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Evaluation of microbial quality of traditional sweets, Zoolbia and Bamieh, during Ramadan in Mashhad, Iran

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ABSTRACT

Introduction: The purpose of this study was to evaluate the microbial quality of two traditional Iranian sweets, (i.e., Zoolbia and Bamieh) made in confectioneries during the Muslim holy month of Ramadan in Mashhad.

Methods: This cross-sectional study was conducted on 75 samples of Zoolbia and Bamieh collected randomly from some confectionaries during Ramadan in Mashhad in 2018. Microbial quality tests, including mold and yeast, *Escherichia coli, Enterobacteriaceae* and *Staphylococcus aureus* enumeration as well as *Salmonella* isolation were performed.

Results: Out of the total samples tested, 22.6% of them were within the specified limit of the standard. The contamination rate of mold and yeast, *Salmonella*, *Enterobacteriaceae, E.coli*, and *S. aureus* were 13.3%, 6.6%, 13.3%, 16%, and 28%, respectively. According to the results, *S.aureus* and *Salmonella* obtained the highest and lowest levels of contamination, respectively.

Conclusion: The results showed significant rates of contamination regarding these two kinds of sweets. Therefore, given the high percentages of contamination of samples, production and distribution practices as well as the workers hygiene must be under more control and attention. The existence of vulnerable groups among consumers adds to the importance of this issue.

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Introduction

Fasting is one of the Muslims practices in the holy month of Ramadan. In this regard, Muslims refrain from eating three main meals (i.e., Breakfast, Lunch, Dinner) for one month and usually eat 2 meals a day (i.e., before sunrise, , Suhoor, , and after sunset, Iftar,) (1). In the month of Ramadan, people abstain from eating for 11-18 hours during the day and every year hundreds of millions of Muslims experience hunger for a month (1).

Zoolbia and Bamieh, two of the famous traditional Iranian sweets, are among the various kinds of foods consumed in the holy month of Ramadan. They are commonly eaten in Iftar meal during Ramadan. Zoolbia ingredients include yogurt, starch, and water, whereas Bamieh is made of water, wheat flour, and egg. The prepared dough is then fried in too much hot oil and dipped in special syrup (2).

Various microbial and chemical characteristics are determined to evaluate the quality of these products. The determination of the peroxide value, the presence of chemical colors evaluated by chemical tests and the estimation of fungi level, such as *Enterobacteriaceae*, *Escherichia coli*, and *Staphylococcus aureus* by microbial tests are important in quality control (2).

In developed countries, the presence of

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pathogens in food has been a matter of great concern due to the several reports about rates of food poisoning, 6.5 to 33 million cases of human diseases, and 9,000 deaths per year (3).

Foods contaminated by chemical and biological agents are responsible for 70% of human diseases (4). In the UK, confectionary products account for more than 30% of food-borne diseases; meanwhile, the highest contamination is related to *S. aureus*. The probability of *E. coli*, mold and yeast contamination in traditional sweets is due to the methods of preparation and designing (5).

In this study, considering the importance of food safety and the sensitivity of people who fast due to the changes in their diet, contamination by microbial agents, such as *Salmonella*, *Enterobacteriaceae*, *E.coli*, *S. aureus*, and mold and yeast in Zoolbia and Bamieh as traditional sweets, were evaluated. Food safety and the sensitivity of people who fast due to the changes in their diet are of remarkable importance. Therefore, this study aimed to evaluate the levels of contamination by microbial agents, such as *Salmonella*, *Enterobacteriaceae*, *E.coli*, *S. aureus*, and mold and yeast in Zoolbia and Bamieh as traditional sweets.

Material and methods

This cross-sectional study was conducted on 25 samples of Zoolbia and Bamieh (in three rounds, n=75) collected randomly from some confectionaries located in five districts under the supervision of hygienic centers during Ramadan in Mashhad in 2018. Microbial quality tests, including mold and yeast, *Escherichia coli, Enterobacteriaceae* and *Staphylococcus aureus* enumeration as well as *Salmonella* isolation were performed. Sampling was carried out during a month and finally, 75 samples were collected**.**

The samples were sent to the laboratory of Nutrition, Faculty of Medicine, Mashhad, Iran, under sterile conditions in a cold box for less than 4 h and microbiological tests were carried out.

Microbiological analyses

Isolation of E. coli

E. coli was determined by the method of standard No 2395. For this purpose, red- purple colonies of Enterobacteriacea were transferred into the Brilliant Green Bile Broth (Merck, Darmstadt, Germany) and incubated at 44˚C for 24-48 h. A loopful of each gas positive tubes were streaked onto Eosin Methylene Blue agar (Merck, Darmstadt, Germany) and incubated at 37 ˚C for 24 h. Differentiation of E. coli was carried out by Indole Methyl Red Voges-Proskauer Citrate Utilization Test. A control positive sample (PTCC 1338) was provided from the Iranian organizations for science and technology (6).

