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Microbiological and Chemical Quality of Afyon Clotted Cream

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Abstract: To determine the microbiological and chemical quality of Afyon clotted cream (*Afyon kaymagi*), a total 30 clotted cream samples were analyzed. As a result, total aerobic plate count was $\geq 10^4$ and $\geq 10^8$ cfu g^{-1} in 70 and 10% of the samples, respectively. Micrococci/staphylococci, enterobacteriaceae and coliform bacteria were 10^5 cfu g^{-1} in 20% of the samples. *Pseudomonas* sp. were 10^5 cfu g^{-1} in 10% of the samples. Enterococci and mould/yeast counts were $\geq 10^4$ cfu g^{-1} in 20 and 10% of the samples, respectively. Coagulase-positive staphylococci, *Escherichia coli*, *Bacillus cereus* and sulphite reducing anaerobic bacteria were not determined. The fat ratio was <60% in 20% of the samples. The fraudulent practice of the use of cow milk to substitute buffalo milk was commonly detected in *Afyon kaymagi*. Although, its chemical and hygienic quality is poor, it poses a minimal risk to public health with respect to pathogenic bacteria.

Key words: Clotted cream, microbiology, chemical, quality, APC

INTRODUCTION

There is an increasing research interest and investment in buffalo milk in various countries, owing mainly to its attractive nutrient content and also taste (Amarjit and Toshihiko, 2003). Compared with cow milk, buffalo milk has a higher content of fat, crude protein, lactose, total solids, vitamins and minerals, which impart a rich flavor and taste and make it a highly suitable ingredient for the manufacture of a wide variety of milk products, including cheese, butter fat, ice cream, yoghurt (Fundora *et al*., 2001) and clotted cream in Turkey. There are some differences in buffalo milk composition around the world.

There are almost 104,956 water buffalo (*Anatolian*) in Turkey, which produce nearly 38,000 tons of water buffalo milk and this milk is used in the region where, it is produced. *Anatolian* water buffalo milk, which is produced mainly in the Afyon region in Turkey, is generally used for the production of clotted cream and the Turkish name is *Afyon kaymagi*. Generally, clotted cream and milk are mixed together in a two step heating technique (at 85-90 and at 75-80°C) (Adam, 1955; Izmen and Eralp, 1957; Izmen, 1959). To summarize, traditional water buffalo clotted cream is produced in Afyon as follows; pre-heating-step: as soon as the evening milking is completed, the milk should be heated very slowly to 95°C for 30 min, cooled, then left to settle overnight, heating step: after the following morning's milking, the center cream layer of the evening before milk is perforated and the morning milk is added to the evening before milk from this perforation. Then, the mixture of evening and morning milk is heated very slowly for a second time for 45 min. Then, it is cooled to room temperature and refrigerated overnight. The clotted cream layer is separated by cutting it away from the liquid layer and finally inverted before sale. In traditional clotted cream production, approximately, 80% of milk fat passes to the clotted cream and 350-450 g of clotted cream is produced from 2.5 L milk. The yield is approximately 14-18% milk fat. However, the procedure applied, weather and temperature variations can affect the yield.

Standard and good quality of dairy products production depends on high quality of fresh milk. To obtain high quality raw milk also depends on several factors: good animal health status, hygienic milking and milk handling includes tools, equipment and stuff on farms, cooling of milk and transport of milk to a milk processing plant as soon as possible after milking (Siirtola, 2000). Therefore, these factors above are valued for standard, good-quality Afyon clotted cream production, too. In addition these factors, hygienic practices during production, suitable packaging techniques, storage and sale at refrigerated temperature

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affect on quality of Afyon clotted cream. Traditionally, buffalo milk and Afyon clotted cream were generally produced as a family enterprise in villages. Today, they are produced mostly by small or medium scale companies, besides for village families in Afyon Province and its vicinity. However, village families or farmers generally do not employ appropriate milk hygiene and cleaning of barns. For milk to reach the processor and ultimately the consumer still in good condition, the criteria listed above must be followed right from the farm level to the processing factory and thereafter to the retailers and consumers.

