Microbial Contamination of Cream-filled Pastries in the Confectioneries of Zanjan, Iran

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A R T I C L E I N F O	**A B S T R A C T**
Article type: Original article *Article History:* Received: 16 Jul 2018 Accepted: 22 Oct 2018 Published:27 Oct 2018 *Keywords:* Cream-filled Pastries, Food-borne Pathogens, Iran, Microbial Contamination, Zanjan	**Introduction:** Non-standard food hygiene can lead to the microbial contamination of food products. Food-borne pathogens cause more than 200 illnesses ranging from mild diarrhea to cancer. Cream-filled pastries are milk-based bakery products with high production and consumption rates in the confectioneries in Iran. These products have soft, spongy texture made of various ingredients. The present study aimed to assess the microbial contamination of cream-filled pastries as a highly consumed food product and evaluate the relation between microbial contamination and sanitary conditions in the confectioneries in Zanjan, Iran. **Methods:** In total, 62 samples of cream-filled pastries were collected from the confectioneries in Zanjan city, Iran. Preparation, dilution, and microbiological examination of the samples were conducted in accordance with the instructions of the Institute of Standards and Industrial Research of Iran regarding the total count enumeration of Escherichia coli, coliforms, Staphylococcus aureus, Salmonella, Bacillus cereus, yeasts, and molds (No. 2395 and 8923-1, 2946, 2461, 6806, 1810,2324, and 10899, respectively). **Results:** Microbial analysis indicated that 40 out of 62 samples had a total microbial count of below the standard limit for cream-filled pastries. In addition, 20, 24, 4, and 47 specimens had higher counts than the recommended limit of the Iranian national standards for E. coli, S. aureus, Salmonella spp., and coliforms, respectively. Also, 30 samples of cream-filled pastries had substandard contamination with molds and yeasts. **Conclusion:** Results showed low hygienic conditions of processing, storage and distribution of cream-filled pastries. Therefore, it is strongly recommended that all the principles of food hygiene be observed by the confectioneries in Zanjan, Iran.

Introduction

Microbial contamination of foods and food-borne diseases are considered to be among the foremost economic and public health concerns throughout the world including Iran (1,2). Unsafe foods are a threat to the global health, particularly in the case of vulnerable populations, such as pregnant women, infants, children, the elderly, and patients with underlying diseases (2). Dairy, meat, and bakery products are most frequently affected by contamination, thereby causing food-borne diseases (3,4).

Cream-filled pastries are milk-based bakery products with high production and consumption rates in the confectioneries of Iran. These products have a soft, spongy texture made of various ingredients, including wheat flour, milk, eggs, cream, jelly, fresh fruits, and coloring and flavoring agents. Due to their base composition and wetness, cream-filled bakeries are the optimal media for microbial growth (3,5-7).

Substandard food hygiene may lead to the microbial contamination of food products. Food-borne pathogens have been shown to cause more than 200 diseases, ranging from mild diarrhea to cancer (8). Escherichia coli, Staphylococcus aureus, Salmonella enterica serotypes, campylobacter, molds, and yeasts are among the most important food pathogens, which threaten public health. Consumption of the foods that are contaminated with these pathogens causes diarrheal diseases. Statistics suggest that approximately one per 10 individuals acquires food-borne diseases, and the mortality rate has been estimated at 420,000 cases per year (2). In some cases, food pathogens could cause chronic disorders, declining development and productivity, especially in developing and held back countries (5).

Microbial contamination of confectionery products is important in point of view economic and hygienic aspects which limit the shelf-life of these food products and cause food-borne diseases (7,8). In this regard several domestic and international studies have recommended the monitoring of microbial quality of these products (1,3,4,8,9-13). The findings have also denoted the partial contamination of cream-filled pastries with food pathogens and food spoilage

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microorganisms. Microbial contamination of bakery products have many potential sources, including the primary contamination of raw materials and the subsequent secondary contaminations, equipment and final products in the production process, transportation and distribution of the products, and non-observance of good manufacturing practices (GMP) by laborers (14,15).

