
Microbiological Characteristics and Mineral Content of Local Smoked cheese produced in Yemen

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Abstract: This study aimed to investigate the microbiological quality and mineral content of smoked cheese collected from different retail markets from Ibb and Taiz areas in Yemen. The microorganisms were determined as follows: Mesophilic bacteria, Proteolytic, Halotolerant, Coliforms, *Staphylococcus aureus*, Yeast and Mold as well as the presence of *Salmonella* spp, *Escherichia coli*. Cheese made in Ibb and Taiz areas had significant differences ($P < 0.05$) in mesophilic, proteolytic, halotolerant, *Staphylococcus aureus* microorganisms, yeast and mold. *Salmonella* spp was not detected. pH, NaCl, fat and moisture content were also analyzed There was no significant differences for chemical composition except in moisture content. Minerals including Cd, Cu, Fe, Mn and Pb were analyzed using atomic absorption spectrometry. The order levels of the metals in the Smoked cheese samples was determined to be $Mn < Pb < Cd < Cu < Fe$, with mean concentration 0.47, 0.53, 0.73, 1.56 and 7.84 $\mu\text{g/g}$, respectively. The investigation showed high microorganism counts, high levels of metals and poor quality of Smoked cheese, according to the Yemen Standardization (YSMO: 1556/1998).

Keywords: Smoked, Cheese, Mineral levels, Taiz

1. Introduction

Smoked cheese is the traditional cheese of Yemen, also called taizy cheese, the most commonly consumed cheese in the Yemen, taiz located in the southern Yemen. The smoked cheese is produced from mostly raw goats and powder milk in farms or mostly private houses.

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 (1). The smoked cheese is popular with consumers and, while large quantities of cheeses are sold immediately after production, its texture is semi-hard or hard and it is sold in disks, at room temperature. The fresh cheese product has a characteristic with a nice brown color all over surface of the cheese and imparted a characteristic aroma.

Most traditional cheeses are usually produced under poor hygienic conditions with different manufacturing technologies that are dependent on the geographical location (2). It has to be noted that there is no standardized processing method for smoked cheese production. Raw milk contaminated with foodborne pathogens and introduced into dairy processing plant constitute a risk to human health

if used unpasteurized for the production of some types of cheeses or in case of cross contamination with pathogens (3). 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 to 20 years (4, 5-6).

Cheese produced from raw milk was based on the assumption that pathogenic organisms, even if present initially in the milk or curd, would eventually die in the low pH, low water activity and high salt (7,8- 9,10). Humans have been found to serve as contamination source of cheese with pathogenic bacteria like *S. aureus* (11).

Cheese are exposed to different types of contamination during manufacture processing with different kinds of heavy metals such as cadmium, nickel, lead, copper and mercury (12). It causes many health problems such as weakness, heart failure, induced cancer diseases (13) and also affects the kidney. National Oncology Centre (NOC), located in the capital, Sana'a. 2008 reported that 360000 cancer cases in Yemen at present, with 22000 new cases each year and 12000 annual cancer-related deaths. Most of these cases and kidney failures were related to consumption foods and water, which contaminated with minerals. The

heavy metal content of cheese is variable due to factors such as differences between species, geographical area, characteristics of the manufacturing practices and possible contamination from the equipment during the process (14, 15-16). For cheese samples, Cd, Cu and Pb levels were systematically higher than in milk, suggesting an effect of concentration at the stage of curdling in the cheese process and whey removal (17). Metals such as Cu and Fe are essential micronutrients and have a variety of biochemical functions in all living organisms. While Cu and Fe are essential, they can be toxic when taken in excess; both toxicity and necessity vary from one element to another (18).

There has been no study carried out on the metals aspects of smoked cheese. The purposes of this study was to determine some of microbiological characteristics and minerals contents of traditional smoked cheese which has sold in local market without refrigerated.

2. Materials and Methods

2.1. Collection of Smoked Cheese Samples

Twenty six samples were collected from different of public markets of the (Ibb and Taiz cities) Yemen during January and May 2012. The samples were transported in sterile plastic bags to the laboratory under aseptic and refrigerated conditions. All samples were stored at 4 °C and processed within 24 h of collection.

