Assessment of Aflatoxin M₁ Levels and Microbiological Quality in Egyptian White Soft Cheese

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Abstract: The quality evaluation of white soft cheese in the Egyptian markets is an urgent need to create awareness among population about the existing situation and to protect the consumer’s health and rights. Thus, the aim of this work was to determine AFM₁ levels and evaluate microbiological properties in commercially available white soft cheese collected from local markets as well as to evaluate the manufactured control samples using the Hazard Analysis Critical Control Points (HACCP) system. Results revealed that AFM₁ concentration was detected in almost half of the samples (46.66%) with an overall mean of 14.39 ng/kg and was detected in 24 samples at levels below 1.0 ng/kg and in 15 samples at levels ranging from 10 to 50 ng/kg, whereas about 3 samples were higher than 50 ng/kg. All positive samples of white cheeses exceeded the Egyptian regulations (free from AFM). The microbiological analysis revealed that all samples contained high total viable count (TVC), which ranged from 4.20 to 8.30 log₁₀ CFU/g as well as high counts of moulds and yeast ranging from 2.30 to 3.95 log₁₀ CFU/g. The pathogenic bacteria coliforms and staphylococci were detected in 13.33 and 33.33 % of the collected cheese samples respectively. On the other hand, the standard manufactured control samples prepared according to the HACCP system were free of AFM and pathogens. Six critical control points (CCPs) were identified during manufacture of soft white cheese. In conclusion implementation of the HACCP system in dairy industries, suggests an efficient mean for limiting mycotoxin and pathogen contamination in Egyptian white cheese.

Key words: White Soft Cheese • Microbiological Quality • Pathogens • Aflatoxin M₁ • HACCP System

INTRODUCTION

Cheese is a vital fermented dairy product, which has a major role in human nutrition for centuries. White soft cheese is one of the common delicious cheeses consumed in Egypt. There are many varieties of white soft cheese depending on the technique of manufacture, salt percentage and many other factors. Cheese is an excellent tasty, 99% digestible energy food, which is suitable for all age groups and contains high quality proteins [1]. White soft cheese is made mainly from pasteurized milk by different methods and stored at low temperature with or without brine [2].

Milk and dairy products represent fundamental items in the human diet and can be the principal way for aflatoxins (AFs) to be ingested [3, 4]. Aflatoxin M₁ (AFM₁) is a hepatocarcinogen found in the milk of animals that consume feeds contaminated with aflatoxin B₁ (AFB₁) [5], whereas AFB₁ is metabolically bio transformed into a hydroxylated form (4-hydroxy derivatives) in hepatic microsomal mixed function oxidase system to produce AFM₁ [6]. The occurrence of AFM₁ in milk and milk products is a potential threat to the health of consumers of dairy products and represents a worldwide concern mainly because these products are widely consumed by children, the major consumers, who are more susceptible to the adverse effects of mycotoxins. The International Agency for Research on Cancer classified AFM₁ as Group 2, a probable human carcinogen [7].

For this reason, many investigators have studied the occurrence of AFM₁ in milk and dairy products. Several investigators reported that AFM₁ is detected in 19.5, 26.5
and 53.5% of cheese samples manufactured in France, Germany and the Netherlands, respectively [8] and in 91% of the Grana Padano cheese samples [9]. On the other hand, the presence of AFM$_1$ in milk and milk products in Egypt was reported [10, 11]. Thus to reduce the risk of exposure, many countries have set or proposed maximum permissible levels of AFM, in milk, whereas the European Communities have set a maximum admissible level of 0.05 ppb in raw milk, heat-treated milk and milk for the manufacture of milk based products [12]. In addition, regulatory limits for dairy products such as cheese have also been introduced by few countries, where some countries adopted a tolerance limit for AFM$_1$ in cheese between 0.250 and 0.500 ng/g [13].

Microorganisms may be introduced during cheese processing by cross-contamination from milk or from infected humans handling the food. These microorganisms reflect unsuitable procedures and practices during manufacture, especially the use of unheated milk as starting materials or heat treatment with low temperature [14]. The number and types of micro-organisms present in dairy products depends on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured such as the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages and quality of rinsing water [15].