Isolation of S. aureus

Totally, 25 g of each sample was weighed and homogenized with 225 mL of Buffered Peptone Water (BPW) (Merck, Darmstadt, Germany) for about 10 min. The samples were further diluted with BPW, and 0.1 mL of each dilution was spread on the surface of Baird Parker agar (Merck, Darmstadt, Germany), supplemented with egg yolk–tellurite emulsion and incubated at 37 ˚C for 24 h. A control positive (PTCC 1431) was provided by the Iranian organizations for science and technology (7).

Isolation of Salmonella

Totally, 25 g of each sample was weighted and homogenized with 225 mL of BPW. These samples were incubated at 37˚C for 24 h. Upon the completion of the pre-enrichment process, 0.1 mL was transferred into the Rappaport-Vassiliadis broth and 1 mL was transferred into the Selenite Cystine Broth. Each broth was incubated at 42 ˚C and 37 ˚C for 24 h, respectively. Then, the positive cultures were finally streaked onto Hektoen Enteric agar (Merck, Darmstadt, Germany) and Brilliant Green Agar (Merck, Darmstadt, Germany) and incubated for 24–48 h at 37˚C. Subsequently, the positive samples were confirmed by inoculating into Triple Sugar Iron agar tubes. A control positive (PTCC 1609) was provided by the Iranian organizations for science and technology (8).

Enumeration of Enterobacteriaceae

Totally, 25 g of each sample was weighed and homogenized with 225 mL of BPW, and 0.1 mL of each dilution was spread on the surface of Violet Red Bile agar (Merck, Darmstadt, Germany) and incubated at 37 ˚C

for 24 h. Then, the positive cultures were finally inoculated in the Brilliant Green Bile Broth (Merck, Darmstadt, Germany), and they underwent an incubation process at 35°C for 48 h. Furthermore, the colonies on the VRBA were counted with colony counter. A control positive (PTCC 1291) was provided from the Iranian organizations for science and technology (9).

Enumeration of mold and yeast

Totally, 25 g of each sample was weighted and homogenized with 225 mL of BPW. After preparation of serial dilution, 100 µL of each dilution was streaked onto the plates of Potato Dextrose Agar (PDA), (Merck, Darmstadt, Germany). Petri dishes were incubated in the dark at 22-25 °C for 5 days and colonies were counted with colony counter (10).

Statistical analyses

The data were analyzed in SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to determine whether there were any statistically significant differences between the means of groups.

Results

The results of isolation and enumeration of bacteria in Zoolbia and Bamieh showed that 17 (22.6%) samples had acceptable microbial quality; however, 58 (77.3%) samples did not have good microbial quality (Figure 1).

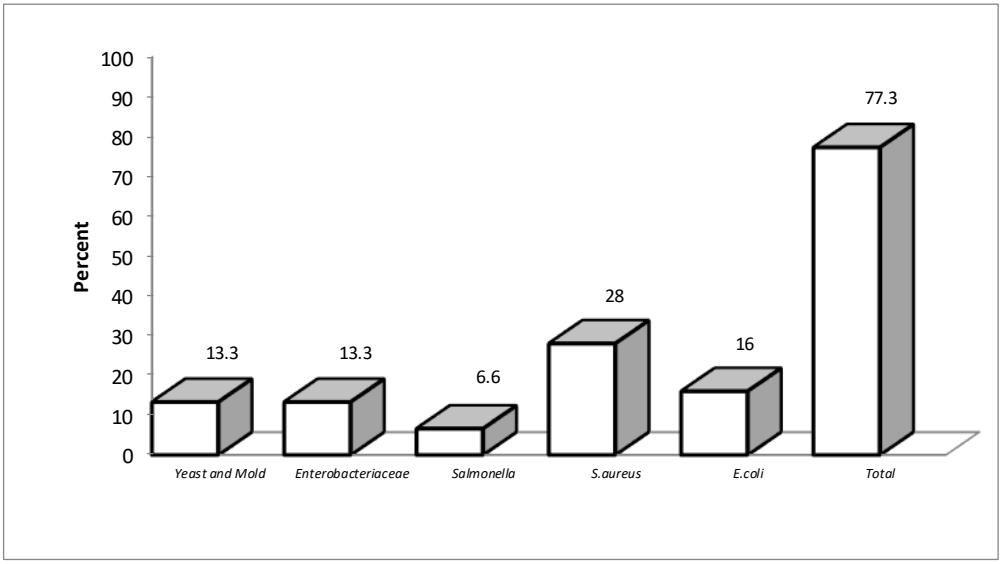

Figure 1: Microbial contamination (percentage) of Zoolbia and Bamieh in Mashhad city

Mold and yeast contamination rate was obtained 13.3% (10 out of 75) (Table 1). The results of this study also confirmed the contamination of 10 (13.3%) samples with *Enterobacteriaceae* (Figure 1). In the evaluated samples, the prevalence of *Salmonella* was reported to be 6.6% and had the least amount of contamination among pathogens. According to the results, 12 (16%) samples of Zoolbia and Bamieh were identified to be contaminated by *E. coli*. In this study, the highest contamination rate was related to *S. aureus* with the prevalence rate of 28%.

