

Evaluation of Microbial Contamination of *Sohan* Produced in Qom, Iran, with Reference to National Standards

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Background & Aims of the Study: *Sohan* is one of the confectionery products produced in Qom, Iran. Microbial contamination of confectionary items is crucial in terms of hygienic and economic issues. This sort of spoilage shortens the storage time and causes an outbreak of food poisoning. Due to the high utilization of these products, it is vital to implement microbiological management to improve shelf life and maintain quality. The present study aimed to evaluate the levels of microbiological contamination in *Sohan*.

Materials and Methods: In this study, the *Sohan* products of Qom were classified according to the Code of Health, standard logo, as well as ISO 9001:2008 and 22000:2005 certifications. Then, 1 to 2 boxes (out of 7 boxes) were purchased from an official representative shop in Qom. The diagnostic and enumeration tests for Enterobacteriaceae, *Escherichia coli* (*E. coli*), *coagulase-positive staphylococcus*, as well as molds and yeasts, were performed in accordance with the national standards of 2461-1, 2946, 6806-3, and 10899-2, respectively.

Results: Results of this study showed that 71.4% of the samples contained Enterobacteriaceae, and 14.2% of the samples contained *coagulase-positive staphylococci* higher than the determined standard levels. In addition, no case of contamination with molds, yeasts, and *E. coli* was observed among the samples.

Conclusion: Findings of the present investigation indicated the necessity for the precise implementation of Good Hygiene Practices in the factories manufacturing this product.

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Background

Confectionery products comprise the main part of a balanced diet (1), which is industrially and corporately produced and supplied. Confectionery products, as defined by the Standard Institute of Iran, refer to the products

that mainly contain sugar and oil (2). *Sohan* is one of the confectionery products produced in Qom, Iran. Main ingredients of this product are flour, wheat germ, sugar, oil, eggs, saffron, and cardamom. This item is produced in different forms and decorated by pistachio and almond (3). Daily production of *Sohan* in Qom is about 25 tons, 20 tons of which are exported outside

the province, and the rest are sold in this province. This product is also exported to countries in Europe and Central Asia (4).

Most of the product is produced in small factories and the rest in large industrial centers. It is necessary for manufacturers to receive the hygiene code. In any case, only a couple of manufacturers managed to obtain this code. The standards defined for *Sohan* are national and of voluntary type, which some of the manufacturers have managed to obtain. In addition, a few manufacturers have managed to achieve hazard Analysis and Critical Control Point (HACCP) and ISO-9001 and 22000 certificates. *Sohan* is produced throughout a four-stage process of which the machines conduct the two first stages and two final stages are performed through hand involvement that increases the possibility of contamination with pathogens transmitted by humans, such as *Staphylococcus aureus* and different kinds of Enterobacteriaceae (2).

Decay of confectionary items includes physical, chemical, and biological spoilage. Microbial contamination of confectionary products is vital in terms of hygienic and economics issues. This kind of spoilage shortens the storage time and causes an outbreak of food poisoning (1). Due to the high consumption of these products, it is important to implement microbiological control in order to enhance shelf life and maintain quality (2). The contamination could have diverse sources, such as workers' hands, ingredients or

equipment, and different materials utilized in the production process. This product has not been investigated from a microbial perspective in Iran up to now; therefore, this study aimed to evaluate the microbial contamination of *Sohan* produced in different workshops and factories.

Materials & Methods

Chemicals and mediums

The chemicals and mediums employed in the tests were used according to the Iran national standards (no. 2461-1, 2946, 6806-3, and 10899-2) (5-8).

Sample collection

Sohan sampling was carried out in accordance with the standard sampling of agricultural products orally consumed (no. 2836). The *Sohan* products based on the possession of a hygiene code, standard logo, HACCP, ISO 9001:2008 and 22000:2005 certificates, as well as production and expiration date, were classified into general categories. Since it was not possible to mention the brands of the manufacturers, the samples were determined with a researcher-defined code from A to G (Table 1). Then, from each category, 1 to 2 boxes (out of 7 boxes) were purchased from different areas of Qom in November 2014 and transferred to the laboratory without opening the packages. The tests were performed in accordance with the

Table 1) Specifications of *Sohan* samples produced in Qom

Sample codes*	A	B	C, D	E	F	G
Number of boxes	1	1	2	1	1	1
Health Code	✓	×	✓	✓	✓	✓
ISO 9001:2008	✓	✓	×	✓	×	×
ISO 22000:2005	✓	✓	×	×	×	×
Hazard Analysis and Critical Control Point	✓	✓	×	×	×	×
Standard logo	✓	✓	×	×	×	×
Production date	×	✓	×	✓	✓	×
Expiration date	✓	✓	×	✓	×	×

* Since it was not possible to mention the brands of the manufacturers, the samples were determined with a researcher-defined code from A to G.

national standards.