Considering the high consumption of confectionery products in Zanjan city (Iran) and risk of microbial contamination in cream-filled pastries, which increases the possibility of food-borne diseases, as well as the scarce data in this regard in Zanjan city, the present study aimed to assess the microbial contamination of cream-filled pastries as frequently consumed food products and evaluate the relation between microbial contamination and sanitary conditions in the confectioneries in Zanjan, Iran.

Materials and Methods
Sampling
In total, 62 samples of cream-filled pastries were randomly collected from the confectioneries in Zanjan via cluster sampling during June-July 2017. The samples were immediately transferred to a food microbiology laboratory in sterile, labeled polyethylene bags under cold chain conditions. All the samples were preserved at the temperature of 4ºC until microbiological analysis.

Microbiological Analysis
Preparation, dilution, and microbiological examination of the samples were conducted in accordance with the instructions of Institute of Standards and Industrial Research of Iran regarding the total count enumeration of Escherichia coli, coliforms, S. aureus, Salmonella, and Bacillus cereus (No. 2395 and 8923-1, 2946, 2461, 6806, 1810, 2324, and 10899, respectively) (16-23).

Sample Preparation
At this stage, 10 grams of each sample was added to 90 milliliters of sterile 0.1% buffered peptone water (BPW) (Merck, Darmstadt, Germany) and homogenized with a stomacher for two minutes (Interscience-Bag Mixer 400, St., Nom., France). Serial dilution was prepared in tubes containing nine milliliters of peptone water (0.1%). In addition, instructions were followed to count various microorganisms in the samples.

Total Count (TC)
Total viable counts were performed based on transferring one milliliter of the prepared serial dilutions to the plate count agar (PCA) (Merck, Darmstadt, Germany). Afterwards, the plates were incubated at the temperature of 37◦C for 48 hours (16).

Enumeration of Coliforms and Escherichia coli
At this stage, one milliliter of each of the prepared dilutions was placed on violet red bile agar culture medium (Merck, Darmstadt, Germany) and incubated at the temperature of 35◦C for 24 hours. The plates containing 30-300 red-to-purple colonies were enumerated as coliforms. Simultaneously, to detect the E. coli bacteria, the most probable number (MPN) test was run in nine tubes containing Lactose broth (Merck, Darmstadt, Germany), as well as the Durham tubes. After 24-48 hours of incubation at the temperature of 37◦C, 100 microliters of the suspension was inoculated from the tubes containing gas on Eosin methylene blue agar (EMB) (Merck, Darmstadt, Germany) in order to confirm the suspected positive E. coli. Centered blue-black colonies with metallic green sheen were identified as E. coli after 24 hours of incubation at the temperature of 37◦C (19).

Enumeration of Staphylococcus aureus
According to the Iranian national standards for the detection and enumeration of coagulase-positive Staphylococci, 100 microliters of the prepared dilutions were spread on Baird-Parker agar medium (Merck, Darmstadt, Germany) and incubated at the temperature of 37◦C for 48 hours. The plates containing black colonies with bright halos were selected to count S. aureus. In addition, the coagulase test was used to confirm the bacteria (20).

Enumeration of Molds and Yeasts
In accordance with the Iranian national standard (No. 10899), 100 microliters of the prepared serial dilutions were spread on Potato Dextrose agar containing chloram- phenicol (5 mg/l) and incubated at the temperature of 25◦C for 3-5 days (21).

Enumeration of Bacillus cereus
Mannitol egg yolk polymyxin (MYP) agar was used as the selective culture media for the identification and enumeration of B. cereus. For this purpose, 100 microliters of the prepared serial dilutions were spread on MYP agar and incubated at the temperature of 30◦C for 48 hours. B. cereus colonies were those with light pink background colonies with a zone of whitish precipitate. The culture for the detection of B. cereus (ATCC 9634) was provided by the Iranian Research Organization for Science and Technology (IROST) and used as the positive control to confirm the test results (22).