Microorganisms such as coliforms, yeast and moulds enter as post-pasteurization contaminants of fresh cheese such as cottage and white cheese and thus may be subjected to spoilage [16]. Many of the common enteric pathogens such as Salmonella, Escherichia coli O157: H7 and Campylobacter are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats. Thus effective cleaning procedures, including removing faecal material from udders prior to milking and good manufacturing practices during cheese making process can reduce the risk [17]. The presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the cheeses and can produce mycotoxins which represent a potential health risk [18]. The major toxigenic species of fungi are those belonging to genera Aspergillus, Fusarium, Acremonium and Phomopsis [19].

In Egypt, the available data on the content of AFM$_1$ in raw milk and cheese are rare. Thus, the aim of this work was to determine AFM$_1$ levels and evaluate the microbiological quality of commercially available white soft cheese collected from local markets as well as to evaluate the manufactured control sample using the HACCP system.

**MATERIALS AND METHODS**

**Sampling:** Forty-five samples of white soft cheese were collected in clean dry and sterilized plastic containers from low-priced markets (Group I, $n=15$), vendors (Group II, $n=18$) and expensive markets and big known companies (Group III, $n=12$) in Cairo, Egypt during the period from April to May 2012. Three standard control samples were manufactured using the HACCP system under laboratory scale [20] at the Department of Dairy Science, National Research Centre. Raw cow milk was obtained from the herd of the faculty of agriculture, Cairo University (Cairo, Egypt). Rennet was purchased from (Hala, Hansen lab, Denmark).

**Manufacture of White Soft Cheese:** The milk was strained on the microbial quality of milk used, heat treatment of milk, the conditions in which the products are manufactured such as the temperatures and duration of storage, feeding of the animals, season, area, general sanitation in the plant, quality of starter cultures, occurrence of phages and quality of rinsing water [15].

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In Egypt, the available data on the content of AFM$_1$ in raw milk and cheese are rare. Thus, the aim of this work was to determine AFM$_1$ levels and evaluate the microbiological quality of commercially available white soft cheese collected from local markets as well as to evaluate the manufactured control sample using the HACCP system.
**Immunoaffinity Clean Up:** The filtrate was passed through an immunoaffinity column (AFM, VICAM, USA) for clean-up by 5mL of purified water at a rate of about 2 drops/s. AFM was eluted with 1.0 mL methanol HPLC grade (BDH Laboratory supplies, Pool, England, UK) at a rate of 1-2 drops/s and then evaporated to dryness under a stream of nitrogen. Samples were quantitatively analysed using HPLC.

**HPLC Analysis:** The HPLC system (Waters, Milford, MA, USA) consisted of a model 1525 binary pump, model 1500 Rheodyne–manual injector, model 2475 multi-wavelength fluorescence detector and data workstation with Breeze software, version 3.3 (Waters, Breeze Software, Milford, MA, USA). AFM was eluted with methanol-water-acetonitrile (60:20-20, v/v/v) at a flow rate of 1 mL/min. Recoveries of AFM was carried out by analyzing spiked blank white cheese samples (n=3) with a mixture of AFM, standard to give a concentration of 0.05, 0.10, 0.25 and 0.50 µg/kg. The spiked samples were extracted as mentioned previously and the recovery recorded 86.00 % for 0.50 µg/kg. Peak identity assigned as AFM was confirmed by comparing test chromatograms with standards, with regard to retention time (Fig. 1).

**Microbiological Analysis:** White soft cheese samples were microbiologically examined according to ICMSF [21]. The samples were examined for total viable count (TVC), coliform, staphylococci, moulds and yeast (log10 CFU/g) according to APHA [22]. Aseptically, approximately 25 g of white soft cheese was 10-fold diluted in 225 mL of sterile sodium citrate solution (2% w/v) for 1 min. Serial decimal dilutions were made and the following analysis were carried out on agar plates in duplicates. 1) Plate Count Agar (PCA, Oxoid) was used for enumeration of TVC; 2) Coliform bacteria count was determined using MacConkey Agar; 3) Staphylococci were enumerated by plating on Baird-Parker Agar (BPA, Oxoid; 4) Mould and yeast were enumerated using Potato Dextrose Agar (PDA, Difco). Pure cultures of the microorganisms were identified using the standard procedures [23].

**Statistical Analysis:** Statistical analysis was performed using SPSS statistical program for windows (Version 16) (SPSS Inc., Chicago, IL, USA). All data were statistically analyzed using analysis of variance.

