Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition

Shahid Sadoughi University of Medical Sciences
School of Public Health
Nutrition Department

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2024; 9(2): 200-206 Website: jnfs.ssu.ac.ir

The Study of Food Pathogens in Yazd Traditional Confectionary Products

Mitra Kimiaee; MSc[1], Mahboobeh Madani; PhD[1*], Mohammad Hossein Mosaddegh; PhD[2] & Seyed Mohammad Moshtaghioun; PhD[3]

[1] Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran; [2] Shahid Sadoughi University of Medical Sciences, Pharmacy School, Yazd, Iran; [3] Department of Biology, Faculty of Science, Yazd University, Yazd, Iran.

ARTICLE INFO

ORIGINAL ARTICLE

Article history:
Received:18 Jul 2022
Revised:22 Oct 2022
Accepted: 22 Oct 2022

Corresponding author
mmadani66@gmail.com
Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.

Postal code: 8451731167
Tel: 98 09134097629

ABSTRACT

Background: Food poisoning could be caused by eating the sweets contaminated with microorganisms, which has always been considered as one of the major problems of people in developing countries, including Iran. The aim of this study is to investigate microbial variety and fungal load in the traditional sweets offered in confectionery stores in Yazd province in 2020. **Methods:** In this study, 170 samples of traditional sweets, referred by confectioneries in Yazd province, were randomly selected and tested for microorganisms contamination such as *Enterobacteriaceae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, molds, and yeasts according to Iranian national standards. **Results:** The highest rate of microbial contamination in samples was related to "Pistachio Loz" (52.94%) and the lowest rate was related to "Hajibadam" (11.76%). The infection rates of *Enterobacteriaceae*, *Escherichia coli*, molds, and yeasts were 17.06%, 8.83%, 20.59%, 3.53%, respectively. No infection was observed with *Staphylococcus aureus* and *Salmonella* in the samples. **Conclusions:** Due to the high level of microbial contamination of traditional sweets, especially "Pistachio Loz" offered in Yazd, more control measures are needed in preparation and distribution of such sweets.

Keywords: *Food contamination; Microbial load; Fungal contamination; Traditional confectionery products; Yazd*

Introduction

Foods contain many pathogens (bacteria, viruses and parasites), which can be transmitted to humans by eating or drinking (Newell *et al.*, 2010). Food-borne disease is one of the important causes of death in undeveloped countries. Symptoms could happen in a few minutes to a few weeks and include vomiting, diarrhea, fever, etc. (Bula-Rudas *et al.*, 2015, Newell *et al.*, 2010). Many types of microorganisms or toxins produced by them, cause food-borne diseases with different mechanisms (Mozafari *et al.*, 2002). The US Centers for Disease Control and Prevention (CDC) estimated that 75 million people suffer from food-borne diseases each year (Newell *et al.*, 2010, Ostadrahimi, 2010).

Since 1990, three major food-borne bacterial groups, including *Salmonella*, *Escherichia coli*,

This paper should be cited as: Kimiaee M, Mosaddegh MH, Moshtaghion SM, & Madani M. ***Prevalence of Methicillin-Resistant Staphylococcus Aureus and Antibiotic Residue in Pasteurized and Raw Farm Milk in Tehran.*** *Journal of Nutrition and Food Security (JNFS)*, 2024; 9(2): 200-206.

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[DOI: 10.18502/jnfs.v9i2.15414]

and *Campylobacter* had been studied in food industry (Meng and Doyle, 2002, Newell *et al.*, 2010). Countries such as the United Kingdom and the United States, reported that 20-40% of illnesses were caused by eating contaminated food (Bolton *et al.*, 2008). Contamination with mold and yeast may reduce the shelf life of confectionery products, causing unpleasant odors, tastes, and discoloration. Fungal contamination of confectionery raw materialsand confectionery containers and distributors could also lead to contamination of various types of sweets (Azhdari and Hashemzehi, 2020).

