

Microbial Contamination of Pastry Cream: Evidence From Hamedan, Iran

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Background & Aims of the Study: Given the importance of microbial contamination in creating food-borne diseases, this study was conducted to assess level of microbial contamination of pastry creams in Hamedan, Iran.

Materials and Methods: Totally, 80 samples were randomly collected from the confectioneries and analyzed for microbial contamination according to Iranian national standard microbial tests.

Results: Data indicated that 49 (61.2%) samples were contaminated, mostly comprised of Coliforms (92.5%). Moreover, the infection was seen to be higher in jelly roll compared to puff pastry. Yeast contamination was about 82.5 percent, which could accelerate the decay of such products. However, yeast contamination of puff pastries was higher than jelly roll. The microbial contamination with *Staphylococcus aureus*, total viable count and molds were 57.5%, 35% and 37.5%; respectively.

Conclusion: Nevertheless, *Salmonella*, *Escherichia coli* and *Listeria monocytogenes* were not found in any of the samples. Abundance of microbial contamination in the puff pastry samples might put consumer's health at risk.

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Background

Outbreaks of food poisoning have always been a problem all around the world. Many people suffer from food-borne diseases per year (1). Confectionery products are an important source of food poisoning in Iran. Pastry creams have high potential for being contaminated with various microorganisms such as *Staphylococcus aureus* and various Enterobacteriaceae due to their ingredients and method of production (2). High consumption of confectionery products worldwide makes it necessary to apply microbial control over pastry creams from both health and industrial

perspectives (3). Bacteria are the most common cause of food-borne illnesses. Compared to chemical and physical contaminations, these microorganisms are more important in food poisoning. They seldom create any changes in color, odor or taste of the products. Microorganisms are everywhere. Soil, water, air, human, animals and surfaces which come in contact with food, might cause some levels of contamination. If these microbes find the opportunity to grow and multiply, they will cause decay and undesirable chemical changes as well as food poisoning (4).

Escherichia coli, *Staphylococcus aureus* and *Salmonella* are the most important microbial agents in either food poisoning or intestinal

infection that could put general health at risk. Annually, millions of people around the world suffer from food poisoning caused by dairy, confectionary and meat products. It should be noted that in some cases such agents could lead to chronic health effects such as joints diseases, immune system disorder or renal failure (5,6). Contaminations come from various sources during production, transportation and distribution of the products.

Aims of the study:

High consumer acceptance of confectionery products as well as outbreaks of food-borne diseases caused by pastry creams necessitates this study aimed at determining level of microbial contamination in such products supplied in Hamedan province, Iran.

Materials & Methods

Sample collection

In this cross-sectional study, conducted over a period of nine months in 2014-2015, out of 40 popular confectioneries in Hamedan, Iran, 80 samples of puff pastry and jelly roll were randomly selected. Marking the specimens in sterile conditions and along with ice packs (4 ° C), the samples were transferred to food hygiene laboratory, faculty of para-veterinary sciences, Bu-Ali Sina University. All tests were run in triplicate (7).

Microbial examination

Microbial tests on the samples and sampling were implemented based on the Iranian national standards (7-14). Initially, the samples were homogenized in a bag mixer (ONETECH S400, South Korea), and then diluted by adding 90 ml of sterile peptone water into each 10 grams sample. Eventually, microbial counting was performed after serial dilutions of the samples.

Total viable counts (TVC): based on the pour plate count method, 1 ml of each dilution was inoculated into the melted plate count agar (PCA), and incubated at 35 ° C for 2 days. For ultimate count, plates containing 30-300 colonies were selected (8,9).

Coliforms and Escherichia coli counts (E .coli): one ml of sequentially-obtained dilution was cultured at melted Violet Red Bile agar (VRBA) in over lay form, and was incubated at 35 ° C for 24 h. To count Coliforms, those plates containing 20-200 purple colonies, ≥ 0.5 mm diameter, and with bile acid deposition were counted. From these colonies, 10 were selected and inoculated in brilliant green lactose broth (BGLB) containing Durham tube, and later incubated at 37 ° C for 24 h. Turbidity and gas producing tubes were confirmed as coliform. Total coliform count was calculated by multiplying the number of positive tubes (divided by 10) in counted colonies (10).

In order to detect *E. coli*, samples from the positive BGLB tubes inoculated into EC broth containing Durham tubes, and incubated at 45.5° C for 48 hours. Gas containing EC tubes were inoculated on eosin methylene blue agar (EMB) and incubated at 35 ° C for 24 h. Positive colonies were examined microscopically and IMViC-tested (Indole, Methyl Red, Voges Prauskoer, Citrate) (11).