**RESULTS**

**Product Description:** The major characteristics of white cheese were the snow-white colour, pleasant and slightly acid taste and rich in flavour. The texture was firm, smooth and creamy and some irregular small mechanical openings were desirable. The order and relations of all steps of white cheese production including ingredients, intermediate and end products as well as the CCPs produced a final product with appropriate standards. Six CCPs were identified during manufacture of soft white cheese these included: milk reception, pasteurization, addition of salt and rennet, tray filling and incubation, coagulation and cheese cutting. Pasteurization is the most important CCP, because some pathogens and bacteria such as Mycobacterium can survive under the ripening conditions and be of high risk for public health.

**Aflatoxin M1 Analysis:** The retention time of the AFM peak was 4.235 min (Fig. 1). The recovery of the method for three replicate samples spiked at 0.05, 0.10, 0.25 and 0.50 µg/kg was 82.70, 83.50, 82.42 and 86.00% respectively (Table 1). A summary of the results of the study was shown in Table (2), whereas AFM concentration was detected in almost half of the samples (46.66%) with an overall mean of 14.39 ng/kg. The overall means of AFM level were quite low; nevertheless, group I samples were significantly higher (20.56ng/kg) than those of group II and III samples (10.85and 5.19 ng/kg respectively) (Table 2). The number of positive cheese samples for AFM, in group I, II and III was nine out of fifteen (60.00%), nine out of eighteen (50.00%) and three out of twelve (25.00%), respectively. Data also showed that the maximum AFM concentration is recorded in group I (59.62ng/kg). Data in Fig (2) revealed that AFM concentration is detected in 24 samples at levels below 1.0 ng/kg and in 15 samples at levels ranging from 10 to 50 ng/kg, whereas only three samples were higher than 50 ng/kg.

**Microbiological Quality:** The results of microbial counts (TVC, total coliform group, staphylocoeci, yeasts and moulds) of the cheese samples were presented in tables (3, 4, 5 and 6). The TVC (Table 3) in group II white soft cheeses (5.74 log10 CFU/g) was lower than those of the group I and III white soft cheeses samples (7.31 and 7.21 log10 CFU/g) respectively.

The coliform count (Table 4) in white soft cheese samples were not detected in group III samples (obtained from expensive markets and companies). Meanwhile, coliform count for group I and II ranged from 1.08 to 2.00 and 2.04 to 5.18 log10 CFU/g respectively. The percentage of contamination of coliform count recorded 20.00 and 16.66% for group I and II respectively.
Table 1: Recoveries of aflatoxin M₁ in white soft cheese

<table>
<thead>
<tr>
<th>Spiked level (µg/kg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>82.70±1.1</td>
</tr>
<tr>
<td>0.10</td>
<td>83.50±0.6</td>
</tr>
<tr>
<td>0.25</td>
<td>82.42±1.6</td>
</tr>
<tr>
<td>0.50</td>
<td>86.00±1.5</td>
</tr>
</tbody>
</table>

Results are mean values ± SD obtained from three independent measurements.

Table 2: Aflatoxin M₁ frequency and levels in white soft cheese samples

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>No. of samples (n)</th>
<th>Positive samples (n)</th>
<th>Contamination rate (%)</th>
<th>Aflatoxin M₁ concentration (ng/kg)</th>
<th>Overall Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>9</td>
<td>60.00</td>
<td>1.76±0.49</td>
<td>59.62±0.37</td>
</tr>
<tr>
<td>Group II</td>
<td>18</td>
<td>9</td>
<td>50.00</td>
<td>20.40±1.63</td>
<td>44.75±0.55</td>
</tr>
<tr>
<td>Group III</td>
<td>12</td>
<td>3</td>
<td>25.00</td>
<td>2.00±0.89</td>
<td>20.77±0.33</td>
</tr>
<tr>
<td>Total groups</td>
<td>45</td>
<td>21</td>
<td>46.66</td>
<td>1.76±0.49</td>
<td>59.62±0.37</td>
</tr>
</tbody>
</table>

Results are mean values ± SD obtained from three independent measurements.
Overall means superscripts with different letters are significantly different (P < 0.05).
Collected samples: Group I: small low-priced markets Group II: venders Group III: expensive markets and companies.
*Overall mean of all group ND: Not detected.

