



ORIGINAL ARTICLE

Evaluation of Microbial Contamination and Chemical Qualities of Cream-filled Pastries in Confectioneries of Chaharmahal Va Bakhtiari Province (Southwestern Iran)

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Abstract

Objectives: High consumption of bakery products such as cream-filled pastries may cause serious health risks and food poisoning to humans. Therefore, investigation of the microbial and chemical qualities of bakery products containing cream is necessary. The purpose of the present study was to investigate the chemical qualities and microbial contaminations of cream-filled pastries collected from confectioneries located in six cities in Chaharmahal Va Bakhtiari province (Southwestern Iran).

Methods: Microbial tests and chemical characteristics (fat and acidity level) were done on 228 cream-filled pastries samples that were collected randomly from various confectioneries.

Results: After microbial tests, it was found that 33.33% of all samples were contaminated by microbial agents. The microbial tests showed that Shahrekord (10.09%) and Broujen (9.21%) cities had high levels of contamination and in Koohrang (1.31%) it was low compared with the other four cities. High contamination of coliforms (61.84%), staphylococci (48.68%), and yeast (27.63%) were observed in almost all samples. The chemical analysis showed maximum amounts of fat content and titratable acidity in cream-filled pastry samples obtained from Lordegan and Shahrekord cities, respectively.

Conclusion: The findings of the present work demonstrated that the microbial contamination and chemical quality of cream-filled pastries produced in confectioneries of Chaharmahal Va Bakhtiari province were not in acceptable ranges. These problems may be related to fecal contamination of cream samples

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or lack of hygiene by handlers and it is necessary to observe the standards of hygiene and to develop safe food handling techniques and aseptic pastry manufacturing systems in some confectioneries of Chaharmahal Va Bakhtiari province.

1. Introduction

Cream-filled pastries are bakery products that are consumed a lot in the food and confectionary industries; however, they are a common cause of food poisoning in humans [1,2]. Food poisoning affects healthy people around the world and the most common symptoms include nausea, vomiting, abdominal pain, cramps, and diarrhea [3].

Milk and milk-based products like desserts and cakes containing milk or cream are rich nutrient media for microbial growth. Nonconformity of standards of food hygiene by food staff may lead to food contamination [2,4]. *Staphylococcus aureus*, *Salmonella*, *Campylobacter*, *Escherichia coli*, molds, and yeasts can contaminate bakery products, in particular cream-filled pastry, and they are the major micro-organisms causing food-borne diseases in humans [5,6]. In addition, it is clear that the increase of fat content and acidity levels of bakery products like cream-filled pastries, cream pies, and cream puffs may be related to the risk of obesity and heart disease [2,7].

E. coli is a gram-negative, facultative anaerobic bacterium that belongs to the *Enterobacteriaceae* family and is one of the important causes of food poisoning [8,9]. *S. aureus* is a gram-positive, nonmotile, non-sporeforming facultative anaerobe which is significant in food industries and causes a range of illnesses, especially foodborne diseases, via enterotoxins [10,11].

Microbial and chemical characteristics of cream-filled pastries in food and confectionary industries must be evaluated. In the present study, the chemical qualities and microbial contamination of cream-filled pastries (*S. aureus*, *E. coli*, and yeast) collected from confectionaries in Chaharmahal Va Bakhtiari province (Southwest of Iran) were examined.

2. Materials and methods

2.1. Study area and sampling

In the present study, six cities (Shahrekord, Ardal, Farsan, Lordegan, Koohrang, and Brougen) of Chaharmahal Va Bakhtiari province (Southwestern Iran) with a population of > 1,000,000, were subjected to sample collection. A number of 228 cream-filled pastries samples were collected randomly from 34 different confectioneries from July 2013 to September 2013. All samples were transported to the laboratory immediately in cool conditions and stored at 4°C and were analyzed within 2 hours of collection. In addition, the cream of

three samples from each confectionery were suspended in sterile polyethylene bags and labeled for further tests.