In developing countries such as Iran, the prevalence of food-borne infections is much higher than developed countries. Dairy, confectionery, and meat products in foods are the most common cause of food poisoning (Nikniaz *et al.*, 2011). Traditional sweets, due to the type of ingredients, methods of preparation and decoration, are likely to be contaminated with pathogens such as *Escherichia coli*, *Enterobacteriaceae*, mold and yeast. Sweets have a variety of nutrients that are well known all over the world. Due to the high consumption of these products, it is necessary to use microbial control in order to increase shelf life and maintain the quality of these products (Smith *et al.*, 2004).

The results of a study conducted by Sultan Dalal *et al*. on microbial contamination of fresh sweets in south of Tehran indicated that 72.7% of the sweets in the study were contaminated with *Enterobacteriaceae* (40%), yeasts (33%) and *Staphylococcus aureus* (12%), which were the highest types of microbes detected (Soltan Dalal *et al.*, 2010). Another study in Gorgan investigated the contamination of sweets containing cream. The results confirmed that 56% of the samples were infected with *Enterobacteriaceae* and 43.3% were infected with *Staphylococcus aureus*, and the highest contamination of sweets was in summer (Shabani *et al.*, 2014).

The study of microbial quality control regarding sweet samples in Leeds, England, showed that 28% of the total samples were infected with *Escherichia coli* (Azhdari and Hashemzehi, 2020). In evaluation of sweets containing cream and ice creams, 50% of the samples were infected with *Escherichia coli* and 30% with *Staphylococcus aureus* (Khoramrooz *et al.*, 2015). Due to the increase in food poisoning caused by the consumption of contaminated sweets in recent years, few studies were performed in this regard. The present study was conducted to investigate the microbial and fungal variety and load in Yazd traditional confectionery products such as Baqlava, Quttab, Pashmak, Nan Berenji, Loz and Hajibadam. These products have different ingredients and moisture levels, which are definitely effective on the type and level of contamination.

Materials and Methods

This cross-sectional study was conducted in 2020 in Yazd, and chemicals and culture media used in this study were prepared and applied according to the Iranian National Standard numbers 2395 and 9899 from the German company (Merck) (Institute of Standards, 2020). Sample preparation and dilutions were performed using the Iranian National Standard No. 8923-1 and 8923-4. The traditional sweet samples were taken from Yazd province confectioneries. One hundred and seventy samples received by Food, Cosmetics and Hygiene Supervision Department of the Food and Drug Administration of Yazd Shahid Sadoughi University of Medical Sciences were used in this study. The methods used in microbiological experiments were in accordance with the Institute of Standards and Industrial Research of Iran standards of 10899-3, 1810, 6806-3, 2964, 2461-1, 2461-2 (Khoramrooz *et al.*, 2015, Soltan Dalal *et al.*, 2010). Microbial tests including *Enterobacteriaceae* count, *Escherichia coli* count, *Staphylococcus aureus* count, *Salmonella* count, mold and yeast count were evaluated.

Samples included various Yazd traditional confectionery products which were investigated

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[DOI: 10.18502/jnfs.v9i2.15414]

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as soon as possible after entering the laboratory. Five grams of samples were mixed with 45 ml of ringer solution, and they were cultured using specific culture media (for each type of microorganism). They were incubated at different temperatures (each sample was incubated for a certain period according to the type of bacteria tested). The samples were taken out afterwards, and their contamination was determined by national standard values (Institute of Standards, 2020). Samples with hihger than standard contamination were declared unusable, and samples without contamination or the ones with less than standard contamination were accepted (**Table 1**).

Enterobacteriaceae were counted according to the Iranian national standard numbers, 2461-2 and 2461-1. *Escherichia coli* was examined in accordance with the standard number 2946. *Staphylococcus aureus* coagulase positive test was performed according to the Iranian National Standard Number 6806-3. *Salmonella* was also examined according to the standard number 1810. Mold and yeast tests were performed according to the number of 10899-3.

Data analysis: Data were analyzed using SPSS 22 software and Chi-square and Fisher tests.

Table 1. Microbial tests and standard allowable limits for dry and semi-dry sweets.

