

Microbiological Changes and Determination of Some Chemical Characteristics for Local Yemeni Cheese

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Abstract

The changes in microbiological parameters during the storage of local Yemeni cheese were studied. Both the smoked and the non smoked cheese were produced by using traditional techniques in some regions of Yemen. Microbiological examination was carried out at 2, 4 and 7 days of storage for serve cheese and compared with control cheese prepared in laboratory. During storage period, the total viable bacteria count, lactic acid bacteria, staphylococci, coliforms, yeast and molds increased and reached to 11.97, 10.36, 11.8, 13.4, 11.8 and 8.9 Log cfu/g, respectively, in some samples and then decreased at the end of storage period. Also, the number of coliforms, staphylococci, yeast and molds in cheese samples were higher than limits allowed by the national standards for Yemeni soft cheese. Pathogenic flora as *Salmonella* and *Listeria* were detected in some samples and disappeared at the end of storage period. . The hygienic quality of smoked cheese was best than non smoked cheese. At the final days of storage a sharp drop in pH values changing from 5.4 to 4.1 was noticed. The average contents of chemical composition of smoked and non-smoked cheeses were that, moisture 46.55 and 57.27%, fat 21.29 and 20.67 %, protein 14.87 and 16.98% salt 5.01 and 3.75 % respectively.

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Keywords: Smoked and non smoked Yemeni cheese, microbiological changes, storage period, chemical composition.

1. Introduction

Smoking of foods is one of the oldest methods of food preservation but, presently, foods are smoked for sensory quality rather than for preservative effect. In general, smoking infuses the high-protein food with aromatic components, which lend flavor and color to the food and also play bacteriostatic and antioxidant roles (Bratzler *et al.*, 1969; Poutler, 1988; Horner, 1992). The most common smoked varieties of cheese are Seretpanir (Iran), Caramakase (Germany), Bandal (India), and Provolone (Italy). In a study at Michigan State University in the 1960s, it was found that smoked cheeses sold at 10 cents per pound more than similar nonsmoked cheeses and increased sales by 45% (Kosikowski and Mistry, 1997). There are reports that phenolic compounds found in smoke inhibit growth of microorganisms on smoked Cheddar cheese (Wendorff *et al.*, 1993). Rheological and fracture properties are of great importance for the producer, the market and the consumer. These properties differ depending on the type of cheese, the stage of maturation and also depending on the composition of the cheese as content of water, fat, salt, pH, protein degradation and environmental factors such as temperature (Walstra and Peleg, 1991). Historically there have been

outbreaks of infection associated with the consumption of cheese and the predominant organisms responsible have included *Salmonella*, *Listeria monocytogenes*, Verocytotoxin producing *Escherichia coli* (VTEC) and *Staphylococcus* sp. (Razavilar, 2002; Karim, 2006; Tamagnini *et al.*, 2005)

Smoked cheese is the most popular cheese in Yemen. It is made primarily from raw goat's milk by some villagers under unsanitary conditions. The product is considered as a semi-hard cheese with about 40% moisture content and characterized as a salted cheese with an attractive light brown color imposed by smoking (Al-Zoreky, 1998). There is no standardized technique for the manufacture of Smoked cheese, only using traditional methods in the different geographical locations in Yemen without species starters. It is true that it is potentially unsafe and could cause problems in the future if its production conditions are not improved. These types of cheese were marketing during 7 days after production.

The aim of the present study was to evaluate the changes of the main groups of microorganisms during the storage, handling and determination some chemical characteristics of local Yemeni cheese. Also we sought to investigate, the processed smoked cheese in healthy conditions and its comparison to their traditional products.

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2. Materials and Methods

2.1. Cheese making and samples collection

These types of cheese are made from the milk of cows, sheep or goat by some villagers without heat treatment. Clot milk is extracted from the stomach of young goats, which are no older than two weeks to be used as the milk curdles. A small amount of clot milk is added to fresh milk, mixed and left several hours until formation strength curd, then salt is added. The blocks of cheese are exposed to smoking by using types of wood Althahya or Almziz or Alhamer or other woods used for this purpose.