Afyon Province is a famous city for its traditional meat and dairy products, including clotted cream. In spite of its popularity, little data is available on the hygienic and chemical quality of Afyon clotted cream. It is generally produced by traditional methods and sold in local markets. Nowadays, consumer awareness of the microbiological and chemical quality of this product has increased in Turkey. In addition, there is the allegedly common fraudulent practice of substituting a less costly type of milk. Therefore, the present study was designed to determine the microbiological and chemical qualities of Afyon clotted cream.

MATERIALS AND METHODS

A total of 30 Afyon clotted cream samples were purchased in original packages of 250 g from markets, local shops or market places in Afyon Province, Turkey. All of the samples were transferred to the laboratory under cold storage and analyzed.

Microbiological analyses: To determine the hygienic and pathogenic flora of the clotted cream samples, the total aerobic bacteria, coagulase-positive staphylococci, enterobacteriaceae, coliform and enterococci count were detected by the drop-plating technique, while *Bacillus cereus* and sulphite-reducing anaerobic bacterial counts were carried out using the spread-plating technique.

For this purpose, 10 g samples of clotted cream were aseptically transferred into sterile plastic bags containing 90 mL of sterile peptone water (Oxoid CM 9, UK) and homogenized for 1-2 min (Bag mixer, Interscience-400). Following homogenization, 10-fold serial dilutions were done in sterile peptone salt water up to 10^{-6} and inoculated into specific culture media as shown in Table 1 for determination of total Aerobic Plate Counts (APC), Micrococci/staphylococci (BP), Coagulase Positive Staphylococci (CPS), enterobacteriaceae (VG), coliforms (VL), *E. coli*, enterococci (SB), moulds/yeasts (RO), *Pseudomonas* sp. (CFC), *B. cereus* (CSM) and sulphite-reducing anaerobic bacterial counts (SPS) (Hauschild *et al*., 1977).

For the isolation of *Pseudomonas* sp., oxidase tests were done and oxidase positive colonies grown on CFC media plates were presumed positive for *Pseudomonas* sp.

For the isolation of coagulase positive staphylococci, up to five typical colonies (black or grey) grown on BP agar were selected and transferred to tubes contained Brain-Heart Infusion Broth (BHI-Oxoid CM 225, Basing stoke, UK). The tubes were incubated at 37°C for 24 h. Post-incubation, coagulase tests were done according to the method of Thatcher and Clark (1978). For this purpose, Coagulase Plasma EDTA (Difco 0803-46-5) was used.

For the isolation of *E. coli*, presumptive colonies on VL agar were selected and directly streaked onto Endo Agar Base (EAB) and incubated for up to 48 h at 37°C.

Table 1: The mediums used for the microbiological analyses and incubation conditions

		Incubation conditions		
Microorganisms	Mediums	Temperature (°C)	Time	Conditions anaerobic/aerobic
Total aerobic plate count	Plate Count Agar (Oxoid CM 509, Basing stoke, UK)	30	48-72 h	Aerobic
Micrococci/staphylococci	Baird-Parker Agar (Oxoid CM 275, Basing stoke, UK)	37	24-48 h	Aerobic
Enterobacteriaceae	Violet Red Bile Glucose Agar (Oxoid CM 485, Basing stoke, UK)	37	24-48 h	Anaerobic
Coliform	Violet Red Bile Agar (Oxoid CM 107, Basing stoke, UK)	37	24-48 h	Anaerobic
E. coli	Endo Agar Base (Oxoid CM 479, Basing stoke, UK) MR-VP Agar (Oxoid CM 0043, Basing stoke, UK) Simmons Citrate Agar (Oxoid, CM 155,Basing stoke,UK) Tryptone Water (Oxoid, CM 87, Basing stoke, UK)	37	24-48 h	Aerobic
Enterococci	Slanetz and Bartley Medium (Oxoid CM 377, Basing stoke, UK)	37	24-48 h	Aerobic
Pseudomonas sp.	Pseudomonas Agar Base (Oxoid CM 559, SR 103) Identification Sticks Oxidase (Oxoid, BR00 64A, Basing stoke, UK)	30	24-48 h	Aerobic
B. cereus	Bacillus cereus Selective Agar Base (Oxoid CM 617, Supl. SR 99, Basing stoke, UK,)	30	24-48 h	Aerobic
Mould/yeast	Rose Bengal Chloramphenicol Agar (Oxoid CM 549, Supl. SR 78, Basing stoke, UK)	25	3-5 day	Aerobic
Sulphite-reducing anaerobic bacteria	Perfringens Agar Base (Oxoid CM 543, Basing stoke, UK), (Supl. A-SR 76, B SR 77, Gas Generating Kit- BR 38)	37	24 h	Anaerobic