2.2. Microbiological Characteristic

Cheese samples were analyzed for microbiological aspects according to standard methods. In general, the samples were obtained from the core or interior portions of the cheese, although small amounts of surface material may have also been incorporated into some of the samples.

Cheese samples of 10 g were homogenized with 90 ml sterile 0.1% peptone water with 0.1% Tween 80 for 2 min, then serial 10-fold dilutions were prepared and plated in duplicate (19).

(I) Determination of total of mesophilic bacteria and coliform were on plate count agar at 30 °C for 48h and on Violet Red Bile agar at 30 °C for 24h, respectively (20). For the determination of *Escherichia coli*, coliform colony on EC broth in sterile tubes with Durham's tubes at 44.5 °C for 24h. The gas formation in Durham's tubes was then assessed as positive (21). (II) Proteolytic microorganisms grown on plate count agar supplemented with skimmed milk reconstructed at 10% at 30 °C for 48 h (22). (III) Halotolerant microorganisms determined on Mannitol Salt agar at 35–37 °C for 48 h. (V) *Staphylococcus aureus* counts enumerated on Baird–Parker agar supplemented with egg yolk and potassium tellurite at 35–37 °C for 48 h (19).

(IV) Yeast and mold were grown on Potato Dextrose agar acidified with 10 ml/l of 10% tartaric acid (23) and the pH was adjusted to 3.5. Then from each sample 0.1 ml dilutions were deposited on the surface of Potato Dextrose

agar. After incubation at 25°C for 5 days counts were performed on the plate. (VI) Smoked cheese samples were examined for the presence of *Salmonella* spp. a sample of 25 g was homogenized with 225 ml of nutrient lactose broth, and incubated at 35–37 °C for 24 h, then 1 ml of pre-enrichment broth was subcultured in 10 ml of tetrathionate broth and another 1ml in 10 ml of Rappaport–Vassiliadis broth, incubated at 41°C for 24h. Subcultures were streaked onto bismuth–sulfite agar and xylose–lysine–desoxycholate and the plates were incubated at 35–37 °C for 24–48 h (19).

2.3. Chemical Analysis

The pH of cheese was measured using a pH meter with a glass electrode (LabTech, 300-India). Salt and fat content were determined according to IDF methods (24). Moisture content was determined according to (20). Triplicate tests were performed for each analysis.

2.4. Metal levels Analysis of Smoked Cheese

1g of smoked cheese samples was ashed. The ashes were digested with 3 ml of nitric acid (HNO₃, 65%) added to 15 ml of distilled water on the sample and mixed. The residue was filtered through Whatman filter paper and then the sample was diluted to 10 ml with distilled water. Metal concentrations were then determined in acidic samples using flame atomic absorption spectrometry (Nova, 300, Germany), for Cd, Cu, Fe, Mn, and Pb, according to (25, 26).

2.5. Statistical Analysis

The results of microbiological and minerals contents are given as means and standard deviations (SD), with 26 being the number of smoked cheese samples. Two times were carried out for each analysis. All the statistical analysis was performed at 95% level of significance using (SASS 9.1) program.

3. Results and Discussion

3.1. Microbiological Characteristics of Cheese in Different Areas

Due to increasing interest in traditionally produced raw milk cheeses that makes questions about the safety of these products more important. The aim of this study was to survey the presence of potential pathogen microorganisms and metals contents during the sale of smoked cheese on local markets of Yemen, which has been produced by using raw milk.

The results of some microbiological properties and chemicals analysis of smoked cheese are presented in Tables 1, 2. For all cheese samples, the pH ranged from 4.0 to 5.5. The moisture content ranged from 44.6 to 59.7%. The fat and salt content were in the range of 10 to 17 and 3.00 to 7.1 respectively. The comparisons of samples among

areas showed significant differences ($P < 0.05$) in the water content in Ibb and Taiz only.

Table 1. The Microbiological accounts of 26 samples of Smoked cheese in different areas (log cfu/g).