Table 3: Enumeration of total viable count from white soft cheese samples

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>No. of samples (n)</th>
<th>Positive samples (n)</th>
<th>Rate of positive samples (%)</th>
<th>Total viable count log₁₀ CFU/g</th>
<th>Overall Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>3</td>
<td>100.00</td>
<td>4.23±0.66</td>
<td>6.14±0.70</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>15</td>
<td>100.00</td>
<td>7.00±0.11</td>
<td>8.30±1.10</td>
</tr>
<tr>
<td>Group II</td>
<td>18</td>
<td>18</td>
<td>100.00</td>
<td>4.20±0.98</td>
<td>7.60±0.45</td>
</tr>
<tr>
<td>Group III</td>
<td>12</td>
<td>12</td>
<td>100.00</td>
<td>7.00±0.33</td>
<td>7.60±0.65</td>
</tr>
<tr>
<td>Total groups</td>
<td>45</td>
<td>45</td>
<td>100.00</td>
<td>4.20±0.98</td>
<td>8.30±1.10</td>
</tr>
</tbody>
</table>

Results are mean values ± SD obtained from three independent measurements.
Overall means superscripts with different letters are significantly different (P<0.05; P<0.10).
Collected samples: Group I: small low-priced markets Group II: venders Group III: expensive markets and companies.
*Overall mean of all group ND: Not detected.

Table 4: Enumeration of coliforms from white soft cheese samples

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>No. of samples (n)</th>
<th>Positive samples (n)</th>
<th>Rate of positive samples (%)</th>
<th>Coliform count log₁₀ CFU/g</th>
<th>Overall Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>3</td>
<td>20.00</td>
<td>1.08±1.35</td>
<td>2.00±0.70</td>
</tr>
<tr>
<td>Group II</td>
<td>18</td>
<td>3</td>
<td>16.66</td>
<td>2.04±1.25</td>
<td>5.18±0.92</td>
</tr>
<tr>
<td>Group III</td>
<td>12</td>
<td>0</td>
<td>0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Total groups</td>
<td>45</td>
<td>6</td>
<td>13.33</td>
<td>1.08±1.35</td>
<td>5.18±0.92</td>
</tr>
</tbody>
</table>

Results are mean values ± SD obtained from three independent measurements.
Overall means superscripts with different letters are significantly different (P < 0.10).
Collected samples: Group I: small low-priced markets Group II: venders Group III: expensive markets and companies.
*Overall mean of all group ND: Not detected.
### Table 5: Enumeration of staphylococci count in white soft cheese samples

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>No. of samples n</th>
<th>Positive samples n</th>
<th>Rate of positive samples %</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Overall Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
| Group I       | 15              | 3                  | 20.00                      | 1.00±0.03| 2.15±1.26| 0.43±0.962*
| Group II      | 18              | 9                  | 50.00                      | 2.30±1.01| 4.30±0.32| 1.63±1.893b
| Group III     | 12              | 3                  | 25.00                      | 2.00±1.36| 3.90±0.70| 0.98±1.950c
| Total groups  | 45              | 15                 | 33.33                      | 1.00±0.03| 4.30±0.32| 1.05±1.632  |

Results are mean values ± SD obtained from three independent measurements.
Overall means superscripts with different letters are significantly different (P < 0.05).
Collected samples: Group I: small low-priced markets
Group II: vendors
Group III: expensive markets and companies
* Overall mean of all group ND: Not detected

### Table 6: Enumeration of mould and yeast counts from white soft cheese samples

<table>
<thead>
<tr>
<th>Sample groups</th>
<th>No. of samples n</th>
<th>Positive samples n</th>
<th>Rate of positive samples %</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Overall Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Group I</td>
<td>15</td>
<td>6</td>
<td>40.00</td>
<td>2.30±1.25</td>
<td>3.08±0.92</td>
<td>1.08±1.49a</td>
</tr>
<tr>
<td>Group II</td>
<td>18</td>
<td>9</td>
<td>50.00</td>
<td>2.30±1.32</td>
<td>3.00±1.27</td>
<td>1.27±1.410a</td>
</tr>
<tr>
<td>Group III</td>
<td>12</td>
<td>9</td>
<td>75.00</td>
<td>2.47±0.57</td>
<td>3.95±1.35</td>
<td>2.36±1.685b</td>
</tr>
<tr>
<td>Total Groups</td>
<td>45</td>
<td>24</td>
<td>53.33</td>
<td>2.30±1.25</td>
<td>3.95±1.35</td>
<td>1.49±1.500</td>
</tr>
</tbody>
</table>

Results are mean values ± SD obtained from three independent measurements.
Overall means superscripts with different letters are significantly different (P < 0.10).
Collected samples: Group I: small low-priced markets
Group II: vendors
Group III: expensive markets and companies
*Overall mean of all groups ND: Not detected