Five samples of fresh cheese processed by a traditional procedure were collected from several local markets in Taiz (samples A and B from Al-bab alkabeer and samples C, D and E (non-smoked) from Albarh). Samples were transported to the laboratory and kept overnight before analyses. Samples L1 and L2 (non-smoked) were prepared in the laboratory of Biotechnology and Food Technology Department, Faculty of Agriculture and Veterinary by a standard protocol (kosikowski and Mistry, 1997). One type of local wood material, most commonly used in cheese smoking, namely Althahya, was used in this study. The curd cheese (L1 and L2) was pressed and divided into small blocks. The blocks of cheese were subjected to heat and wood smoke by placing them on the top grate with suitable space between blocks of cheese and wood. The smoking was continued until the surface of the cheese blocks had a nice brown color all over and imparted a characteristic aroma and flavor.

2.2. Microbiological analysis

All microbiological analysis was performed according to American Public Health Association (APHA, 2002). Cheese samples (10 g) were mixed with 90 ml of warm (40°C) sterile 2% Na citrate and homogenized in a Stomacher for 3 min. Sequential decimal dilutions of the homogenate were prepared in sterile peptone water and plated in duplicates onto specific media. Plate Count Agar (Himedia, India) was used for the total aerobic bacteria count. The petri dishes were aerobically incubated for 24-48 hours at 37°C. Potato dextrose agar of pH 3.5 adjusted with 10% tartaric acid (PDA) (Himedia, India) was used for moulds-yeast counts. MRS Agar (Biolab, UK) was used for the lactic acid bacteria count. All colonies created after incubation at 35°C for 48-72 hours of plates planted with two layers were counted. Violet Red Bile Agar (Himedia, India) was used to coliform count. *Salmonella* detection was carried out after enrichment of sample in Selenit cystine broth (Himedia, India) and incubated at 37°C for 18-24h. After the enrichment step, the cultures were surface streaked onto *Salmonella* /*Shigella* agar (Oxoid, UK) and colorless colonies with black centers were counted after 48h of incubation at 37°C. Enumeration of *Staphylococcus* sp. was performed on Staph. Agar 110 (Biolab). Yellow colonies were counted after 24h of incubation at 37°C. For *Listeria* detection each sample (25 g/ml) was taken and placed in a stomacher bag to which 225 ml of sterile *Listeria* Selective Enrichment Broth (Oxoid) was added and homogenized with a stomacher and incubated at 30°C for 48 h, the cultures were surface streaked onto Tryptic soy agar (Oxoid CMO131) and

incubated at 30°C for 24-48 h. All Cheese samples were kept in cleaned poly styrene and stored at room temperature for 7 days in the same sales conditions. Above tests were carried out after 2, 4 and 7 days of cheese manufacture, except detection of *Salmonella* and *Listeria* were carried out after 2 and 7 days.

2.3. Chemical Analyses

Moisture and NaCl contents were determined in cheese according to AOAC. 14th.16, 260 (1984) and AOAC. 14th.16, 272 (1984), respectively. The pH values (Inolab 720) in cheese were measured according to the 14022 AOAC (1975) method. Fat and Nitrogen content were determined in cheese according to AOAC. 14th.16, 284 (1984) and AOAC. 14th.16, 284 (1984), respectively.

3. Results

The changes of different microbial groups investigated during the storage of local Yemeni cheese are shown in (Figure 1- Figure 5) and Table 1. All the microbiological analyses were carried after 48 hrs of cheese production.

As results in figure 1 demonstrate, during the storage, smoked and non-smoked cheese (sample A, C, D and E) the total aerobic bacteria counts increased from 8.91, 8.86, 9.4 and 9.4 Log cfu/g to 11.97, 10.1, 9.8 and 10.1 Log cfu/g, respectively, within 48 hrs. Furthermore, 4-7 days after production, the total count of bacteria was reduced to 9.04, 9.3, 9.3 and 9.79 Log cfu/g, respectively. As to sample B the total aerobic bacteria counts gradually increased to the value 9.8 Log cfu/g. In samples A, B, C, D and E the total counts of moulds and yeast gradually increased to 6.6, 7.6, 5.6, 8.9 and 6.5 Log cfu/g, respectively, after 168 hrs of production (Figure 2).