Parameter /Areas	Ibb	Taiz
Mesophilic	7.98±0.76 *a	5.08±0.23*b
Proteolytic	4.06±0.62 *a	2.66±0.17 *b
Halotolerant	6.04±0.43 *a	4.72±0.84* b
<i>Staphylococcus</i>	5.83±0.48 *a	4.16±0.61* b
Coliform	5.22±0.71*	4.04±0.51*
<i>E.coli</i>	4.91±0.24*	3.06±0.08*
Yeast and molds	4.14±0.71 *a	6.12±0.42* b
<i>Salmonella spp</i>	nd	nd

□ Mean counts log cfu/g± standard deviation for smoked cheeses. nd not detected.

Means with different letter superscripts (a and b) differ significantly of Ibb and Taiz cheese ($P < 0.05$).

Table 2. The Chemical Characteristics of Smoked Cheese Samples.

Parameter	Areas	Mean ±SD	Minimum	Maximum
Moisture (% w/w)	Ibb	51.26±5.18 ^a	44.6	59.5
	Taiz	54.61±4.48 ^b	47.0	59.7
pH	Ibb	4.46±0.50	4.0	5.4
	Taiz	4.60±0.51	4.0	5.5
Salts (% w/w)	Ibb	4.40±1.20	3.0	7.1
	Taiz	4.20±0.92	3.6	6.5
Fat (%)	Ibb	14.00±1.73	11.0	17.0
	Taiz	13.30±2.05	10.0	17.0

Means with different letter superscripts (a and b) differ significantly of Ibb and Taiz cheese ($P < 0.05$).

The difference in microbiological characteristics of cheese between different areas may be attributed to variations in processing environment, handling, raw milk, storage and place of distribution. Furthermore, the milk composition could be influenced by feeds and different lactation stages (27, 28).

The total mesophilic bacteria, yeast and mould counts in smoked cheese was significantly different between Ibb and taiz areas (Table 1), but are lower compare to Carra traditional Turkish cheese, which made from raw milk (29). They found the mean counts of aerobic mesophilic bacteria as 1.87×10^8 and yeast and moulds 4.80×10^7 , respectively. Similar to our results, (30) found the aerobic mesophilic bacteria was 8.0, 6.7 and 5.9 log/g in raw goat milk cheeses, treated by high-pressure at different stages of maturation. The number of yeast and molds in our study could be supported by the explanation of (31) that low pH, moisture content and temperature contribute to the growth of yeast. Table 2 shows, smoked cheese has low pH, relatively low

moisture content and high salt.

In the smoked cheese 21 out 26 samples (80.7%) of ibb and taiz areas were also found to be contaminated with *S. aureus* with mean of 5.83 and 4.16 log cfu/g respectively, was always lower than the count of halotolerant, contaminated which probably due to the poor hygienic processing conditions. In the earlier study raw milk was established as the main source of contamination with *S. aureus* for four contaminated final products and food handlers were a potential source of contamination for another final product. Contamination of milk and cheese with *S. aureus* by food handlers was also reported in other studies (11). (32) reported three out of 41 raw milk cheeses in United States, are contaminated with *S. aureus* ranging from 10^2 cfu/g to 10^4 cfu/g. The *S. aureus* counts also lower than that in local Yemen cheese (33). *S. aureus* numbers in the five samples of cheese made from raw milk should not exceed 10^3 cfu /g and the maximum permissible *S. aureus* number in 2 of 5 samples is 10^4 cfu /g according to the European Union (34).

Coliform counts in smoked cheese was slightly higher than those found in white turkey cheese and raw milk cheese which were 4.3×10^3 and 2.7×10^3 cfu/g, respectively (35, 33). The high counts of *S. aureus* and coliform in smoked cheese that due to of insufficient hygienic conditions during the manufacturing, selling period and use of raw milk. The ccounts of *E.coli*. differed between Ibb and Taiz areas with mean 4.91 and 3.06 log cfu/g respectively, these differences could be due to the random arrive of *E.coli* in cheeses, normally by contamination during cheese making. In comparison with Carra cheese the *E.coli* 3.65 log cfu/g was higher than Taiz area, but low than Ibb area (29). The author suggests that may be caused by the higher salt content and low moisture content, which agreement with our results (Table 3). *E. coli* can be a food safety hazard; therefore, it is very essential to find preventive means for inhibiting growth and survival to avoid contamination of *E. coli* in cheeses.

