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Bacteriological Quality and Incidence of Some Pathogenic Bacteria in Fresh White Cheese Sold in Tripoli, Libya

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Abstract

The aim of this study was to evaluate the bacteriological quality and safety of fresh white cheese sold in Tripoli, Libya. The study lasted for approximately 7 months (November 2011-May 2012), during this period 87 fresh white cheese samples were collected from seven different areas (4 to 5 factories from each area with the rate of 3 duplicates). The samples were tested for temperature at receiving, pH and acidity, total aerobic counts, total coliform counts and the detection of incidence of some pathogenic bacteria including: *Escherichia coli*, *Escherichia coli* O157:H7, *Staphylococous aureus*, *Salmonella* spp., *Aeromonas hydrophila* and *Listeria monocytogenes*. Results indicated that the average temperature, % acidity, and pH of the tested samples were (15.80°C ± 5.8, 0.21 ± 0.02% and 5.81 ± 0.06) respectively. Data indicated that 70.1% of the samples exceeded the Libyan standard for white cheese No. 366 in respect to temperature, while pH of all samples was within the limits of such standard. On the other hand, means of total aerobic counts, total coliform counts, and the numbers of *Staphyloccoccus aureus* were (38×10^7, 74×10^5, 35×10^4 and 53×10^3 cfu/g) respectively of the study.

Keywords: White cheese; Bacteriological quality; Pathogenic bacteria; *E. coli*; *Staphyloccocus aureus*

Introduction

Cheese are mostly divided according to moisture content as very hard, hard, semi soft and soft cheese. Fresh white cheese is considered soft cheese and according to moisture content and nutrients it is subject to the growth of different microorganisms and pathogens. The presence of *E. coli* in fresh white cheese is an indication of low quality milk, poor production practices or contamination after processing [1-3]. Thus, Identification and enumeration of *E. coli* is widely used as an indicator for evaluating the safety of fresh white cheese. The presence of *E. coli* in fresh white cheese was confirmed by O'Brien et al. [1] in Ireland, Tekinsen and Ozdemir [2], Aygun et al. [4] in Turkey, Araujo et al. [5] in Brazil, Hamid and El-Owni [6] in Sudan and Abo-aza et al. [7] in Syria. Obviously, the magnitude of its presence differs from region to another. Hamid and El-Owni [6] and Karim et al. [8] found that fresh white cheese contains 2.3×10^6 cfu/g and 1.5×10^5 cfu/g, in Sudan and Syria respectively. Similarly, In Antakya, Turkey; Aygun et al. [7] found *E. coli* in fresh white cheese at a rate of 1.02×10^4 cfu/g. Moreover, in a study in Brazil [5] indicated that the range of *E. coli* was 1.0×10^3 to 1.8×10^8 cfu/g. Milk and milk products are important cause of out breaks by *Staphylococcus aureus* in many areas around the world and soft white cheese was reported to be the primary source for such pathogen yet its presence differs from study to another. The percentage of its presence in white cheese varied from 4%, O'Brien et al. [1] to 30.9% Moraes, et al. [9]. Furthermore, it was isolated from 20.6%, 16.3%, 15%, and 14.16% of white cheese by Araujo et al. [5]; De Luca, et al. [10], Karim et al. [8] and Al-abidy and Sharif [11] respectively. Thus, different studies were oriented to the enumeration of Staph. aureus in fresh cheese in different countries. For instance, in Turkey, Irkin [12], Aygun et al. [7] and Tekinsen and Ozdemir [2] reported an average of 1.5×10^3 cfu/g, 2.5×10^3 cfu/g and 1.2×10^6 cfu/g, respectively. On the other hand, Abo-aza et al. [1] and Hamid and El-owni [6] reported the presence of such pathogen in white cheese In Syria and Sudan up to 4.8×10^4 cfu/g and 1.1×10^4 cfu/g respectively. Salmonella is considered the main causative of food poisoning, which contribute to about 90% of food outbreaks. Different investigators in many areas pointed the presence of Salmonella in fresh white cheese [2,4,6,13,14]. *Aeromonas hydrophila* is usually destroyed by pasteurization and its presence in white soft cheese is mostly an indication of post pasteurization contamination. Yet, *Aeromonas* spp. were detected in 17.7% of fresh white cheese samples from Rio de Janeiro, Brazil, 54.4% of those isolates were reported to be *A. hydrophila* [5]. Listeriosis is a very serious food poisoning illness caused by *Listeria* spp. especially *L. monocytogenes*, which was reported to be responsible for about 30% fetal, cases [15-18]. The main objective of this study was to evaluate the bacteriological quality and the presence *of E. coli, E. coli* 0157: H7, *Staph. aureus, Salmonella* spp., *A. hydrophila* and *Listeria monocytogenes* in fresh white cheese sold in Tripoli, Libya. Additionally to investigate the potential effect of sample source on the existence of those microorganisms.