Methods

For each sample, 4.0 g of *Sohan* (without pistachio) from each box was precisely weighed and added to 36 ml of buffered peptone water to obtain 0.1 dilution. Then, the dilutions of 10^{-2} and 10^{-3} were prepared using an initial dilution. All experiments were performed in triplicate for each sample, and the obtained results were recorded by repeating the entire experiment (3).

For Enterobacteriaceae isolation, the most probable number (MPN) technique and colony count were used, and the utilized media involved Enterobacteriaceae enrichment broth (Quelab Cat no. 651702), Violet Red Bile Glucose Agar (Quelab Cat no. 651702), and Nutrient Agar (Merck Cat no. 105450) incubated at 37°C for 24 h. For further confirmation, the oxidase and glucose fermentation tests using Glucose Agar Medium (Quelab Cat no. 651135) were performed (Iran national standard no. 2461-1).

For the isolation of *Escherichia coli* (*E. coli*), MPN technique, Lauryl Sulfate Broth (Quelab Cat no. 652406), *E. coli* Broth (EC Broth, Merck Cat no. 110285), peptone water, and indol free (Quelab Cat no. 652106) were applied as the mediums, and the indole reagent was performed for confirmation (Iran national standard no. 2946). The MPN technique was used for the identification of *coagulase-positive staphylococci*. The utilized media were Giolitti and Cantoni broth (Quelab Cat no. 836930), Baird Parker Agar (Merck Cat no. 105406), and Brain Heart Broth (Quelab Cat no. 180239).

The Rabbit plasma was performed (Merck Cat no. 113306) as a confirmatory test (Iran national standard no. 6806-3).

The spread plate technique was performed for the isolation and enumeration of molds and yeasts. The used media included DG18 Agar (i.e., Dichloran 18% mass fraction glycerol agar) (Liofilchem Cat no. 620238) incubated at 25°C for 7-5 days (Iran national standard no.10899-2) (9). After determining the contamination of the product, the obtained values were compared with the standard limits (Table 2).

Table 2) Standard limits for microbial contamination of *Sohan* (no. 2395) (10)

Bacteria	Limit contamination
Enterobacteriaceae	$10^2/g$
<i>Escherichia coli</i>	Negative per gram
<i>Coagulase-Positive staphylococcus</i>	Negative per gram
Molds	$10^2/g$
Yeasts	$10^2/g$

Results

Table 3 shows the microbial contamination of various *Sohan* in this study. The results revealed that the Enterobacteriaceae contamination of five samples (71.4%) exceeded the contamination standard limited level of *coagulase-positive staphylococcus*. Furthermore, *E.coli*, mold, and yeast contamination were not detected among the samples.

According to Table 3, the most astounding Enterobacteriaceae contamination was observed in sample C (with no specification or details on the packages), which was illegally

Table 3) The microbial contamination rates of various *Sohan*

Sample	Enterobacteriaceae	<i>Coagulase-Positive staphylococcus</i>	<i>Escherichia coli</i>	Molds and yeasts
A	46×10^2 MPN/g	Not observed	Not observed	Not observed
B	74×10^{-1} MPN/g	Not observed	Not observed	Not observed
C	11×10^3 MPN/g	Not observed	Not observed	Not observed
D	15×10^1 MPN/g	Not observed	Not observed	Not observed
E	36×10^{-1} MPN/g	Not observed	Not observed	Not observed
F	11×10^2 MPN/g	36×10^{-1} MPN/g	Not observed	Not observed
G	24×10^2 MPN/g	Not observed	Not observed	Not observed

MPN: Most probable number

produced. Furthermore, the lowest level of Enterobacteriaceae contamination was observed in sample E (with health code and ISO-9001 certificate). The most abnormal amount of *staphylococci* was observed in sample F. The highest overall contamination rate was detected in sample F (with health code, as well as no other information and details).

Discussion

The present study aimed to investigate the contamination of *Sohan* in seven factories in Qom. Obtained findings of this study showed high levels of Enterobacteriaceae (i.e., enterobacterial contamination) in *Sohan* (71.4%). Source of this contamination could be detected in the individuals working in the manufacturing plants and instruments used in the process of production. The workers could contaminate the products by touching the ingredients or final products. In addition, lack of hygiene in washing the instruments could be another source of contamination. The second factor was the contamination with *coagulase-positive staphylococcus* (14.2%). This bacterium is the main cause of food poisoning in humans and might be transmitted by hands, injuries, acnes, and abscesses on the hands or faces of the workers.