Type of pollution	Permissible limit to pollution	
	Dry	Semi-dry
Enterobacteriaceae	Negative	10^2
Escherichia coli	Negative	Negative
Staphylococcus	Negative	Negative
Salmonella	Negative	Negative
Yeast	10^2	10^2
Mold	Negative	10^2

Results

Analysis of the data showed that 8.83% of the total samples were infected with *Escherichia coli*, 17.06% with *Enterobacteriaceae*, 20.59% with mold, and 3.53% with yeast. Moreover, no infection was detected with *Staphylococcus aureus* and *Salmonella*. (**Figure 1, Table 2**)

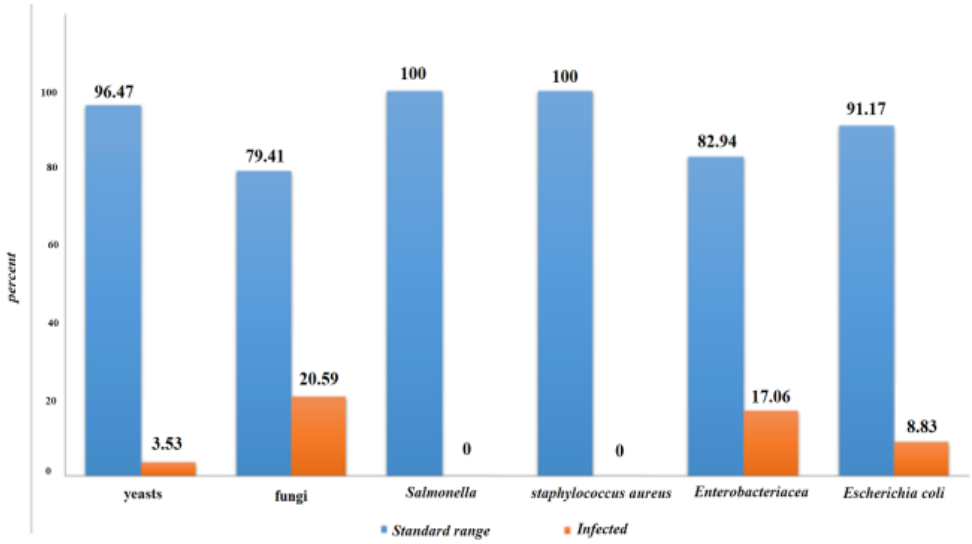


Figure 1. Distribution of different types of contamination in samples of traditional sweets in Yazd.

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Table 2. Distribution of microbial contamination in Yazd traditional sweets (n = 100).

Test name	Result
E.coli confirmation test	The growth rate of bacteria in 91.17% of the samples were within the standard range and 8.83% were higher than the standard range.
Enterobacteriaceae confirmation test	The growth rate of bacteria in 82.94% of the samples were within the standard range and 17.06% were higher than the standard range.
Staphylococcus aureus confirmation test	The growth rate of bacteria in 100% of the samples was within the standard range
Salmonella confirmation test	The growth rate of bacteria in 100% of the samples was within the standard range
Mold confirmation test	The growth rate of bacteria in 79.41% of the samples were within the standard range and 20.59% were higher than the standard range.
Yeast confirmation test	The growth rate of bacteria in 96.47% of the samples were within the standard range and 3.53% were higher than the standard range.

The collected samples were from different types of traditional sweets in Yazd province, and the highest level of microbial contamination was related to pistachio nuts, and the lowest level of contamination was attributed to traditional HajiBadam sweets. Examples of detected fungi and yeasts were presented in **Figure 2**.