Materials and Methods

The study lasted for approximately 7 months (Nov. 2011-May 2012). During that period 87 fresh white cheese samples were collected from local small factories in 7 areas around Tripoli, Libya (4-5 factories in each area) and transferred aseptically in cooled ice box to the laboratory for further analysis. Fresh white cheese in the studied areas were produced from raw unpasteurized milk by adding rennet enzyme at 40°C and remove the whey immediately after coagulation

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and subjected to selling. The samples were subjected to measuring temperature at receiving, pH (WTW, 300) at 25°C as mentioned by Graham [19] and titratable acidity as specified by Hooi et al [20]. Different microorganisms were enumerated using standard methods for the examination of dairy products [19-21].

Detection of Pathogenic Bacteria

E. coli was isolated and detected as specified by Davidson et al. [22], Hary and Pall [23]. Meanwhile, *E. coli* O157:H7 was detected as specified by Meng et al. [21], Hary and Pall [23]. *Salmonella* were detected using API 20 E System and identified using BD Phoenix, ISO 6579, Hary and Pall [23]. *Staphylococcus aureus*: *Staphylococcus aureus* was detected as specified by Lancette and Bennett [24]. *A. hydrophila* was detected and identified using API 20 E System as specified by Palumbo et al. [25] and ISO 6579. *L. monocytogenes* was isolated and detected as specified by Gasanov et al. [26], Hary and Pall [23].

Statistical Analysis

The data obtained were analyzed statistically using SAS System as specified by Abosalah and Awad [27].

Results and Discussion

Results in Table 1 showed that overall means at receiving point in the factories of temperature, pH, and titratable acidity were 15.8 ± 5.9°C, 5.81 ± 0.007 and 0.21 ± 0.03% respectively. The relatively high temperature of receiving of some samples coincides with high acidity and low pH, beside low quality unpasteurized milk used and the possibility of contamination with spoilage and pathogenic microorganisms including mastitis causing types is consider hazard to consumer. The effect of sample source on temperature at receiving as well as pH, and titratable acidity could be clearly seen from data presented in Table 2. Results indicated that source Z was the highest in temperature (16.7 ± 6.81°C) and source A was the lowest 14 ± 6.74°C. Yet, statistical analysis indicated that source of samples has no significant effect (P<0.05) on temperature of receiving. Concerning pH and % of titratable acidity statistical analysis showed that samples source has significant effect (P<0.05) on acidity and pH. Source H was the lowest in acidity (0.18 ± 0.01%) and highest in pH (6.10 ± 0.02) while source Z showed the highest in acidity (0.24 ± 0.01%) and lowest in pH (5.83 ± 0.02). This could be related to high temperature of receiving since source Z was the highest in temperature of receiving and could be of highly contaminated with spoilage bacteria. Results of the present study are in agreement with data reported by Karim et al. [8] in Syria. Table 3 presents means and variation of total aerobics, total coliform and *Staphylococcus aureus* in all samples. The mean number of total aerobic counts, coliforms, most probable number of thermo-tolerance and *Staphylococcus aureus* were 38×10^7 cfu/g, 74×10^5 cfu/g, 35×10^4 cfu/g and 53×10^3 cfu/g respectively. The highest number of *E. coli* in this study could be related to low quality of milk used, unhygienic production during manufacturing, contamination after processing and the use of unpasteurized milk. Results of the present study are relatively lower than those reported by Moraes et al. [9] of 18×10^6 cfu/g and higher than those reported by Oksuz and Aici [28] of 10^2-10^5 cfu/g. The overall mean of *Staphylococcus aureus* of all samples studied were 53×10^3 cfu/g. Results of this study were close to the finding of Irkin [12] of 15×10^3 cfu/g, but relatively higher than the results of Karim et al. [8] of 2×10^3 cfu/g and lower than the results of Aygun et al. [4] of 25×10^5 cfu/g. Figure 1 presents the number and percentage of pathogenic bacteria in all samples. The number and percentage of samples which contain *E. coli*, *E. coli* O157:H7, Salmonella, *Staphylococcus aureus, A. hydrophila* and *Listeria monocytogenes* were 72 (82.7%), 31 (35.6%), 7 (8%), 62 (71.2%), 21 (24.1%) and zero consequently. The high percentage of *E. coli* (82.75%, P<0.05) in this study could be related to the use of low quality milk, unhygienic production, use of unpasteurized milk and contamination after processing. Generally speaking, results of the present study were relatively better than the finding of O'Brien et al. [1] and Araujo et al. [5], who reported that 97% and 97.7% of their samples contained *E. coli* respectively and higher than the results of Karim [8], which showed only 70%. *E. coli* O157:H7 were present in 31 (35.6%) of samples studied. Results of this study were higher than the results of Mora et al. [29], which indicated that only 7.8% from the fresh white cheese samples studied in Lima, Peru contain *E. coli* O157:H7. Salmonella was isolated from 7 samples (8.04%) of all samples studied, one of the isolates was identified as being *S. cholera* and the other was *S. paratyphi* A (Tables 4 and 5). Results of this study were relatively lower (P<0.05) than the findings of Todd [30] in Mexico, and Karim et al. [8] in Syria who reported that Salmonella was isolated from 13% and 18% of cheese samples respectively. On the other hand, these results