Results of the tests showed no contamination of *E. coli* in the studied samples. This bacterium is categorized under fecal coliforms and is considered one of the factors leading to food poisoning in human beings. Moreover, none of the samples was identified with mold contamination. This contamination could be caused by the air, instruments, and fungal contamination of *Sohan* ingredients, particularly sugar, flour, and pistachio. Lack of yeast contamination in the samples could be attributed to low levels of water activity (a_w) since this kind of contamination is mainly observed in the products that contain high

levels of active water.

Several microbial studies have been conducted on different types of sweets in Iran, as well as other countries. In one of the studies carried out on cream-filled pastries in Tabriz, Iran, (11), the findings indicated 48.8%, 31.2%, 27.5%, and 70% contamination with *E. coli*, *staphylococcus aureus*, molds, and yeasts, respectively (11). Results of another study conducted by Hosseini et al. in 2008 and 2009 showed that in the total 216 samples of creamy sweets in Tehran, Iran, 83% of the samples were inedible, and Enterobacteriaceae was the most important and frequently observed contamination factor (12).

In a study performed by Khezri et al. on the contamination of creamy sweets in 2007, the contamination rates were reported as 69%, 10.5%, 26%, and 9% with Enterobacteriaceae, *staphylococcus aureus*, *E. coli*, and molds, respectively (13). In a study carried out by Shadan et al. (2004) on creamy sweets in Zahedan, Iran, it was shown that 53.83%, 60.5%, and 5.9% of the samples contained coliforms and *E. coli*, *staphylococcus aureus*, as well as molds and yeasts, respectively (14).

In an investigation conducted by Pishkar et al. (2003) on creamy and dry sweets in Shahrekord, Iran, the obtained estimates showed that 26% of the creamy and 16% of dry sweets contained coliforms higher than the standard levels for consumption. In addition, 11% and 10% of the creamy sweets, as well as 8% and 5% of the dry sweets, contained *E. coli* and *staphylococcus aureus* higher than the standard levels, respectively (15).

Examination of Soltan Dalal et al. (2008) on 121 samples of creamy sweets sold in the confectionaries in southern part of Tehran showed that 4%, 33%, 12%, 5%, and 2% of the samples were contaminated with Enterobacteriaceae, yeasts, *staphylococcus aureus*, molds, and *E. coli* (16). The microbiological studies carried out by Kačaniová et al. (2011) and Uhaniaková et al. (2013)

reported that all of the confectionery products in Slovakia were produced according to the food codex of this country (17, 18).

In a study performed by Costanzo Anunciaçao et al. in 1995, it was demonstrated that more than 50% of the sweets preserved in the room temperature were contaminated with *staphylococcus aureus* (19). Kamat et al. (1998) also reported that 87% of creamy sweets produced in India contain foodborne bacteria (20). Todd et al. (1996) announced that 35-47% of foodborne diseases in Poland, Portugal, Bulgaria, and Sweden resulted from the consumption of contaminated confectionery products (21).

Most of the studies in this field examined sweet products; nevertheless, the present study focused on one type of confectionery products (i.e., *Sohan*) that might explain the differences observed in the findings of this study and those of other investigations. Sweets products are exposed to microbial contaminations, particularly molds, more than confectionery products, due to containing water and process of production (2).

Controlling the ingredients, process, and environment (i.e., personnel, equipment, and air) are considered the critical factors in the elimination of microbial contamination. Constant supervision, Good Manufacturing Practice (GMP), and Good Hygiene Practices (GHP) are among the most convenient methods for eliminating this contamination (17). As the findings of the present study implied, there was no relationship between the contamination levels of the samples and their specifications; however, the brands with certification, except for one case, showed acceptable conditions.

Sohan is heated during the process of production; therefore, it is unlikely that the source of contamination is the ingredients or process. Nevertheless, the two final stages of *Sohan* production are conducted without heating and through hand involvement;

therefore, the contamination might be explained by the lack of GHP. In this way, considering HACCP, as well as following standards in *Sohan* production line, can significantly reduce the product contamination.

Conclusion

The highest level of general contamination in the present study was observed in the brand with the hygiene code that indicates the requirement of higher food hygiene control. There is no expiration date for this product; therefore, it is suggested that the standard institution should directly be involved in the determination of an expiration date for the product.

Footnotes

Conflict of Interest

The authors declare that there is no conflict of interest.

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