2.2. Microbiological analysis

The microbiological tests and bacterial counts were done on suspended samples according to specific standard instructions for coliforms, *E. coli*, *Staphylococcus aureus*, and yeast [12,13]. Each cream sample (10 g) was separately added into sterile 0.1% buffered peptone water (90 mL) and homogenized in a sterile stomacher polyethylene bag for 2 minutes at 220 g in a Stomacher (Interscience-Bag Mixer 400, St., Nom., France) and then was serially diluted in 0.1% peptone solution (Sigma–Aldrich, Pool, UK). One hundred-microliter aliquots of three serial dilutions were spread-plated in triplicate on the surface of plate count agar (Merck, Darmstadt, Germany), violet red bile agar (Merck), Baird–Parker agar base with egg-yolk tellurite emulsion, Saboroud dextrose agar (Merck), and Yeast Extract Glucose Chloramphenicol agar (Merck), for the enumeration of total aerobic bacteria, coliforms, *Staphylococci*, and yeast, respectively. For bacterial growth turn over the plates and plate count agar, violet red bile agar, and Baird–Parker plates were incubated at 30°C for 1–2 days and Yeast Extract Glucose Chloramphenicol agar plates were incubated for 48 hours for 5 days at 20–25°C for yeast total count. Confirmatory cultures and tests including culturing of isolated bacteria on eosin methylene blue agar, gram staining, and standard biochemical tests such as the oxidative/fermentative utilization of sugars, catalase, and coagulase tests were performed for isolation and differentiation of *E. coli* from *Salmonella* and other coliforms, as well as *S. aureus* from coagulase negative staphylococci. The average number of visible colonies obtained from plate counts and the number of colony forming units (CFU/g) were evaluated.

2.3. Biochemical examination

The titratable acidity (lactic acid%) and fat content of cream-filled pastries collected from confectionaries in Chaharmahal Va Bakhtiari province were examined. The fat content and titratable acidity were determined according to the methods of the Association of Official Analytical Chemists [14]. The fat content of cream samples was measured using the Gerber method (primary and historic chemical test to determine the fat content) via a special calibrated butyrometer. The titrated acidity of cream specimens was measured by a titration of 10 mL melted cream containing 0.5 mL

phenolphthalein by the N/10 sodium hydroxide solution (0.1 normal NaOH).

2.4. Analysis of data

The microbial count data were collected and finally the mean values and standard error of the mean were evaluated in SPSS version 20 (SPSS Inc., Chicago, IL, USA) followed by pair-wise test for comparison of significant differences among means and the impact of different areas on chemical and microbial amount. A p value < 0.05 (5%) was considered statistically significant.

3. Results

The microbial counts of cream-filled pastries which were collected from six cities of Chaharmahal Va Bakhtiari province are shown in Table 1. The microbial analysis of cream-filled pastries collected from six cities of Chaharmahal Va Bakhtiari province (Iran) showed that 76 specimens (33.33%) out of 228 samples were contaminated. Moreover, in Shahrekord, Broujen, Farsan, Lordegan, Ardal, and Koohrang cities 10.09%, 9.21%, 4.82%, 4.38%, 3.51%, and 1.31% of all samples were polluted, respectively (Figure 1). According to the results mentioned in Table 2, the means of total count, coliform count, staphylococci, and yeast counts in Shahrekord and Broujen cities were higher than the four other cities and a statistically significant difference was detected in the total count of these cities compared with other regions of Chaharmahal Va Bakhtiari province ($p < 0.05$). In addition, Koohrang had low levels of microbial contamination (1.31%) with a mean of 5.45 ± 0.9 . A comparison of microbial contamination by Iranian National Standards [15] showed the average numbers of total, coliform, staphylococci, *E. coli*, and yeast were high (Table 3).

The evaluation of chemical tests (titratable acidity and fat content) showed that the maximum and minimum amounts of fat content belonged to the Lordegan and Ardal cities, respectively, and the maximum and minimum range of lactic acid was observed in Shahrekord and Lordegan cities, respectively. The comparison of titratable acidity and fat content of confectionery samples between six cities of Chaharmahal Va Bakhtiari province were not statistically significant ($p > 0.05$). According to Table 3 and Iranian National Standards, titratable acidity (lactic acid) and fat content in 71% and 33% of all samples that were collected from six cities of this province were in unacceptable ranges, respectively.