One suspected *E. coli* colony on the EAB was selected and identified by the indole, methyl red, Voges Proskauer and Simmon's citrate tests (IMViC tests).

The media used for the microbiological analyses and incubation conditions are shown in Table 1.

Chemical analyses: The fat ratio of the clotted cream was determined with the Gerber method.

RESULTS AND DISCUSSION

The microbiological analysis results of clotted cream samples are shown in Table 2. According to analysis results, APC was range of between $<10^2$ and 10^9 cfu g^{-1} levels. APC was detected in between $<10^2$ and 10^6 cfu g^{-1} levels in 70% and between $\geq 10^6$-10^9 cfu g^{-1} levels in 30% of the samples. Enterobacteriaceae and coliform bacteria were determined between $<10^2$ and $\leq 10^5$ cfu g^{-1} levels. They were detected in $\geq 10^3$ cfu g^{-1} levels in 40% of the samples. However, *E. coli* was not determined. Mould and yeast were found $\geq 10^3$ cfu g^{-1} levels in 30% of the samples. Although, micrococci/staphylococci was found 10^5 cfu g^{-1} levels in 20% of the samples, coagulase-positive staphylococci was not detected. Enterococci were found 10^4 cfu g^{-1} levels in 20% of the samples. *Pseudomonas* sp. were found at 10^5 cfu g^{-1} levels in 10% of the samples. However, *B. cereus* and sulphite reducing anaerobic bacteria were not detected in any of the samples tested.

The chemical analysis results of clotted cream samples are shown in Table 3. The fat ratio of the samples was found between 55 and 75%. Fat ratio of the samples was found <60% of the 6 (20%) samples.

In the present study, hygienic and chemical qualities of clotted cream were determined to be generally low (Table 3). The Turkish Food Codex's Cream and Clotted Cream Communiqué states that microbiological analysis limits for clotted creams are as follows; the maximum coliform bacteria counts has to be <3-9 MPN g^{-1}, the maximum *S. aureus* and yeast/mould numbers have to be 10^1-10^2 cfu g^{-1}. *Listeria monocytogenes*, *E. coli* and *Salmonella* sp. have to be absent in 25 g of the product. According to Turkish Food Codex, although coliform bacteria and yeast/mould numbers of 50 and 30% of the analyzed samples were high, *S. aureus* counts and *E. coli* were suitable. In addition, *B. cereus* and sulphide reducing anaerobic bacteria were not isolated in the present study. *Pseudomonas* sp. were detected at $\geq 10^5$ cfu g^{-1} in 10% of the samples may indicate shorter shelf life of the clotted cream. Although, hygienic quality of clotted cream was generally poor, it had poses minimal risk to public health regarding these pathogenic bacteria.