Staphylococcus aureus count: 100 μ l of sequentially-obtained dilution was inoculated to Baird Parker agar, cultured based on the spread plate count method, and incubated at 35 ° C for 2 days. Plates containing 15-150 black colonies with bright halos were selected for the enumeration of *S. aureus*. The colonies were confirmed using coagulase test and inoculation in DNase agar (12).

Salmonella detection: pre-enrichment was performed in lactose broth at 37 ° C for 24 h. For the enrichment, one ml of lactose broth was transferred into both selenite cysteine and tetrathionate broths, and incubated at 35 and 44 ° C for 24 hours; respectively. Later, they were inoculated on Salmonella-Shigella (SS) agar. To approve Salmonella, colorless colonies with black centers were transferred on Triple Sugar Iron agar (TSI), Lysine Iron agar (LIA) and urea broth for approval (13).

Listeria monocytogenes detection: under cold conditions, each sample was enriched in Brain

Heart Infusion broth (BHI), and cultured in blood agar and Palcam Listeria agar (PLA) containing Palcam supplement (Polymixin B, Acriflavin & Ceftazidime). Gray-green colonies surrounded by dark brown on PLA were confirmed using biochemical tests as ability of the bacteria in hydrolysis of sodium hippurate (8).

Mold and yeast count: 100 µl of sequentially-obtained dilution was inoculated on Potato Dextrose agar (PDA) containing chloramphenicol (5 mg/l), cultured based on the spread plate count method, and incubated in a dark place at 25 ° C for 3-5 days (14).

Statistical analysis

The data was done by analysis of variances (ANOVA) using the SPSS software package (IBM SPSS statistics 21). Tukey test with a significance set at P<0.05 was applied to compare differences among mean values. The results were calculated as mean values±standard deviation (SD).

Microorganisms were counted according to log cfu/g and compared with the permissible standard (Table 1).

Results

Average microbial contamination of pastry creams are shown in Table 2. Microbial analysis indicated that 49 samples (61.2%) were contaminated to various microbes. No contamination of Listeria monocytogenes,

Escherichia coli and Salmonella was found in the tested samples. Coliforms had the most contamination comprising 92.5% of the total. Such contamination in jelly roll (100%) was significantly higher than puff pastry (85%) (P<0.05). No significant differences were observed (P<0.05) in terms of TVC, S. aureus, mold, yeast, Salmonella and L. monocytogenes contamination between puff pastry and jelly roll.

About 82.5% of the inedible samples revealed yeast contamination, which unlike coliforms, were higher in puff pastry (85%) compared to jelly roll (80%). Staphylococcus aureus and mold comprised 57.5 and 37.5 % of totally contaminated unusable samples (Table 2); respectively. Total viable count (TVC) was found higher in jelly roll compared to puff pastry.

Table 1) Permissible limits for microbial contamination in pastry cream according to Iranian national standards, no: 2395

Microorganisms	Permissible limits
TVC	5 × 10 ⁴ cfu g ⁻¹
<i>Staphylococcus aureus</i>	N ¹
Coliforms	N
<i>E. coli</i>	N
<i>Salmonella spp.</i>	N ²
<i>Listeria monocytogenes</i>	N
Mold	5 × 10 ² cfu g ⁻¹
Yeast	10 ³ cfu g ⁻¹

N: negative
 N¹: negative in 0.1 g per sample
 N²: negative in 25 g per sample

Table 2) Average (±SD) microbial counts (log cfu g⁻¹) of pastry cream samples from Hamedan

Microorganism	jelly roll			puff pastry		
	mean±SD	Max	Min	mean±SD	Max	Min
TVC	4.06 ± 0.33 ^a	4.47	3.20	3.90 ± 0.29 ^a	4.38	3.25
<i>Staphylococcus aureus</i>	3.07 ± 0.86 ^a	3.93	0	2.70 ± 1.43 ^a	4.04	0
Coliforms	3.34 ± 0.5 ^a	4.07	2.30	2.68 ± 1.20 ^b	3.70	0
Mold	1.90 ± 1.60 ^a	3.60	0	1.85 ± 1.70 ^a	3.77	0
Yeast	3.48 ± 0.90 ^a	4.14	0	3.31 ± 1.19 ^a	4.30	0
<i>Salmonella spp</i>	-	0	0	-	0	0
<i>Escherichia coli</i>	-	0	0	-	0	0
<i>Listeria monocytogenes</i>	-	0	0	-	0	0

Values with different superscript alphabets are significantly different in rows (p<0.05)

Table 3) Percentage of edible and inedible samples based on microbial contamination