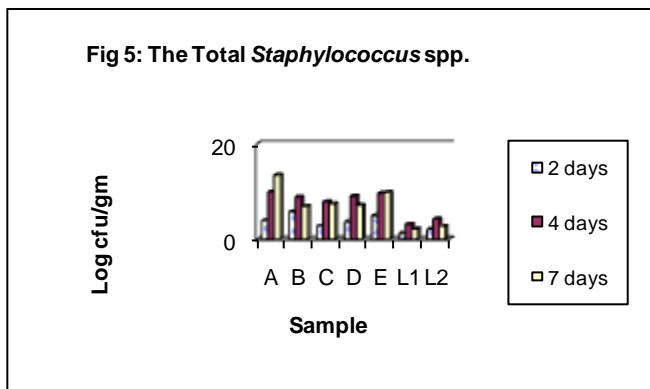
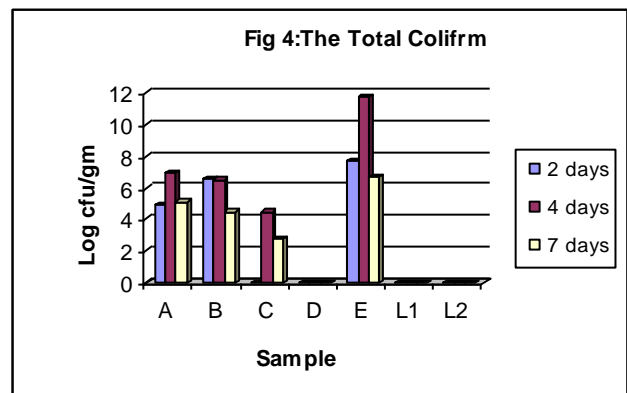
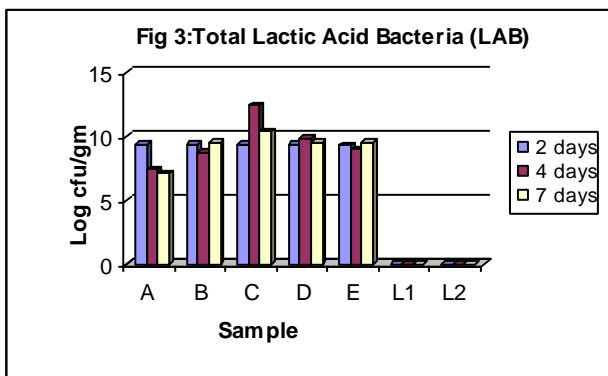
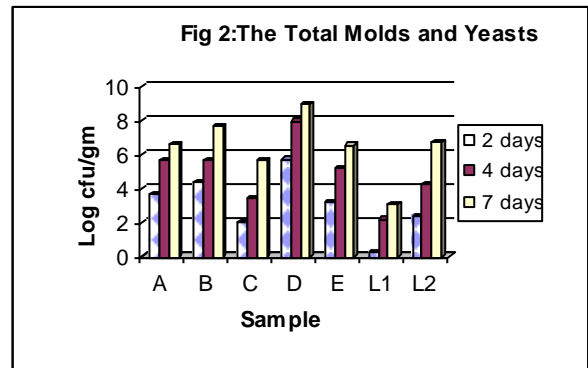
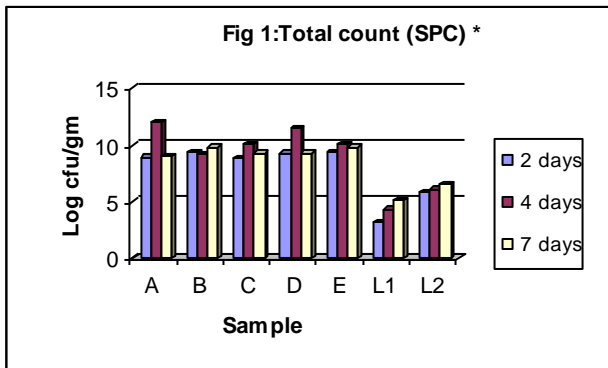
Lactic acid bacteria (LAB) count was 9.39 Log cfu/g in sample A after 48 hrs of production and then dropped to 7.1 Log cfu/g after 168 hrs. However, in samples B, C, D and E a slight increase from 9.37, 9.39, 9.3 and 9.4 Log cfu/g to 9.5, 10.36, 9.56 and 9.9 Log cfu/g, respectively was recorded after 7 days of production (Figure 3).