There are relatively few articles for clotted cream in Turkey and worldwide. In one of these, it was reported from Afyon province that the APC was 10^3-10^{11} cfu g^{-1} in Afyon clotted cream and $>10^6$ cfu g^{-1} in 95.2% of samples. In the same study, coliform bacteria were isolated at 10^3-10^6 cfu g^{-1} in 49.2% and $>10^6$ cfu g^{-1} in 6% of the samples, respectively. It was determined that mean yeast/mould and proteolytic bacteria were 10^4 and 10^7 cfu g^{-1}, respectively. At that time, the hygienic quality of clotted cream was poorer than currently. In another study, Nikodemusz (1984) reported from Hungarian that analyzed 120 pasteurized clotted cream samples and APC were 10^6 cfu g^{-1} in 76.7% of the samples and yeast and mould were detected in 39.2 and 18.4% of the samples,

Table 2: Microbiological analyses results of Afyon clotted cream samples

Microorganism level cfu g^{-1}	Total aerobic plate count n (%)	Micrococci/ staphylococci (%)	Coagulase positive staphylococci	Enterobacteriacea	Coliform	*E. coli*
			n (%)			
$<10^2$	3 (10.0)	9 (30.0)	30 (100.0)	18 (60.0)	15 (50.0)	30 (0.0)
10^2-10^3	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.0)	0 (0.0)
10^3-10^4	0 (0.0)	3 (10.0)	0 (0.0)	6 (20.0)	6 (20.0)	0 (0.0)
10^4-10^5	3 (10.0)	12 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^5-10^6	12 (40.0)	6 (20.0)	0 (0.0)	6 (20.0)	6 (20.0)	0 (0.0)
10^6-10^7	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^7-10^8	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^8-10^9	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Microorganism level cfu g^{-1}	Enterococci	*Pseudomonas* sp.	Mould/yeast	*B. cereus*	Sulphite reducing anaerobe bacteria
	n (%)				
$<10^2$	18 (60.0)	27 (90.0)	21 (70.0)	30 (100.0)	30 (100.0)
10^2-10^3	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^3-10^4	3 (10.0)	0 (0.0)	6 (20.0)	0 (0.0)	0 (0.0)
10^4-10^5	6 (20.0)	0 (0.0)	3 (10.0)	0 (0.0)	0 (0.0)
10^5-10^6	0 (0.0)	3 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^6-10^7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^7-10^8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
10^8-10^9	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 3: Chemical analses results of Afyon cream samples (n = 30)

Clotting cream samples n (%)	Fat ratio (%)
6 (20.0)	55-59
9 (30.0)	60-64
3 (10.0)	65-69
9 (30.0)	70-74
3 (10.0)	75

respectively. In another study, Scwarcbort de Tamsut and Garcia (1999) reported from Venezuela that 100 pasteurized clotted cream samples from 11 different milk plants were analyzed microbiologically. As a result, 70, 95, 91 and 58% of the samples did not meet the international standards because of the presence of aerobic mesophile bacteria, *S. aureus*, coliform and yeast/mould, respectively. Furthermore, pathogenic bacteria such as *Salmonella* Typhimurium, *Shigella sonnei* and enteropathogenic *Escherichia coli* were also isolated. In another study, *E. coli* O157 could not be isolated from 30 unpasteurized clotted cream samples, but *E. coli* was isolated at 1.00-5.99 $\log_{10}$ g^{-1} in 7 (23.3%) of the samples. In the same study, the APC was determined at $\geq 10^4$ cfu g^{-1} in 70% and $\geq 10^8$ cfu g^{-1} in 10% of the samples, respectively, while enterococci were determined at $\geq 10^3$ cfu g^{-1} levels in 20% of the samples.