Test	Means ± S.D
Temperature (°C)	15.80 ± 5.9
Acidity (%)	0.21 ± 0.03
pH	5.81 ± 0.07

Table 1: Means and (SD) of temperature, acidity and pH of all samples.

Test Source	No. of Samples	Temperature (°C)	Acidity (%)	pH
A	13	14.0 ± 6.74[a]	0.20 ± 0.02[b]	5.93 ± 0.1[b]
B	15	15.8 ± 5.93[a]	0.19 ± 0.02[c]	5.95 ± 0.03[b]
C	12	16.5 ± 5.54[a]	0.21 ± 0.01[b]	5.88 ± 0.05[c]
D	11	16.5 ± 5.25[a]	0.23 ± 0.02[a]	5.83 ± 0.03[c]
H	12	15.0 ± 6.0[a]	0.18 ± 0.01[c]	6.10 ± 0.02[a]
E	12	16.5 ± 5.38[a]	0.19 ± 0.01[c]	5.96 ± 0.03[a]
Z	12	16.7 ± 6.81[a]	0.24 ± 0.01[a]	5.83 ± 0.02[c]
Over all mean		15.80 ± 5.8	0.21 ± 0.03	5.81 ± 0.06

[abc]LS Means with the different letter within the attribute are significantly different

Table 2: Means and (SD) of temperature, acidity and pH by sources.

Test	Means	Range	
		Minimum	Maximum
c (cfu/g)Total aerobi	38×10^7	29×10^4	10×10^9
Total Coliforms (cfu/g)	74×10^5	15×10^3	14×10^7
(cfu/g)	53×10^3	50×10^0	87×10^5

Table 3: Means and ranges of total aerobic, total coliform, of thermo-tolerant and in all samples.

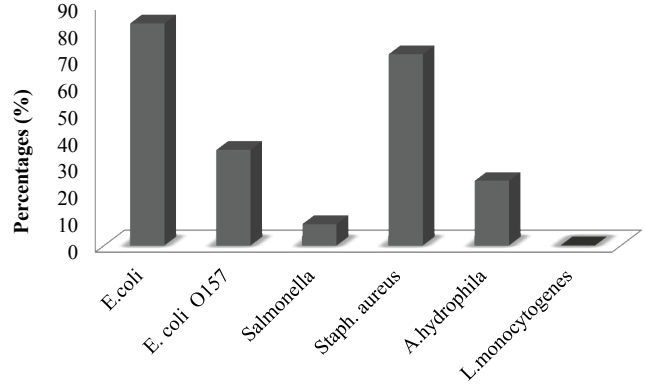

Figure 1: Percentages of pathogenic bacteria in samples.

Salmonella type	Total isolates	% of Isolates
	5	71.4
	1	14.3
	1	14.3

Table 4: Numbers and percentage of spp. in samples.

