions in small primitive production establishments (Bostan et al., 1992; Tekinsen et al., 1993).

In conclusion, the results demonstrate that the hygienic quality of white cheeses sold in dairy shops in Tanta city was low and did not have enough assurance in terms of public health. The raw milk had been shown to be a potential source of cheese contamination, also, equipment were additional probable sources of total aerobic bacteria. To improve the safety of cheese efforts to raise awareness of the importance of hygiene barriers and raw milk quality as well as ensuring proper decontamination of processing equipment

is essential to improve the safety of cheese for human consumption. It is important to initiate good hygiene practice (GHP) applications in farms to produce safe dairy products.

5. REFERENCES

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الخطورة البكتيرية للجبن الأبيض المصنع في بعض المصانع البدائية الصغيرة (محلات الألبان) بمدينة طنطا

جمال ابراهيم هيكل¹، داليا فتحي خاطر¹، صفاء الوكيل²

¹قسم صحة الأغذية- معمل طنطا- معهد بحوث صحة الحيوان، ²معهد بحوث تناسليات الحيوان

الملخص العربي

أجريت هذه الدراسة لتقييم مدى الجودة البكتيرية وسلامة الجبن الأبيض التقليدي المصنع من الحليب الخام، وكذلك معرفة مصادر التلوث المحتملة وحمولتها الميكروبية أثناء إنتاج الجبن في بعض محلات الألبان التجارية بمدينة طنطا-مصر. وقد حيث تم تجميع عدد 120 عينة ومسحة من 3 محلات ألبان مختلفة بمعدل 40 عينة لكل محل، حيث أخذت 5 عينات أو مسحات من الحليب الخام، المنفحة، الشرش، الجبن، الأوعية، الشاش، سكاكين التقطيع وأكياس التعبئة والتغليف؛ وذلك بغرض تحليلها للعد الكلي للبكتيريا الهوائية، وبكتيريا المكورات العنقودية، والكوليفورم، مع عزل بعض ميكروبات التسمم الغذائي مثل ميكروب إي كولاى، السالمونيلا، العنقودي الذهبي، الباسيلس سيرس، والكلوستريديوم برفرنجينز. وقد أفادت النتائج تلوث الجبن بأعداد ميكروبية عالية من البكتيريا الهوائية، والكوليفورم والميكروبات العنقودية وصلت إلى 6.80، 4.41، 4.37 لوج وحدة ميكروبية / جرام على التوالي. وقد لوحظ أنه لم يتم الكشف عن ميكروب الباسيلس سيرس، والكلوستريديوم برفرنجينز في أي عينة جبن، بينما وجدت ميكروبات إي كولاى، السالمونيلا. والمكورات العنقودية الذهبية في 26.7%، 6.7% و6.7% من عينات الجبن المفحوصة على التوالي. كما لوحظ أن جودة الحليب الخام أيضا كانت غير مرضية وبه نفس مسببات التلوث الغذائى المعزولة سابقا؛ وذلك على عكس عينات المنفحة والشرش التي كان تأثيرها على الجودة البكتيرية للجبن هامشيا. وقد خلصت الدراسة عن سوء الجودة الصحية للجبن الأبيض الذي يباع ويصنع في محلات الألبان بمدينة طنطا وأنه له تأثير ضار على الصحة العامة. كما أكدت النتائج أن الحليب الخام والأدوات المستخدمة في صناعة الجبن من مصادر التلوث المحتملة أثناء تجهيز الجبن الطري.

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