Fig. 1: Auto-scaled chromatogram of (A) aflatoxin M₄ standard (50 ng/mL), (B) aflatoxin M₄ in white cheese samples.
HPLC conditions: Column, phenomenex 4µ, 250 x 4.6 mm; mobile phase, methanol–water–acetonitrile (60:20:20, v/v/v); flow rate, 1.0 mL/min

Fig. 2: Aflatoxin M₄ levels in white soft cheese samples
The enumeration of staphylococci in white soft cheese samples were presented in Table (5). Data revealed that staphylococci were detected in 33.33% of white soft cheese samples obtained from various markets. Results also demonstrated that staphylococci contamination rate was higher in group II samples (50.00%) compared to groups I (20.00%) and III (25.00%) respectively. In the same trend, the overall mean of staphylococci in group II (1.63 log_{10} CFU/g) was higher than those recorded in groups I and III.

The results presented in table (6) revealed that mould and yeast were detected in 53.33% of white soft cheese samples while the lowest yeasts and moulds count was documented for group I samples and recorded 1.08 log_{10} CFU/g, whereas the highest count was documented for group III and recorded 2.36 log_{10} CFU/g. The control samples manufactured according the HACCP system recorded an overall mean of 5.11 log_{10} CFU/g for TVC, whereas these samples were free of coliform, staphylococci and moulds and yeast. It was noticed on comparing the sample groups concerning the rate of contamination (Fig. 3), that coliform and AFM, were lower in group III than in the other two groups. On the other hand, moulds and yeast were higher in group III than in group I and II.

DISCUSSION

Due to the increase of AFs in many food consumptions, studies on the presence of AFM, in dairy products have been increasing globally as well as in Egypt. The current results revealed that the maximum AFM, level in white soft cheese samples (59.62 ng/kg) is lower than those recorded by Amer and Ibrahim [24] who detected AFM, at levels of 87.6 ng/kg. It was also noticed that our detected levels exceeded the tolerant limit for AFM, in cheese set in Egypt, by the Ministry of Health and population which established that fluid milk and dairy products should be free from AFM, [25], but were considered lower than the maximum levels (250 ng/kg) for cheese allowed by the European Commission Regulation [26]. These findings are in agreement with those obtained by Elgerbi et al. [27] who indicated that the decrease of AFM, level in cheese might be due to biological activities during the processing of milk to cheese such as the presence of some lactic acid bacteria strains and related genera that have been reported to be effective in removing AFM,[28, 29]. In this respect, *Lactobacillus* species were previously used to control the presence of AFs in Egyptian Ras cheese artificially infected by *Aspergillus parasiticus* [30]. In the same trend, Hathout and Aly [31] reported that lactic acid bacteria were found to prevent the growth of common food spoilage fungi and extend the shelf life of Talbina (Cereal-Dairy product).

AFM, was detected in white soft cheese in 46.66% with a concentration ranging from 1.76 to 59.62 ng/kg, which is considered lower than those detected previously in Brazil (74.7%) with a concentration ranging from 0.02 to 6.92 ng/g [32]. In agreement, Gürses et al. [33] found that approximately 44% of the cheese samples to be positive for AFM, residues. The current data also revealed that percentage of AFM, contamination in sample groups varied according to markets, cheese were obtained from and in differences in the manufacturing steps of the product. These results are in good harmony with those reported by López et al. [34].

It is well known that the natural occurrence of AFM, in milk and milk products depends on the level of AFB1 in feed. These observations are confirmed by Henry et al. [35] who reported that the rate between the amount of AFB1 ingested by cows and the quantity excreted in milk.
is usually 0.2 to 4%, or about 0.3-6.2% with a linear relationship between intake of AFB, in contaminated feed and the AFM, content of milk [36]. Thus it is important to reduce the occurrence of AFB, in feedstuff and prevent factors enhancing mycotoxin production.

Concerning the microbiological quality of cheese samples it could be concluded that the high TVC (8.30 log_{10} CFU/g) in this study might be due to the low quality of milk used in cheese making or could be due to unsanitary conditions during processing and handling of the cheese samples [37]. These findings are consistent with the results of Elowni and Hamid [38]. Our results indicated that the increase in TVC in group II samples could be related to the increase in coliform (5.18 log_{10} CFU/g) and staphylococci (4.30 log_{10} CFU/g) counts.