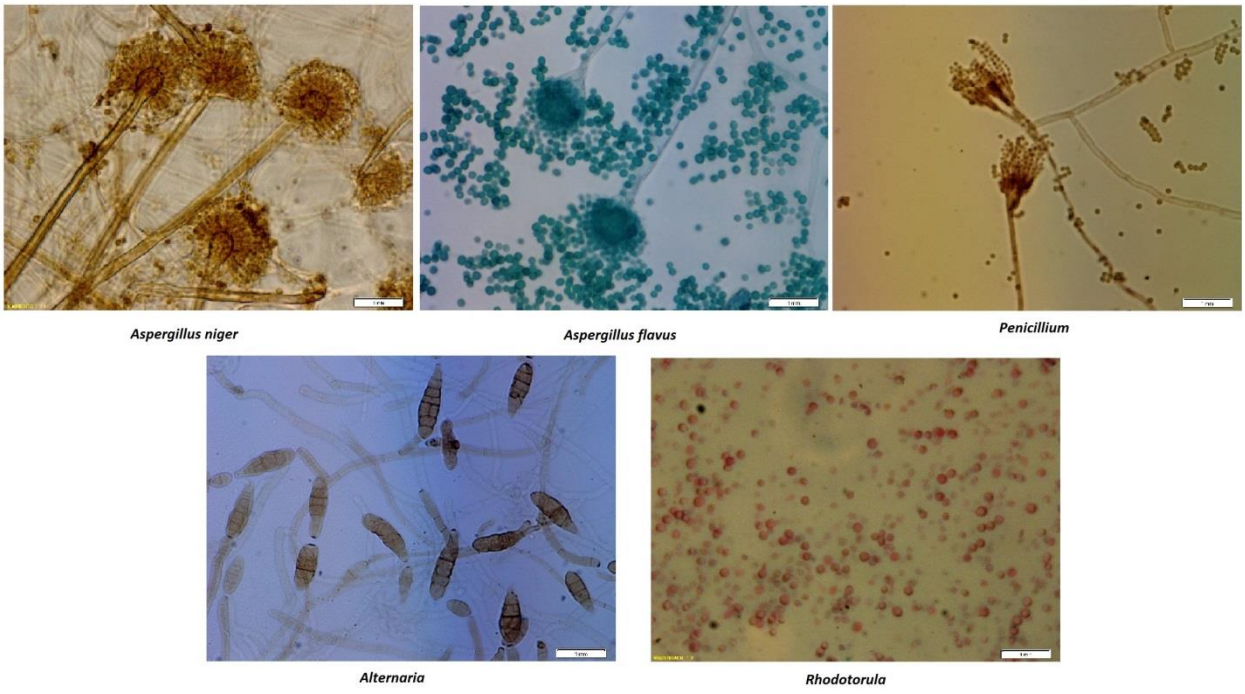


Figure 2. Examples of detected fungi and yeasts.

Discussion

There are numerous reports of diseases and food poisoning due to microbial contamination of food in different parts of the world (Little and De Louvois, 1999) in developed countries. The incidence of these diseases and the number of cases or deaths are continuously recorded by health care centers. There is high frequency of food microbial poisoning In Iran (Jay, 1992). Many cases of gastroenteritis due to the consumption of contaminated food have been referred to hospitals and emergency centers every day. Most of them were due to the lack of performance of hygienic principles in preparing food or storing them (Karim *et al.*, 1995). Fecal

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bacteria including coliforms and enterococci species are also one of the most important infections of food. These bacteria are important in food industry, and in recent years, they have been considered as the best indicator of fecal contamination (Wouafo *et al.*, 1996). The results of the present study showed that 13.2% of the samples were infected with *Enterobacteriaceae*, which can be attributed to the people working in confectionery workshops. Contamination of traditional sweets with *Enterobacteriaceae* family can be due to the preparation of food in contaminated containers and the lack of personal hygiene by confectionery staff, which results in the spread of fecal bacteria during the production and distribution of sweets.

Since these bacteria are present in gastrointestinal tract, urinary tract, and water and soil, compliance with personal and environmental hygiene standards and the use of safe water can play an effective role in reducing this kind of pollution (Malekzadeh, 1995). Considering the importance of *Escherichia coli* as one of the factors involved in food poisoning, various studies were conducted in Iran regarding the contamination of sweets including sweet cream with coliform and *Escherichia coli* bacteria. for example, in a study conducted by Khezri *et al.* in Mashhad, the rate of contamination of sweets containing sweet cream with coliform and *Escherichia coli* was 69% and 26%, respectively (Khezri *et al.*, 2007) . In Fars province, a study by Khalili Tehrani *et al*. showed that the rate of contamination of sweet creams used in confectioneries with *Escherichia coli* was high and 69% of creams were infected with this bacterium (Khalili *et al.*, 2014). In the present study, only 5% of traditional pastry samples were infected with *Escherichia coli*. Because *Escherichia coli* was an indicator of fecal contamination, this opportunistic bacterium caused diarrhea in children and adults by producing enterotoxins. Epidemics of this bacterium were attributed to the consumption of contaminated water and food. This level of pollution indicated lack of personal hygiene and that the personnel did not use gloves in production, distribution, and transportation centers (Malekzadeh, 2003).