A rapid increase in the coliform count to 8.1 Log cfu/g after 7 days of production (sample A). No coliform was detected in sample D and after 2-days of production in sample C, it reached to 4.5 Log cfu/g after 4-days and dropped to 2.8 Log cfu/g at the end of storage. In samples B and E, the coliform count gradually decreased from 6.59 and 11.8 to 4.49 and 6.7 Log cfu/g after 7-days of cheese production, respectively (Figure 4).

A rapid increase in the *Staphylococcus* sp. count from 3.88 to 13.4 Log cfu/g was recorded after 7-days of production (samples A). However, in samples B, C, D and E *Staphylococcus* sp. count increased from 5.7, 2.7, 3.6 and 4.9 to 8.8, 7.8, 9 and 8.7 Log cfu/g within 2-days, then decreased to 6.88, 7.4, 7.1 and 6.5 Log cfu/g after 7-days, respectively (Figure 5).

Table 1 displays the presence of *Salmonella* and *Listeria* in samples during storage period. *Listeria* was detected in all samples after 2 days of production except sample L1, but no *Listeria* observed in samples D and E after 7 days of cheese production. However, *Salmonella* was detected in samples A, B and E during 2-7 days of storage period but no *Salmonella* observed in samples C, D and L1.

Figure 1-5. Microbiological changes in total count (Fig.1) Molds & yeasts (Fig.2) Lactic acid bacteria (Fig.3) Coliform (Fig.4) *Staphylococcus* (Fig.5) during storage of cheese samples.



* A and B: cheese samples collected from Al-bab alkabeer Market

C,D and E: cheese samples collected from Albarh market

L₁ and L₂: cheese samples prepared in the laboratory.

A,B,C,D,L₁ : smoked cheese

E,L₂ : non smoked cheese

Table.1 The presence of *Salmonella* and *Listeria* in samples during storage period

sample	2nd day		7th day	
	Listeria	Salmonella	Listeria	Salmonella
Smoked cheese A	+	+	+	+
Smoked cheese B	+	+	+	+
Smoked cheese C	+	-	+	-
Smoked cheese D	+	-	-	-
Non smoked cheese E	+	+	-	+
Smoked cheese L1	-	-	+	-
Non smoked cheese L2	+	-	+	+

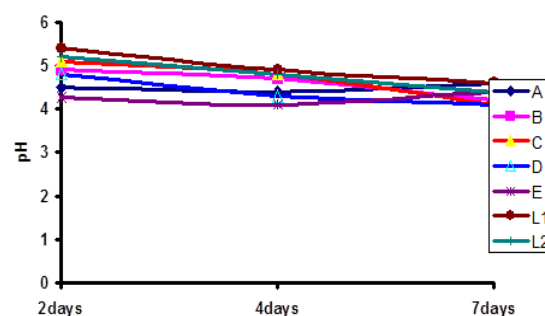
Comparing the results of microbiological examination in figures 1-5, and table 1 with cheese samples produced in laboratory under the optimum conditions, we found that in sample L1 the total plate count, molds and yeast and *Staphylococcus* sp. started increase from 3.2, 0.3 and 1.2 Log cfu/g to 5.1, 3.1 and 2.1 Log cfu/g, respectively during 2-7 days of manufacture. No lactic acid bacteria, *Salmonella* and coliform were isolated at the end of storage period. *Listeria* was detected in sample L1 after 7-days of manufacture. In contrast, in sample L2 (non smoked cheese produced in laboratory under the optimum conditions) the total plate count, molds and yeast and *Staphylococcus* sp. started slowly increase from 5.8, 2.4 and 2 Log cfu/g to 6.5, 6.7 and 2.7 Log cfu/g, respectively during 48-168 hrs of manufacture. Lactic acid bacteria and coliform were not observed.