Table 1. Microbial contamination of Zoolbia and Bamieh based on different regions in Mashhad

Pathogen / Area	1	2	3	4	5
Yeast and mold	0	5	0	0	5
Staphylococcus aureus	3	4	3	6	5
Salmonella.spp	0	0	0	0	5
Enterobacteriaceae	3	4	0	1	2
E.coli	2	4	1	1	4
Total	8	17	4	8	21

Microbial results showed a significant difference between five surveyed regions of Mashhad ($P \leq 0.003$). It can be seen in table1 that regions 5 and 2 with 21 and 17 contaminated samples contain the higher levels of contamination, respectively.

Discussion

Most of the samples in this study were considered contaminated with the aforementioned microorganisms in the Institute of Standards and Industrial Research of Iran (6). Mold and yeast contamination indicates poor storage conditions (11). Air, contaminated dishes, workers, and raw materials, especially flour, can be effective in transmitting molds and yeast (11). The results of this study confirmed the contamination of 13.3% of samples with mold and yeast. According to a study conducted by Nasehinia Nia et al, in 2015, the contamination rates of traditional sweets of Yazd in terms of mold and yeast were 21.7% and 11.4%, respectively (12).

The results of this study also confirmed the contamination of 13.33% of the samples with *Enterobacteriaceae*. In a study conducted in Gorgan by Shabani et al, in 2013, 56% and 43.3% of sweet samples were contaminated with *Enterobacteriaceae* and S*. aureus*, respectively (13).

The highest contamination rate was related to *S. aureus* in this study. *S. aureus* is one of the most common pathogens that are considered as the third food poisoning agent in the world (14). The presence of the low number of *S. aureus* in food is not uncommon; however, food contamination during processing is common due to the carrier state of the human. Therefore, the risk of toxicity with this pathogen is very high through food (14).

According to other studies, about 40% of total food poisonings are caused by *Salmonella* (14). In the evaluated samples, the prevalence of *Salmonella* was reported to be 6.6% which obtained the least amount of contamination among other pathogens.

However, since the absence of *Salmonella* is considered in the food, therefore, this level of contamination is significant. In the studies carried out by Sepahvand et al. (2016) and Petrová et al. (2015), *Salmonella* was not observed in any of the evaluated samples from surveyed confectionaries of Lorestan city (Iran) and Puna (India), respectively (15,16).

The main foods responsible for the transmission of *Salmonella* in Iran are eggs and poultry meat. Eggs are one of the main ingredients of Zoolbia and Bamieh. With regard to the importance of *Salmonella* in food poisoning and the use of eggs in the preparation process, the evaluation of contaminated samples for vulnerable groups, especially children and people during fasting is of great importance (14). On the other hand, since these kinds of sweets are fried in hot oil with more than 100°C temperature, which probably kills *Salmonella*, other contamination sources, including food handlers should be considered.

According to the results, 16% of Zoolbia and Bamieh samples were identified to be contaminated by *E. coli.* There are many reports on the outbreaks of *E.coli* disease throughout the world and their impact on the health of the society has often been disastrous (14). Fecal bacteria like *E. coli* are considered as one of the most important indicators of food safety and they are important in the food industry (17). In a study conducted in Tabriz, 48.8%, 31.2%, 70% of cream sweets samples were contaminated with E. *coli*, *S. aureus*, and mold and yeast (18).

In another study in Mashhad, 27% of creamy pastries samples were contaminated with E. *coli* (19). In a study performed on 216 samples of cream sweet collected from Tehran, 83% of the samples were reported to be non-consumable and the highest level of contamination was related to E. *coli* bacteria (20).

In terms of different regions in Mashhad, regions 5 and 2 showed higher contamination rates compared to the other districts that indicated weaker hygienic practices during making processes of Zoolbia and Bamieh.

Conclusion

Food authorities can be provided with the results of this study in order to be aware of the current conditions and implementation of health plans. Additional studies can also be effective in determining contamination sources of these traditional products. According to the results of this study, more research is needed on traditional Iranian dishes because there is no determined standard regarding most of these traditional products. Therefore, providing practical solutions as well as continuous planning are needed to deal with possible risks regarding health.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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