4. Discussion

The production and consumption of bakery products like cream-filled pastry in Iran are very high and this

Table 1. Microbial counts of cream-filled pastry specimens in six cities of Chaharmahal Va Bakhtiari province (Iran).

City	Total (log 10)				Coliform (log 10)				Staph (log 10)				Yeast (log 10)			
	Contamination No. (%)	Mean \pm SE	Max	Min	Positive No. (%)	Mean \pm SE	Max	Min	Positive No. (%)	Mean \pm SE	Max	Min	Positive no. (%)	Mean \pm SE	Max	Min
Shahrekord	23 (10.09)	6.75 \pm 0.15	7.46	5.2	14 (60.87)	3.72 \pm 2.66	5.41	0	9 (39.13)	1.25 \pm 1.87	5.61	0	6 (26.08)	9.23 \pm 1.9	16.78	0
Farsan	11 (4.82)	6.42 \pm 0.3	8.21	4.95	8 (72.73)	2.53 \pm 2.24	4.95	0	7 (63.64)	1.02 \pm 1.76	4.23	0	5 (45.45)	7.25 \pm 1.23	11.24	0
Ardal	8 (3.51)	6.25 \pm 0.2	7.36	5.64	6 (75)	2.33 \pm 2.11	4.36	0	7 (87.5)	1.20 \pm 0.92	3.95	0	3 (37.5)	6.70 \pm 0.85	8.63	0
Broujen	21 (9.21)	6.54 \pm 0.5	7.22	5.33	11 (52.38)	2.63 \pm 2.02	4.23	0	8 (38.09)	1.31 \pm 1.24	4.67	0	4 (19.05)	8.46 \pm 0.98	9.26	0
Lordegan	10 (4.38)	6.21 \pm 0.1	6.85	5.34	6 (60)	2.20 \pm 1.96	4.00	0	5 (50)	0.89 \pm 0.95	4.55	0	2 (20)	6.55 \pm 0.76	8.30	0
Koohrang	3 (1.31)	5.45 \pm 0.9	6.23	4.65	2 (66.67)	2.25 \pm 2.38	3.78	0	1 (33.33)	0.92 \pm 0.76	4.0	0	1 (33.33)	5.86 \pm 0.53	7.66	0
Total	76 (33.33)	6.27 \pm 0.36	8.21	4.65	47 (61.84)	2.61 \pm 2.23	4.50	0	37 (48.68)	1.1 \pm 1.25	4.50	0	21 (27.63)	7.34 \pm 1.04	10.31	0

Max = maximum; Min = minimum; SE = standard error.

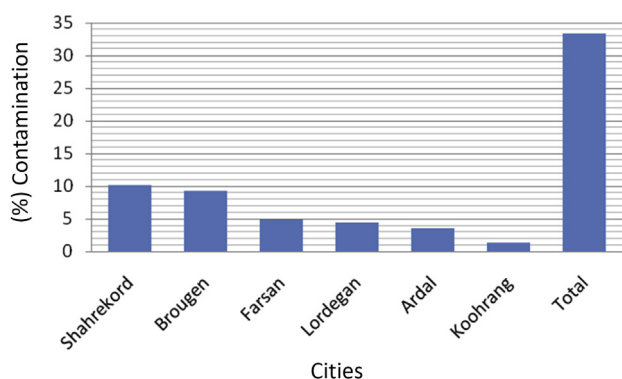


Figure 1. Microbial contamination of cream-filled pastries in six cities of Chaharmahal Va Bakhtiari province (Iran).