Parameter	Maximum Permitted Limit by Libyan Standards	Number/(%) out of Libyan Standards
% acidity	Not more than (1.9%)	0/(0.00)
Temperature (°C)	4-10	61/(70.1)
Total Coliforms (cfu/g)	Not more than 5 cfu/g	87/100
Pathogenic bacteria (cfu/g)	Zero	87/100

Table 5: Numbers and percentage of fresh cheese samples out of the Libyan standards for % acidity and temperature and Pathogenic bacteria.

were higher than those reported by AL-Abidy and Sharif [11] in Iraq and Tekinsen and Ozdemir [2] in Turkey of 5% and 6% respectively. *Staphylococcus aureus* was isolated from 62 (71.2%) of the samples studied. These results were relatively higher than those reported by Karim [8] in Syria (5%) and Araujo et al. [5] in Brazil (20%). *Aeromonas hydrophila* was found in 21 samples (24.1%) studied. These results were very similar compared with the study of Effat [31] in Egypt, who isolated *A. hydrophila* from 23.1% of samples of Demiate cheese and less in Garies cheese. *Listeria monocytogenes* was not detected in any of the samples studied. Results of this study were relatively similar to the finding of Moraes et al. [9], Hamid and Elowni [6]. However O'Brien et al. [1] and Colak et al. [13] reported the presence of *L. monocytogenes* in 5% and 4.8% of soft white cheese produced in Ireland and Turkey, respectively.

The Effect of Sample Source on Pathogenic Bacteria

Figure 2 showed that sources A and Z were the highest in *E. coli* (100%) source E was the lowest (41.6%) and sources B, C and D were almost the same. At the same time source Z was the highest in E. coli O157:H7 (58.3%) and source H and E were the lowest (16.6%) (Figure 3). The effect of sample source on Salmonella is shown in Figure 4. The source Z was the highest of 25% and in source B, D and E were 14.2%, 9.8% and 9.8% and not isolated in sources A, D and H. Figure 5 showed the effect of sample source on *Staphylococcus aureus*. The source E and Z were the highest (100%), source C and D (90.6%) and source H was the lowest. In the case of *A. hydrophila* source B was the highest (40%) and source E was the lowest (8%) (Figure 6). Statistical analysis indicated that the source of samples has no significant effect at 5% level on the presence of *E. coli* O157:H7 and *A. hydrophila* while it has significant effect on *E. coli* and *Staphylococcus aureus*. Several factors could contribute to these differences between samples sources including source of raw milk used in manufacturing of fresh white cheese, acidity of milk, temperature of transportation and storage, hygienic status at production point, use of milk from mastitis animals, the use of unpasteurized milk, contamination during and/or after processing. These factors were also confirmed by Gilmour and Row [32], Bramley and Mckinnon [33], Karim et al. [8], and O'Brien et al. [8], Shata and El-Mgdob [33,34]. Finally, the results of this study were evaluated according to Libyan standards of fresh white cheese [35-37]. As presented in Table 5, data of acidity and temperature at receiving point showed 100 and 70.1% agreement with Libyan standards, respectively [38]. On the other hand, total coliforms and other pathogenic bacteria were 100% out of standards. This is a clear indicator for a very low bacteriological quality of fresh white cheese produced at that period. Mostly source Z was the worst of all sources.

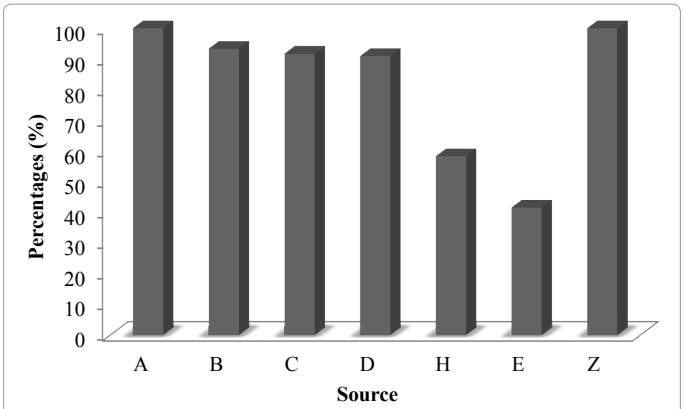

Figure 2: Percentages of *E. coli* in samples according to source.