Proteolytic microorganisms varied significantly between smoked cheese of Ibb and Taiz areas, 7 smoked cheese samples, where their counts were 4.06 and 2.66 log cfu/g. Fluctuations of proteolytic microorganisms of this study were similar to those observed by (36) in Crottin goat cheese made in different seasons.

Salmonella contaminate raw milk from the farm environment, e.g. faeces. Thus, to avoid raw milk contamination at farm, good farm practices (e.g. animal and waste management, water treatment, good hygienic conditions during milking and mastitis control) are essential to prevent the accumulation, survival, and transmission of pathogens (37).

Salmonella spp. was not detected during this study. Similar to our results *Salmonella* has not been detected in any of the 4437 samples of fresh, ripened and semi-hard cheeses made from raw, thermized or pasteurized milk that have been analyzed in two studies undertaken in UK during 2004 and 2005 (38).

Although *S. typhimurium* was survive in 3 out of several smoked cheese sample storage for difference period of time (34). The statistical analysis of variance showed that areas significantly affected ($P < 0.05$) the microbial counts of all bacteria, except coliform and *E.coli*.

3.2. Minerals Levels

The research on trace metals in various milk and dairy products has recently gained a remarkable importance. Because, their presence can represent a qualitative parameter for example a content of production procedures, environmental pollution, sanitary conditions, and quality of animal feeding that can affect milk characteristics, cheese properties, and storage and health aspect. For this reason, some trace elements are the actual importance because of their correlation to environmental pollution, and others release from packaging and alloys of material and tools utilized for milking to dairy productions (39). The traditional smoked cheese which has been produced at home or in small scale and sold in the market without packing, result major heavy metals contaminated smoked cheese from the environment pollution. Figure 1 showed that the highest concentration of heavy metals cadmium and lead in Taiz areas compared to Ibb area, that is due to many of industry factory in Taiz and transportation vehicles.

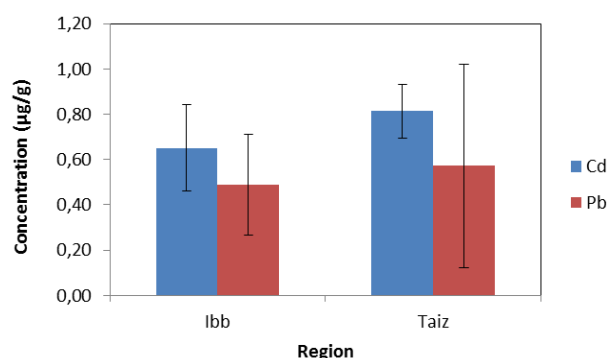


Figure 1. The concentration of heavy metals cadmium and lead in Ibb and Taiz region

The results of minerals metals concentrations were determined on a wet weight basis as µg/g Table 3. This research was conducted for the first time in Yemen for the investigation of the concentration of minerals in local cheeses. The concentration levels (Fe, Cd, Mn, Cu and Pb) measured in the twenty six different of smoked cheese are shown in Table 3.

3.2.1. Iron

The iron values of the samples varied from 1.88 to 19.77 µg/g. Our results were close to those of (16,40) for concentration of iron was found in cheese, ranging from 1–14 g/kg and 7.68–17.8 ppm, respectively. The concentration of iron ranged from 3.9 to 11.9 µg/g of cheese samples were reported by (41).

Table 3. Lead, Manganese, Copper, Ferrous and Cadmium (µg/g) in samples of smoked cheese collected from Ibb and Taiz areas, Yemen.