product provides a suitable environment for bacterial growth and proliferation and possible causes of food poisoning in consumers. In the present study, the microbial counts and chemical characteristics of cream-filled pastry samples collected from six cities of Chaharmahal Va Bakhtiari province were analyzed. This study showed that the microbial and chemical qualities of cream-filled pastries that were collected from different confectioneries of this province were outside of the acceptable range according to Iranian National Standards. The microbial tests showed that 76 specimens (33.33%) out of all samples

were contaminated. In all samples, Shahrekord (10.09%) and Broujen (9.21%) cities had high levels of microbial contamination and Koohrang (1.31%) had low levels of microbial counts compared with the four other cities. After chemical analysis, the maximum amounts of fat content and titratable acidity were detected in collected samples of Lordegan and Shahrekord cities, respectively. High levels of coliforms (61.84%), staphylococci (48.68%), and yeast (27.63%) in samples may be related to fecal contamination of cream samples or handlers and nonconformity of the standards of hygiene in some confectioneries of this province. The microbial and chemical characteristics of cream-filled pastries in Kerman city confectioneries (Southeast Iran) had similar results to the present work and were not in acceptable ranges of Iranian standards [2]. In the present study, 33% and 71% of fat content and titratable acidity were in unacceptable ranges, respectively, while 38% and 24% of fat amount and lactic acid of cream-filled pastry samples in Kerman city confectioneries were outside of the standard range, respectively. Similar to the findings of this study, in six cities of Chaharmahal Va Bakhtiari province the high level of various microbial contamination (95.8%) in pastry cream in Arak city of Iran was reported [16]. In a different study in Croatia, 6.36% of cake samples were contaminated by *Listeria* spp. [17] but did not have high levels of coliform and staphylococci contamination observed in cream-filled

Table 2. Fat and titratable acidity of cream-filled pastries samples collected from different confectioneries of Chaharmahal Va Bakhtiari province (Iran).

City	No. of confectioneries	Fat (%)			Titratable acidity (lactic acid%)		
		Mean \pm SE	Max	Min	Mean \pm SE	Max	Min
Shahrekord	12	26.7 \pm 7.67	32	15	1.82 \pm 0.85	2.24	0.56
Farsan	5	23.3 \pm 10.76	42	13	1.64 \pm 0.46	1.85	0.76
Ardal	3	18.27 \pm 12.35	30	10	1.26 \pm 0.56	1.76	0.88
Broujen	8	36.54 \pm 9.4	52	14	1.58 \pm 0.77	2.02	0.94
Lordegan	4	38.74 \pm 10.37	56	12	1.37 \pm 0.76	1.88	0.36
Koohrang	2	27.6 \pm 8.2	38	11	1.46 \pm 0.95	1.42	0.56
Total	34	28.52 \pm 9.79	56	10	1.52 \pm 0.72	2.24	0.36

Max = maximum; Min = minimum; SE = standard error.

Table 3. Analyzing the unacceptable microbial and chemical ranges of cream-filled pastry samples in Chaharmahal Va Bakhtiari province (southwestern Iran) according to Iranian National Standards and acceptable ranges.

Properties	Acceptable range	No. of unacceptable samples (%)	95% confidence interval
Total count (CFU/g)	> 20,000	72 (95)	86.3–97.6
Coliform (CFU/g)	10	34 (72)	70.5–78.7
<i>E. coli</i> (CFU/g)	0	12 (26)	22.3–32.5
<i>Staphylococcus aureus</i>	0	35 (95)	82.6–95.3
Yeast (CFU/g)	0	18 (86)	80.2–88.6
Titratable acidity (lactic acid%)	0.5–1.5	162 (71)	33.4–64.5
Fat (%)	35–48	75 (33)	65.6–88.6

CFU = colony-forming unit.

pastries of confectioneries in southeast of Iran. Evaluation of microbial contamination of cream-filled pastries in Tabriz city (Iran) by Nikniaz et al [18] showed that coliforms (38.8%), *E. coli* (48.8%), *S. aureus* (31.2%), molds (27.5%), and yeasts (70%) were higher than the standard defined by the Iranian National Standard. Also, in cream-filled pastries produced in Chaharmahal Va Bakhtiari confectioneries, a high level of samples were contaminated by coliforms (72%), staphylococci (95%), and yeast (86%) which were outside the Iranian National Standard range.