Detection of Salmonella
Detection of *Salmonella* strains requires pre-enrichment**,** enrichment of the bacteria, and selective media. For this purpose, 25 grams of the samples were initially prepared in 225 milliliters of 0.1% BPW (Merck, Darmstadt, Germany) and incubated at the

temperature of 37◦C for 18±2 hours(pre-enrichment). Selenite cystine and tetrathionate broth were used for enrichment. One milliliter of the pre-enriched samples in BPW was transferred to the selenite cystine and tetrathionate broth, and the tubes were incubated at the temperatures of 37◦C and 44◦C for 24 hours, respectively. In order to detect and count the Salmonella colonies, Salmonella-Shigella (SS) agar and xylose lysine deoxycholate (XLD) agar were used, and black-centered, colorless colonies were confirmed as Salmonella. Moreover, two other tests were conducted to confirm the *Salmonella* colonies, including culturing the colonies on triple sugar iron (TSI) agar and IMViC test. Salmonella produces hydrogen sulfide gas, and the black and yellow color at the bottom and the red color in slant of the TSI tubes and have (-+-+) reactions in the IMViC test, respectively (16,23).

Statistical Analysis

Data analysis was performed in Microsoft Excel software (2010) to determine the mean values based on the three replications of each experiment. Data were expressed as mean ±SD.

Results

The microbial contaminations in the studied cream-filled pastries are presented in Table 1. According to the microbial analysis, 40 out of 62 samples had a total count out of the standard limit for cream-filled pastries, while 20, 24, 4, and 47 samples had higher counts than the recommended limit of the Iranian national standard for E. coli, S. aureus, Salmonella spp., and coliforms, respectively. Common contamination with both or the three mentioned microorganisms was observed in the cream-filled pastry samples (Table 1). Furthermore, 30 samples had a substandard limit of contamination with molds and yeasts. The mean microbial counts of the tested microorganisms are shown in Table 2. It should be noted that B. cereus was not detected in collected samples.

Table 1. Contamination rate of cream-filled pastries samples supplied from Zanjan confectioneries.

Microbial test	Out of Standard limit and contaminated samplesNo (Frequency %)	Standard limit (CFU/gr)
Total count (TC)	40 (64.51)	5×10^4
Escherichia coli	20 (32.25)	Negative
Coliforms	47 (75.8)	Negative
Staphylococcus aureus	54 (87.09)	Negative
Mold and Yeast	30 (48.39)	Up to 10^3
Salmonella sp.	4 (6.45)	Negative (CFU/25 gr)
Bacillus cereus	0 (0)	Up to 10^2
Contamination with both E. coli and S. aureus	46 (74.19)	Negative
Contamination with both coliforms and S. aureus	20 (32.25)	Negative
Contamination with both Salmonella and S. aureus	4 (6.45)	Negative
Contamination with E. coli, S. aureus and coliforms	20 (32.25)	Negative

Table 2. Microbial counts (Mean±SD) of cream filled pastries samples (log cfu/g)

Microbial test	Mean±SD	Maximum	Minimum
Total count (TC)	5.33±0.05	8.07	3.17
Escherichia coli	3.33±0.12	4.33	0
Coliforms	3.69±0.072	5.63	0
Staphylococcus aureus	4.51±0.11	7.55	0
Mold and Yeast	3.85±0.15	4.1	0
Bacillus cereus	0	0	0

Discussion

According to the results of the present study, the cream-filled pastries obtained from the confectioneries in Zanjan (Iran) were highly contaminated with important food-borne pathogens and spoilage bacteria, including E. coli (32.25%), coliforms(75.8%), S. aureus (87.09%), molds and yeasts (48.39%), and Salmonella spp. (6.45%). However, no B. cereus contamination was denoted.