Coliform was detected in 20.00 and 16.66% of the cheese samples groups (I and II) under study. These results are lower than those detected in Yemeni soft cheese [39] and those reported by Ayad et al. [40] who detected coliforms in 70% of collected Domiati cheese samples. The coliform count in some cheese samples were probably due to the production of milk and cheese under poor conditions [41, 42]. According to the International Standards, white cheese should not contain more than 100 CFU/g coliform bacteria [43]. On the other hand, the absence of coliform in bacteria and group III cheese samples (which was obtained from expensive markets and companies), reflects good hygienic conditions during production and handling and thus is considered in accordance with the recommendations of the Egyptian Organization for Standardization and Quality who recommended that cheese must be free from coliform [2].

Staphylococci are predominantly of animal origin, although isolation of some species may be made from environmental sources. Some strains of *S. aureus* may be able to colonize equipment and the factory environment and *S. aureus* was isolated from a Turkish white cheese [44]. High numbers of *S. aureus* are generally associated with the presence of toxin (>10^8 CFU/g of cheese). In this concern, Shaker et al. [20] stated that the presence of coliforms and *S. aureus* should be taken into account when considering the safety of cheese.

The presence of moulds and yeast in about 53.33% of the cheese samples with counts ranging from 2.30 to 3.95 log_{10} CFU/g indicates poor hygienic conditions. Our results are lower than those reported by Elowni and Hamid [38] who stated that the moulds and yeast count in white cheese samples is 4.46 log_{10} CFU/g. These results are also lower than those reported by Girgis et al. [45] for Ras cheese in the Egyptian market, which ranged between 0.15x10^4 and 8x10^5 CFU/g. In this concern, Fadda et al. [46] reported that the high numbers of yeasts in some acidified dairy products may be attributed to their ability to tolerate low pH values and low water activities and to grow at low temperatures. On the other hand, moulds and yeast usually present in raw milk, do not survive pasteurization; their presence in pasteurized milk and other milk products is caused by re-infection during manufacturing [47] and can periodically cause problems, both economic and sensory. The contamination of milk products, particularly cheeses may be due to the presence of yeasts and moulds in the environment of cheese factories, like walls and shelves of ripening rooms, air, equipment, water, milk, brine, etc. [48]. The presence of wild types of moulds is undesirable as they may produce mycotoxins, which represent a potential health risk [18].

Generally, the presence of different microbial groups especially the pathogens in white cheese could be attributed to the use of raw milk in its preparation. It is known that the microbiology of raw milk is crucial for the production of any high quality dairy food. Most soft, un-ripened cheeses are microbiologically unstable due to metabolic activity of bacteria, mould and yeast contaminants [49]. The sources of yeast and surface bacteria of smear cheeses are the following: milk, cheese brines and the air of the ripening rooms, ripening shelves and the human skin [50]. High numbers of yeasts are frequently observed on processing equipment and in the air of the processing environment [51], such as wooden tables used for dry salting the cheese blocks.

The control samples were free from coliform, staphylococci, moulds and yeast pathogens, whereas TVC were recordable. These results indicated high sanitary practices which ensured good microbiological quality and good shelf life of soft cheeses throughout the milking, manufacturing steps and post-manufacture handling.

From the above, the results revealed that microorganisms seem to be more effectively controlled by developing the quality system (HACCP) in the case of white soft cheese. Good Manufacturing Practices (GMP) and hygienic rules as well as HACCP during handling and cheese processing have become very important. It could be noticed that the presence of AFM, in cheese samples is not related to the contamination of samples by different pathogenic microorganisms. This is because AFM, is present due to the contamination of animal feed by AFB, in farms, whereas contamination with pathogenic bacteria might be due to the use of raw milk in manufacturing and preparation of cheese. This research is very important if
we know that there have been outbreaks of infection associated with the consumption of cheese and the predominant organisms responsible have included *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus* sp. [52]. There were differences in microbiological quality of cheese samples offered by different processing plants and these differences were not only the consequence of different quality, but of the hygienic conditions at milking process. This study strongly suggests the need for a more strict hygienic and technological control during manufacture. Thus it is necessary to incorporate the HACCP and Microbial Risk Assessment (MRA) plans for prevention of contamination of raw milk and cheese. It is also important to control AFB₁ in farm animal feed to prevent the presence of AFM₁ in milk and milk products.

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**REFERENCES**