The findings of the study and previous researches have shown that cream-filled pastries are one of the most important sources of microbial growth and can transmit pathogen agents to people. In addition, according to national standards, this bakery product had low levels of chemical characteristics. Therefore, cream-filled pastries must be produced and stored in appropriate conditions and monitoring microbiologically and biochemically of raw materials, food processing, and food providers according to the national standards are necessary.

Conflicts of interest

The authors declare no conflict of interest associated with this paper.

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References

- Vazirian M, Kashani ST, Ardekani MRS, et al. Antimicrobial activity of lemongrass (*Cymbopogon citratus* (DC) Stapf.) essential oil against food-borne pathogens added to cream-filled cakes and pastries. *J Essent Oil Res* 2012 Oct;24(6):579–82.
- Sami M, Nasri A, Bagheri M, et al. Microbiological and chemical qualities of cream-filled pastries sold in Kerman city confectioneries, southeast of Iran. *Eurasian J Vet Sci* 2013;29(3):138–42.
- Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* 2012 Jul;36(4): 815–36.
- Henson S, Jaffee S. Understanding developing country strategic responses to the enhancement of food safety standards. *World Econ* 2008 Jul;31(4):548–68.
- Gutiérrez D, Delgado S, Vázquez-Sánchez D, et al. Incidence of *Staphylococcus aureus* and analysis of associated bacterial communities on food industry surfaces. *Appl Environ Microbiol* 2012 Sep;78(24):8547–54.
- Lambrechts AA, Human IS, Doughari JH, et al. Bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in some convenient food industries in South Africa. *Pakistan J Med Sci* 2014 Jul–Aug;30(4):755–8.
- Bennett RW. Staphylococcal enterotoxin and its rapid identification in foods by enzyme-linked immunosorbent assay–based methodology. *J Food Protect* 2005 Jun;68(6):1264–70.
- Licandro-Seraut H, Roussel C, Perpetuini G, et al. Sensitivity to vinyl phenol derivatives produced by phenolic acid decarboxylase activity in *Escherichia coli* and several food-borne gram-negative species. *Appl Microbiol Biotechnol* 2013 Sep;97(17): 7853–64.
- Adenipekun EO, Jackson CR, Oluwadun A, et al. Prevalence and antimicrobial resistance in *Escherichia coli* from food animals in Lagos, Nigeria. *Microb Drug Resist* 2015 Jun;21(3):358–65.
- Normanno G, Firinu A, Virgilio S, et al. Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *Int J Food Microbiol* 2005 Jan;98(1):73–9.
- Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* 2010 Jul;2(7): 1751–73.
- Kornacki JL, Johnson JL. *Enterobacteriaceae*, coliforms and *Escherichia coli* as quality and safety indicators. In: *Compendium of methods for the microbiological examination of foods*. Washington DC (WA): AJPB; 2001. p. 69–82.
- Lancette GA, Bennett RW. *Staphylococcus aureus* and staphylococcal enterotoxins. In: *Compendium of methods for the microbiological examination of foods*. Washington, USA: AJPB; 2001. p. 384–403.
- AOAC. Official method of AOAC International. Association of Official Analytical Chemists. 17th ed. Washington DC (WA): AOAC; 2000.
- Institute of Standards and Industrial Research of Iran (ISIRI). Microbiologic characteristics of sweets products. Report No.: 2395; 1993 [In Persian].
- Asadi S, Maram ZR, Kooshk F. Evaluation of microbial contamination of pastry cream in Arak city of Iran. *J Food Safety Hygiene* 2015;1(1):26–9.
- Uhital S, Jakšić S, Petrak T, et al. Prevalence of *Listeria monocytogenes* and the other *Listeria* spp. in cakes in Croatia. *Food Control* 2004 Apr;15(3):213–6.
- Nikniaz Z, Mahdavi R, Jalilzadeh H, et al. Evaluation of microbial contamination in cream filled pastries distributed in Tabriz confectioneries. *J Food Technol Nutr* 2011;8:66–72.