No of samples	Area s	Cd µg/g	Fe µg/g	Cu µg/g	Mn µg/g	Pb µg/g
1	Ibb	0.65	3.23	5.28	0.39	0.43
2	Ibb	0.31	9.56	1.98	0.19	0.95
3	Ibb	0.35	3.17	6.41	0.12	0.80
4	Ibb	0.60	9.37	1.83	0.18	0.49
5	Ibb	0.36	5.62	5.46	1.38	0.19
6	Ibb	0.72	2.53	1.91	0.77	0.61
7	Ibb	0.87	4.27	0.68	0.89	0.64
8	Ibb	0.87	2.78	0.79	0.29	0.36
9	Ibb	0.80	8.46	1.01	0.78	0.48
10	Ibb	0.65	1.88	0.73	0.67	0.35
12	Ibb	0.69	9.20	1.64	0.06	0.45
13	Ibb	0.75	8.69	1.28	0.45	0.15
14	Taiz	0.82	6.42	0.94	0.61	0.45
15	Taiz	0.93	4.95	0.80	0.49	0.37
15	Taiz	0.76	15.48	0.72	0.53	0.22
16	Taiz	0.69	6.87	1.19	0.50	0.49
17	Taiz	0.94	14.36	0.82	0.40	1.08
18	Taiz	0.66	19.77	1.34	1.00	0.23
19	Taiz	0.79	12.12	0.49	0.28	0.21
20	Taiz	0.80	7.93	0.70	0.34	1.25
21	Taiz	0.74	8.03	0.47	0.06	0.30
22	Taiz	0.89	15.50	0.81	0.13	0.13
23	Taiz	0.97	8.25	0.84	0.63	1.58
24	Taiz	0.84	5.63	0.68	0.33	0.70
25	Taiz	0.63	3.25	0.95	0.45	0.35
26	Taiz	0.96	6.40	0.85	0.33	0.50
Mean		0.73±0.18	7.84±4.55	1.56±1.59	0.47±0.32	0.53±0.35

The order of the levels of the elements in the samples was determined to be: Mn < Pb < Cd < Cu < Fe.

3.2.2. Copper

Copper is known to be important and toxic for many biological systems. It may enter the food materials from soil through mineralization by crops, food processing or environmental contamination. The essential role of copper in maintaining normal health in both animals and humans has been recognized for many years. Copper concentration ranged from 0.74 to 6.41 µg/g, and was lower than those found by (17) which reported an average of 5.35–21.34 µg/g.

3.2.3. Manganese

Manganese is a normal component of living things, including both plants and animals, so manganese is present in foods. For nearly all people, food is the main source of manganese. Manganese in the cheese sample ranged from 0.12 to 1.38 µg/g, which was larger than the value reported by (42).

3.2.4. Cadmium

Cadmium is a modern an extremely toxic metal, now it is a very important metal with many applications. Because of it's no corrosive properties, its main use in electroplating or galvanizing. Cd was detected in high concentrations ranging from 0.31 to 0.96 µg/g with the mean values of 0.73 µg/g which was higher than reported in white cheeses by (43, 42). The High Cd content of cheese samples may also be attributed to uncontrolled production and the use of contaminated water in the production process of cheeses due to poor hygiene. Overall cadmium concentrations were comparable to values reported in literature and were below the maximum limit of 500 µg /Kg (44).

3.2.5. Lead

Lead is ubiquitous toxic metal and is detectable in practically all phases of the inert environment and in all biological systems, because it is toxic to most living things at high exposures (45). Specific concern varies with the age and circumstances of the host, and the major risk is toxicity to the nervous systems. The principal route of exposure for people in the general population is food, and sources that produce excess exposure and toxic effects are usually environmental. The maximum lead levels was as 1.58 µg/g, higher values were reported in Jordan white cheeses by (46) and lower results were reported earlier in turkey white cheese by (47). Most of the minerals in this study, the concentration is higher compared with earlier reports, this is due to minerals transferred to Smoked cheese from water, containers and utensils during manufacturing and selling cheese.

4. Conclusion

The present study gives information on raw milk cheese levels of microbiology and heavy metals produced in Yemen. It also provides information on safety and quality of a local and traditional product. The microbiological quality and mineral levels of Smoked cheese sold in the Ibb and Taiz cities non-refrigerated, indicates poor hygienic conditions of cheese products during manufacturing, transport, and marketing, *S. aureus* numbers and *E.coli* in samples of cheese should not exceed 10^3 cfu /g according to the Yemen Standardization, Metrology and Quality Control Organization (YSMO No: 1556/1998). The marked differences in the number of microorganisms, minerals levels and the values of chemical parameters of Smoked cheese samples appear to result also from different selling practices and insufficient sanitary conditions during the cheese manufacturing. For improving the microbiological and minerals quality of smoked cheese, to reduce the health risk, pasteurized milk should be used instead of raw milk. Clean and high quality materials used during manufacturing and selling processes should be carried out under good hygienic conditions.

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