In this study, although micrococcus/stapylococcus were detected in 20% of the samples at 10^5 cfu g^{-1} levels, coagulase positive staphylococci were not detected. However, Amer *et al*. (1986) reported from Zagazig/Egypt that coagulase-positive staphylococci were isolated in 14% of clotted cream samples and Lukasova and Jarchovska (1979) reported from Czech Republic that 30 *S. aureus* strains isolated from clotted cream samples. Although, *S. aureus* itself is not resistant to heat treatment, its toxic compound enterotoxin is resistant to heat treatment. In this study, clotted creams were not analyzed for the presence of stapylococcal enterotoxin. Dairy products such as cream and cheese and cooked meat, are the foods most commonly responsible for staphylococcal toxicity (Wieneke *et al*., 1993). In fact, when foods responsible for staphylococcal food poisoning are analyzed, there is never a positive correlation between staphylococcus counts and staphylococcal enterotoxin levels. The reasons for this are: as the time between food consumption and food analysis is prolonged, the agent may no longer be alive or may reduce in numbers below detectable levels, after producing enterotoxins, microorganisms may die during food processing, enterotoxigenic staphylococcus may be inhibited by nonenterotoxigenic species after enterotoxine production and the bacteria may not be distributed homogeneously within the product (Holmberg and Blake, 1984; Gilmour and Harvey, 1990). In the context of these results, a risk of enterotoxin presence may exist in clotted cream samples.

When the present study and other study results mentioned above are compared, it may be concluded that from Hamzacebi's study from 1973 to today, the hygienic qualities of clotted creams have improved to some degree, but are still deficient. In addition, based on the results of this study and the above mentioned studies, heat treatment of clotted cream and cream may be insufficient for the destruction of spoilage, pathogenic or other microorganisms influencing the hygienic quality of products. It is reported one study that pasteurization control of Afyon clotted cream and determined that peroxidase enzyme was present in 57% of 250 samples and that the enzyme phosphatase was present in 74% of 185 samples. According to these results, it seems that heat treatment applied during clotted cream production was insufficient. An additional factor for the poor hygienic quality of Afyon clotted cream may be cross contamination after heat treatment during different stages. In this study, it was observed that the majority of Afyon clotted cream production is carried out in substandard barn conditions and in poor hygienic environments. In fact, only a minor part of Afyon clotted cream production is carried out in small or medium scale plants, where higher hygiene standards would be expected to apply.

In this study, Afyon clotted cream was also analyzed for chemical quality. According to The Turkish Food Codex's Cream and Clotted Cream Communiqué, the minimum fat content of Afyon clotted cream is stipulated at 60% (w w^{-1}). In this respect, the present study, 20% of samples did not achieve this standard. There are a few studies of fat content of clotted cream in Turkey. One of them, Adam (1955) determined the mean fat value of 23 clotted cream samples to be 65%. In another study, Izmen and Eralp (1957) reported that the mean fat value of 5 clotted cream samples was 61% an another study the mean fat value of 250 Afyon clotted cream samples was 62.7% (39.0-76.0%) and that of 33 samples, 13.2% were <60%. According to the Codex Communiqué cited previously, clotted creams must not contain any fat other than milk fat and that thickeners should not added. To increase the thickness of Afyon clotted cream, starch is sometimes added, although its use is banned. In one study in Turkey, starch was detected in one of 250 Afyon clotted cream samples. Contrary to Turkish regulations, margarine is sometimes added to increase the fat content and to increase the thickness of Afyon clotted cream. The analyzed samples in this study are sold under the name of Afyon clotted cream, which normally appears porcelain white. However, if cow milk is added, the color changes to yellowish. Based on the yellow or yellowish color of analyzed samples, most of the samples were contaminated.

CONCLUSION

According to the present study and other study results cited above, cream and Afyon clotted cream have poor hygienic quality. Although, pathogenic microorganisms were not detected in the present study, some pathogenic bacteria were found in pasteurized cream and other cream studies. According to the present study's results, it appears that the chemical quality of Afyon clotted cream was poor. In addition, the fraudulent practice of the use of a less costly type of such as cow milk to substitute buffalo milk. To improve hygiene standards, adequate heat treatment must be applied during clotted cream production and its production should be performed in modern enterprises where pasteurization control can be ensured, rather than in village enterprises. Also, national standards for clotted cream ingredients should be rigorously enforced through regular testing to prevent the deception of consumers.

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