The results of Nikniaz's study in Tabriz showed that 48.8% of creamy pastry samples were infected with *Escherichia coli*, 38.8% with coliforms, 31.2% with *Staphylococcus aureus*, and 70% were infected with yeasts (Nikniaz *et al.*, 2011). In a study by Faramarzi *et al*. in the west of Tehran, it was found that out of 642 food samples, 4.81% of the sweets were infected with *Staphylococcus aureus*, and 4.81% with *Bacillus cereus* (Faramarzi *et al.*, 2012). Hosseini's study showed that out of 216 samples of sweets containing sweet cream collected from Tehran, which were examined microbiologically, 83% were reported to be unusable and were an important cause of infection with *Enterobacteriaceae* (Hosseini, 1999). In Khezri's study, the rate of contamination of sweets containing sweet cream with coliform and *Escherichia coli* was 69% and 26% (Khezri *et al.*, 2007).

In the present study, the rate of contamination of traditional sweets with mold and yeast was 20.59% and 3.53%. In the studies conducted in Mashhad, the contamination of sweets containing sweet cream with mold was 9%, and the contamination of sweets containing sweet cream with mold and yeast in Zahedan was 5.9% (Khezri *et al.*, 2007, Shadan *et al.*, 2003). The high level of contamination in the present study might be due to poor maintenance conditions. Contamination of traditional sweets with mold, in addition to causing health effects and reduced quality of product, is economically significant for confectionery industry (Smith *et al.*, 2004). Because fungal spores are scattered in the air, they can contaminate sweets. In addition to air pollution, contamination of confectionery containers, distributors, and fungal contamination of confectionery raw materials such as sugar, flour, and especially nuts such as pistachio and almond nuts can also lead to mold contamination of traditional sweets (Caprioli *et al.*, 1994). By observing hygiene, storing, and distributing confectionery products in a suitable and aseptic environment and using preservatives such as sorbates and benzoates, yeast contamination of this product can be reduced to some extent (Azhdari and Hashemzehi, 2020).

Lack of hygiene in confectionery and

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transmission of various microorganisms through contaminated food increases the acquired immunity of individuals against many of these microorganisms; but children, the elderly people with weakened immune systems and some other vulnerable people in the community are always more susceptible to diseases caused by these microorganisms. In addition to children and vulnerable groups, tourists are also more susceptible to infections and food poisoning due to the lack of previous exposure to some microbial strains in food and the lack of acquired resistance to these strains (Caprioli *et al.*, 1994).

A high percentage of traditional sweets distributed in this study did not meet the standards; therefor, following the standards and performing hygienic procedures are very important. In this regard, in order to control primary pollution, improving the level of knowledge of manufacturers in the field of hygiene in all stages of production and distribution to reduce the occurrence of secondary pollution is one of the ways to increase the hygienic level of these products. Observance of these principles in traditional sweets of Yazd is of special importance and should be evaluated in different stages of production and distribution of these products.

Conclusions

The findings of the present study suggest the high prevalence of microbial and fungal contamination of traditional sweets in Yazd, which leads to an increase in the risk of poisoning and diseases transmitted by eating these sweets. Therefore, training experts for correct control of health issues and supervision in the stages of preparation, production, transportation, storage, and supply in order to prevent the transmission of microbial contamination is of great importance.

Acknowledgements

The authors would like to thank Dr Kaveh Kavousi, who read the original manuscript and offered invaluable comments, and Dr Somayeh Moosavi, the manager of food laboratory in Food And Drug Faculty of Shahid Sadughi University of Yazd for their support.

Conflict of interests

The authors declared no conflict of interests.

Authors' contributions

Kimiaee M and Madani M designed and conducted the study; Madani M analyzed data; Kimiaee M wrote the manuscript; Seyed Moshtaghion SM, Mosaddegh MH and Madani M revised the manuscript; and Madani M supervised the study. All the authors read and approved the fnal manuscript

Funding

We have no funding in this study.

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