Total coliforms are considered to the indicator bacteria for the detection and estimation of fecal contamination in food products, particularly water. E. coli is a member of the fecal coliform group is a commonly used bacterium of this group for various purposes. The presence of the fecal coliform strains shows the favorable conditions for the growth of other pathogens, such as Salmonella and campylobacter (24). According to national standards, coliforms including E.colishould not exist in drinking water and food samples (16). Some strains of E. coli may cause serious food-borne diseases, ranging from gastroenteritis to hemorrhagic colitis (25). Given the importance of these bacteria, continuous monitoring for coliform and E. coli contamination is essential. In this regard, several studies have investigated various foods, including confectionery products, in different countries, including Iran. For instance, Jamshidi et al. (2017), Haghparast et al. (2016), Pajohi-Alamoti et al. (2016),

and Khoramrooz et al. (2015) reported the contamination rate of cream-filled pastries with coliforms and *E. coli* 59.1%, 92.5%, 32.6%, and 50% in Arak, Hamedan, Isfahan, and Yasuj cities, respectively (1,11,14,26).

In the present study, the mean contamination rate of cream-filled pastries with *S. aureus* was significantly higher than the recommended limit in Iran. The reported contamination rate with *S. aureus* in our research was higher than the results of Khoramrooz et al. (2015) and Zafarzadeh and Mahfoozi (2015) within 30% and 19%, respectively) in Yasuj and Gorgan cities (Iran), respectively, while it was lower than the results reported rates by Sharifzadeh et al. (2016) in the confectioneries in Chaharmahal and Bakhtiari province in Iran (with a 95%) contamination rate (3,26,27). Several agents have been described as the main causes of high contamination rate in the aforementioned studies, such as the addition of no pasteurized creams to pastries, insufficient cold preservation, use of contaminated equipment and containers, and inadequate personal hygiene of workers during the processing, storage, transfer, and distribution of the products, which is consistent with the findings of the current research (1). Furthermore, the carrier workers have been reported to be the primary sources to contaminate foods and cream-filled pastries with *S. aureus* (14).

In the present study, the contamination rate of cream-filled pastries with molds and yeasts was estimated at 48.39%. The results of the previous studies in this regard have also confirmed the high rate of fungal contamination in cream-filled pastries, which is lower than the reported rate in the current research in some cases. For instance, Jamshidi et al. (2017) and Sharifzadeh et al. (2016) reported this rate to be 13.6-20.5% and 27.63% in Arak and Chaharmahal and Bakhtiari provinces, respectively (1,3). An important economic and serious problem for bakeries is the mold spoilage of bakery products. Contamination of the environment and applied containers for the processing, long-term storage, and distribution of products, as well as cross contamination, are known as the main reasons for contamination of confectionery products by molds and yeasts (5,7). Moreover, all the cream-filled pastries containing fruit parcels or nuts as ingredients were observed to have higher mold and yeast contamination, which is related to microbial quality of the used raw materials (7). In the current research, Salmonella contamination was observed in 6.45% of the cream-filled pastries samples obtained from the confectionaries in Zanjan. In this regard, the findings of Haghparast et al. (2016), Sultan Dalal et al. (2010) and Pajohi-Alamoti et al. (2016) reported 22.1%, 0%, and 0% contamination rate in the cream-filled pastries in Isfahan, Tehran, and Hamedan cities (Iran) (11,12,14). Contaminated eggs, milk, and dairy products (e.g., cream), Salmonella carriers (e.g., workers, food handlers, and product distributers) have a key role in presence of these bacterial strains in bakery products (4,14).

Conclusion

According to the results, the highest rate of microbial contamination in cream-filled pastry samples was associated with S. aureus, coliforms, molds and yeasts, E. coli and Salmonella, respectively. Furthermore, poor hygiene during the processing, storage, and distribution of cream-filled pastries were among the main causes of contamination. Therefore, it could be concluded that consider to quality of raw materials, observance of good hygienic practices (GHP) and good manufacturing practices (GMP) by confectionaries, revising the health inspection rules, continuous monitoring of all the stages of production, implementing health education programs by public health authorities is necessary that could effectively raise the awareness of food producers, distributors, and consumers.

Conflict of interest

All authors declare that there are not any conflicts of interest.

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