Factor	Puff pastry			Jelly roll		
	Percentage	inedible samples	edible samples	Percentage	inedible samples	edible samples
TVC	20	8	32	50	20	20
<i>Staphylococcus aureus</i>	60	24	16	55	22	18
Coliforms	85	34	6	100	40	0
Mold	45	18	22	30	12	28
Yeast	85	34	6	80	32	8
<i>Salmonella spp.</i>	0	0	40	0	0	40
<i>Escherichia coli</i>	0	0	40	0	0	40
<i>Listeria monocytogenes</i>	0	0	40	0	0	40

Discussion

The results of this study indicated that puff pastry and jelly roll -collected from confectionaries of Hamedan, Iran- were highly contaminated to coliform and yeast. According to Iranian national standard of pastry cream No. 2395, Coliforms' count per gram of the samples should be negative. These samples were probably inedible. (8). Coliforms presence in confectionery products, especially fecal coliforms such as *E. coli*, can cause serious poisoning and intestinal infections for consumers. Therefore, monitoring Coliform contamination is critically important from microbiological perspective. Major reasons behind high Coliform contamination in the samples could be due to not using pasteurized cream and inadequate cooling systems. Moreover, coliforms' presence in the samples is more related to not observing personal hygiene in the production and distribution of confectionery sweets. In the studied samples, fecal coliforms and *E. coli* weren't observed. In a study by Khezri et al. (2007) in Mashhad (Iran), the contamination of cream cake to coliform bacteria and *E. coli*, was reported to be 69% and 26%; respectively (15). The reason behind jelly rolls being more contaminated to

coliform compared to puff pastry can be due to contaminated equipment and utensils like knives and cutting tools.

Out of all, 85% of puff pastry and 80% of jelly roll samples showed more than 103 cfu/g yeasts. Similarly results of Nikniaz (2011) in Tabriz (Iran) showed that 38.8% of puff pastry samples were contaminated to coliforms, 31.2% to *Staphylococcus aureus* and 70% to yeast (16).

It could exercise a decisive role in the occurrence of spoilage and the off-flavor (17). Keeping pastry creams for a long time in confectioneries, and inappropriate transport and supply conditions are to be blamed for high levels of yeast contamination. A study conducted by Asadi et al. (2015) in Arak (Iran) reported the contamination of pastry creams to yeast at 95.8 which is consistent with our findings (18).

About 24 samples (60%) of puff pastry and 22 samples (40%) of jelly rolls evaluated in this study were contaminated with *S. aureus*. It is one major agent causing food intoxication. Primary sources of such contamination can be workers' purulent pimple of the hands or face and improper handling of pastry materials by the confectionaries staff. Presence of the bacteria in such products increases the risk of toxicity and makes these products

inconsumable. Sami et al.'s work (2013) on the puff pastry in Kerman (Iran) revealed *S. aureus* contamination to be 20% which echoes our findings (19). In this study, no cases of *Salmonella* spp. and *L. monocytogenes* were found, which indicates healthy ingredients and lack of secondary contamination. Another study was conducted by Kotzekidou (2013) during 2001-2010 in Greece on the pastry cream which microbial contamination level of *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *E. coli*, Enterobacteriaceae and TVC were 20, 12.5, 28.6, 25, 35.3 and 48.8%; respectively (20).

In the present study, none of the samples were contaminated with *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes*. In a study published in 1999 by the Italian Ministry of Health, the main cause of an outbreak of acute poisoning with symptoms of gastroenteritis caused by *Salmonella* spp. contaminated which was reported to be the result of using contaminated eggs in fresh pastry (21). Al-Jafaeri et al. (2013) showed that the contamination level of pastry cream with *S. aureus*, *E. coli* and *Salmonella* spp. were 48, 4 and 8%; respectively (22). Several studies in different countries have shown that milk and other dairy products are potential source for the growth of bacteria, especially in hot weather. Thus to avoid increasing contamination, the temperature in the quickly putrescent products should be controlled appropriately (23,24). Contamination of puff pastry to yeast occurs in pastry products with high levels of active water, which both in terms of creating a piece of pink and white color on sweets and consequently effect on the appearance of the sweets and in terms of fermentation corruption of confectionary products and consequently effect on odor and taste of sweets are important (25,26). Because of increased health risks and reduced product quality, puff pastry and jelly roll's contamination to mold is economically very important to the industry. Since mushroom spores are dispersed in the air, they can cause

pastries contamination. In addition to contaminations caused by air, container, and distributors, contamination of raw materials such as sugar and especially flour can also lead to mold infection in puff pastry and jelly roll (4).

Conclusion

Based on these results and high levels of microbial contamination of puff pastry and jelly roll, the major contamination sources of this type of food may be due to microbial contamination of raw materials such as cream, lack of personal hygiene of workers, and microbial contamination of utensils used for pastry production. It can be suggested that the raw materials be examined separately for microbial contamination.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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