The increase in count during the first week of storage was accompanied by a sharp drop in pH values changing from 5.4 to 4.1 (Figure 6) which is a consequence of the production of acid by the microorganisms. Chemical analysis results of smoked and non-smoked cheese samples are presented in Table 2. As shown that, the average moisture of smoked cheese samples was 47.2% changing between 44.6 and 51.73 %. However, moisture of non smoked cheese sample was 58.02%. The average of fat content of smoked cheese samples was 21.2% changing between 20.05 and 22.95% and fat content of non smoked cheese sample was 19.75%. The protein content of smoked cheese samples changed from 12.02-15.02%, the average was 13.9%. However, the protein content of non smoked cheese sample was 15.27%. The salt content of smoked cheese samples changed from 4.6-5.72%, the average was 5.2%. However, the salt content of non smoked cheese sample was 3.21 % (Table 2).

4. Discussion

Smoked cheese is a regional cheese produced in Taiz. The cheese is produced by local traditional methods to meet family needs and consumed in some regions in Yemen. The production of this cheese is seasonal and restricted to a very specific area, or because it is not possible to produce it industrially, relatively small

quantities are made. There are no statistics available on production. In our study microbiological quality of cheese was not good and the total aerobic bacteria counts varied between 8.86 and 11.97 Log cfu/g. also, the total counts of moulds and yeast reached to 7.6 and 8.9 Log cfu/g in some



samples (Figure 1 and Figure 2).

Fig: 6 Changes of pH value during storing cheese samples.

Table 2. Some chemical composition of the local Yemeni cheese samples.

sample	Moisture %	Fat %	Protein %	Salt %
Smoked cheese A	46.44	22.95	13.72	5.72
Smoked cheese B	44.6	21.8	14.9	5.02
Smoked cheese C	45.9	20.07	15.02	4.6
Smoked cheese D	51.73	20.05	12.02	5.44
Non smoked cheese E	58.02	19.75	15.27	3.21
Smoked cheese(L1)	44.09	21.6	18.7	4.3
Non smoked cheese (L2)	56.9	21.6	18.7	4.3

These results might be due to the poor sanitary conditions during cheese processing. In fact, local Yemeni cheese usually produced under traditional conditions and is handled at various stages, thus, various types of microorganism may be contaminated during cheese making and subsequent handling on the other hand. Total aerobics increased during storage period and a slight decrease was noted at the end of storage time. These results were similar to those reported by (Pazakova *et al.*, 2001) in sheep cheese.

The counts of lactic acid bacteria (LAB) exhibited an increase during storage period in some cheese samples (Figure 2) and decreased in other samples. Because, LAB have been used for centuries in the fermentation of foods,

not only for flavor and texture development but also for their ability to produce antimicrobial compounds such as organic acid, hydrogen peroxide and bacteriocin, which prevent the growth of spoilage and pathogenic bacteria. Similarly, in Caprino d'Aspromonte (Caridi *et al.*, 2003a) and Pecorino del Poro (Caridi *et al.*, 2003b) cheeses, coccal-shaped LAB decreased towards the end of ripening, while the lactobacilli increased. In another study performed on Kashar, the count of lactic acid bacteria was reported to decrease from 8.24 log cfu/g to 3.10 log cfu/g after 90 days of ripening (Cetunkaya and Soyutemiz, 2006). Furthermore, development of non-starter LAB throughout ripening was reported by several authors (Buffa *et al.*, 2001; Beuvier *et al.*, 1997; Ortigosa *et al.*, 2001). No LAB observed in samples L1 and L2 (Figure 2) this is due to effects of heat treatment of milk and high temperature of smoking process. It can be concluded from our study that smoking did not negatively affect the growth of non-starter lactic acid bacteria during storage of smoked cheese. The results agree with those reported by Farkye (2004).

According to our results the presence and increasing of viable coliform and moulds and yeast population during storing period was higher than accepted limits in Yemen for raw cheese. These heavy contamination levels indicate that all samples of cheese may cause serious health risks. This must be due to water in Yemen which is not hygienic enough. Cross contamination may also have occurred during processing and handling. The increasing constant of mould and yeast during storing time could be considered for the fact that yeast and mould count could metabolize lactic acid and lower pH value (Turkoglu *et al.*, 2003). Other authors reported that highest counts being generally reached in all the microbial groups in first week of storage in other varieties of cheese (Tornadizo *et al.*, 1995; Souza *et al.*, 2003; Abdalla and Mohammed, 2010).