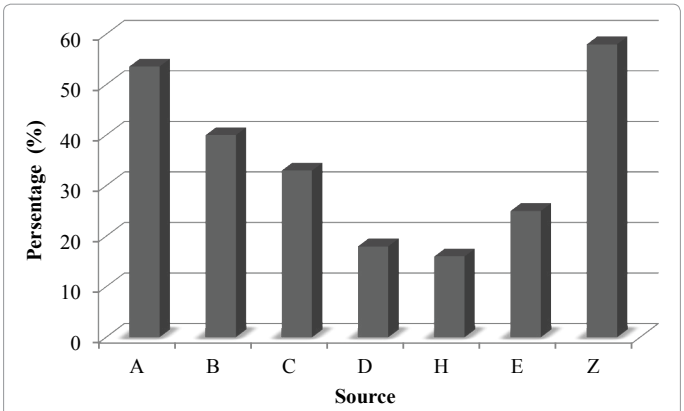

Figure 3: Percentages of *E. coli* O157:H7 in samples according to source.

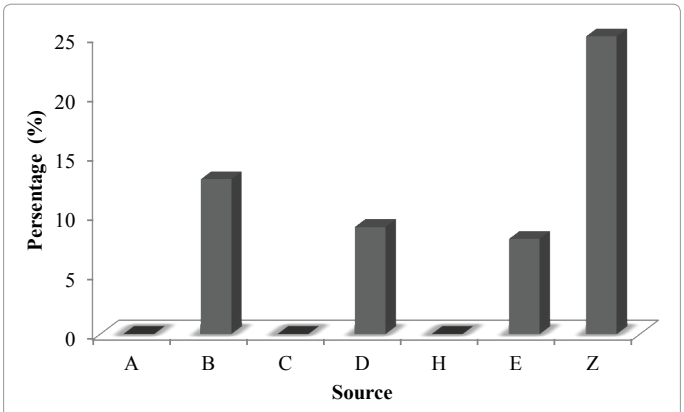

Figure 4: Percentages of Salmonella in samples according to source.

This could be due the area is crowded, undeveloped, poor management, poor sanitation procedures and further far from milk supply.

Conclusion

The relatively high microbial count accompanied with a rise in cooling temperature and the higher incidence of several pathogenic bacteria in the tested cheese samples reflecting the poor sanitation and hygienic practices in those sources and signaling an alarming health threatening problem with the potential occurrence of infection and food poisoning outbreaks among white cheese consumers in Tripoli during the period of this study.

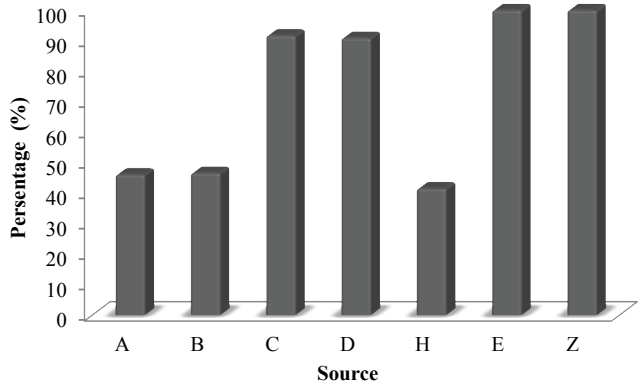

Figure 5: Percentages of *Staphylococcus aureus* in samples according to source.

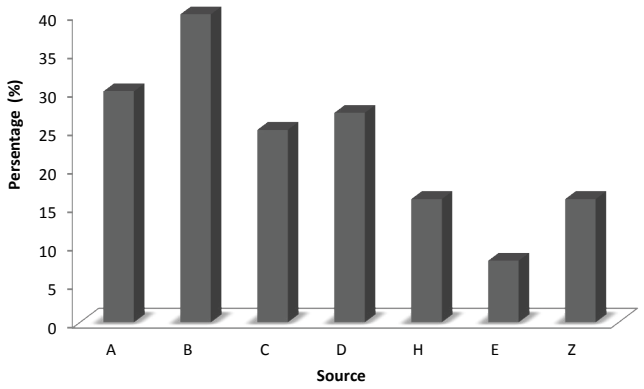

Figure 6: Percentages of *A. hydrophila* in samples according to source.

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