The level of indicator and pathogenic microorganisms including *Staphylococcus* group bacteria found in our study were higher than standard limits accepted in Yemen for raw cheese. *Staphylococcus* sp. is often found in raw milk and in the environment of the cheese plants (equipment and personal). This organism is salt-tolerant and is able to grow under a wide range of conditions; low acid production may allow *Staphylococcus* to grow and produce enterotoxins (Olerta *et al.*, 1999). Table 1 displays the presence of pathogenic bacteria including *Salmonella* sp. and *Listeria* sp. in cheese samples and. These variations may be due to the differences in production and

handling conditions. The absence of these microorganisms at the end of storing time in some samples (D and E) due to the role of LAB which, which prevent the growth of the pathogenic bacteria. In samples cheese L1 and L2 the presence of *Listeria* sp. and *Salmonella* sp. may be due to cross contamination during handling or as Ramsaran *et al.* (1998) reported that the surviving pathogens may grow to high cell counts during the ripening and storage of soft cheese and this effect is more pronounced at the cheese surface, because the rapid increase in the surface pH of smear cheeses favors the growth of *Listeria* sp., which resides in ecological niches in cheese factories. The number of research studies conducted on *Listeria* sp. and *Salmonella* sp. contaminations in smoked cheeses was limited. It has been reported that *Listeria* and pathogenic bacteria was only recovered from 12.5% of smoked cheese samples (Al-Zoreky, 1998). Kinderlerer *et al.*, (1995) reported that the presence of *P. roqueforti*, especially the strains that possess high proteolytic and lipolytic activities, tends to inhibit the survival of pathogenic microorganisms, such as *E. coli* and *Staphylococcus* sp. Some fungal metabolites in mould-ripened cheeses were reported to contain natural *Listeria* inhibitors. *G. candidum* produces two components, d-3-phenyllactic acid and d-3-indolactic acid, which can inhibit *L. monocytogenes* (Dieuleveux *et al.*, 1998).

In our study, various factors contribute to the decline of these microorganisms during storage, they include smoking process, increase in concentration of NaCl and inhibition of these bacteria by lactic acid bacteria by causing decrease in pH. When we compared these results to the cheese samples that manufactured in laboratory under hygienic conditions we found that was best quality than locally cheese. The hygienic quality of smoked cheese was best than non smoked cheese this is due to effects of antibacterial and antioxidant effects of the smoke components such as formaldehyde, carboxylic acids, and phenols (Goulas and Kontominas, 2005).

When the results of our chemical analysis are compared with previous studies (Table 2), the moisture content is seen to be higher than that found by (Al-Zoreky, 1998) but consistent with the results of (Souza *et al.*, 2003) in Serrano cheese. The fat and protein percentage were similar to (Mirzaei *et al.*, 2008) and (Turkoglu *et al.*, 2003) in other varieties of cheese but lower than the findings of researchers (Kamber and Celik, 2007; Kocak *et al.*, 1996; Arici and Simsek, 1991). The salt contents in cheese samples were similar to that found by Cetunkaya and Soyutemiz (2006), Kamber and Celik (2007) and higher than the findings of other researchers (Mirzaei *et al.*, 2008; Souza *et al.*, 2003). It is important to point out that the salt content of the different cheeses is fairly irregular due to the salting technique used. Because this non-standard production style is excessive the compositions and quality of cheese vary depending on the experiences and working conditions of the masters performing the production, in addition to the types of milk (raw or reconstituted) which used in manufacture of cheese.

5. Conclusion

With this research it is determined that smoked cheese offered to the market for consumption in Yemen was low

quality and contaminated with pathogen. This contamination may cause important public health risks. We concluded that standardization of the smoked cheese production, the use of high quality raw materials, production in modern enterprises and hygienic conditions will be effective in prevention of the probable dangers in terms of public health.

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