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Microbiological and Chemical Quality of Sohan: An Iranian Traditional Confectionary Product

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Abstract

Background: Sohan is a traditional Iranian confectionery product prepared mainly by mixing and cooking of wheat flour, sugar, malt, oil, natural flavoring additives and coloring materials. Regarding to high consumption and popularity of Sohan as well as importance of its safety and hygienic status, we investigated microbial and chemical profiles of Sohan in retailers throughout Qom province of Iran during 2013.

Methods: Hundred Sohan samples were collected from Qom retail markets over a 6-month period in 2013. American Public Health Association (APHA) and International Union of Pure and Applied Chemistry (IUPAC) methods were used to determine Sohan's microbial and chemical profiles, respectively. Statistical analysis was carried out by analysis of variance (ANOVA) using SPSS statistical software version 16.0.

Results: The obtained mean count of Enterobacteriaceae, *Staphylococcus aureus*, molds and yeasts were $2.14\times10^2\pm623.6$, $5.38\times10^2\pm593.5$, $1.56\times10^2\pm221.5$ and $2.51\times10^2\pm164.9$ CFU/g, respectively. Also, *Escherichia coli* was found in 10% of Sohan samples. The mean of peroxide and acidity values was 2.59±1.2 mEq/Kg and 0.33±0.2%, respectively. The present research represented that microbial and fungal contamination was significantly higher ($p<0.01$) during the warm months (July, August and September).

Conclusion: According to this study, all Sohan samples had peroxide values and acidity in normal range, but the large number of food borne pathogens was detected in Sohan samples especially during warm months, represented potential health hazard to consumers.

Introduction

Sohan is a traditional Iranian confectionary product produced mainly in Qom province since ancient times in which it was known as Qom Halva. Similar confection was produced with different names such as Helva, Halwa, Halvah, Halawa, Halua, Halvaa, Chalwa, etc. in other parts of the world especially in the Eastern Mediterranean countries and the Middle East (Davidson, 1999). The major components of Sohan are wheat flour, sugar, malt, oil (vegetable oil, butter, and ghee) and water. Its additive derivatives may be pistachios, saffron, almonds, cardamom and cinnamon.

For Sohan preparation, at first, sugar is mixed with boiling water and saffron is solved. Then wheat flour, malt and oil are added and exposed to low temperature. When the dough spreads over a greasy tray, it is pressed with a metallic hammer to flatten and scatter nuts such as almonds and pistachios (Fig. 1). After cooling, Sohan is put in the sealed containers. Sohan is produced in various flatted and morsel types, such as honeyed, sesamed, almonded, pistachioed and

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Gaz sweet. This product is very delicious and calorie-rich compared with other traditional Iranian confections. Sohan is strongly popular souvenir which can be exported to other countries.

Many illness outbreaks are attributed to food borne pathogens such as *Salmonella*, *Staphylococcus aureus*, Enterobacteriaceae, mold and yeast contamination. These food borne infection and intoxication are caused by viable microorganisms or toxins via improper and unhygienic environment, raw materials, handling and abuse time-temperature during storage and launching them to the markets (Ayaz et al., 1986; Boughattas and Salehi, 2014; D'aoust, 1994; Kotzekidou, 1998; Sengun et al., 2005).

Regarding high consumption and popularity of Sohan and its safety and hygiene importance, an attempt has been devoted to determine the microbial and chemical profiles of Sohan in Qom retailers in 2013.

Materials and methods

Sample collection

A total of 100 samples within their original packages were randomly collected from Qom retail markets in Iran with 38 different brands over six months (July to December, 2013).

Sample preparation

To determine the microbiological quality of Sohan, 10 g of each sample was taken under the aseptic conditions and transferred into 90 ml peptone water (0.1%) by Bag-Mixer blender stomacher (400cc, Interscience, France). Ten-fold serial dilutions of samples were prepared, and double culturing was done for enumeration and identification of microorganisms by surface plate method.

Microbiological analysis

The American Public Health Association (APHA) method was used to isolate and enumerate *Staphylococcus aureus*, including enrichment of 1 g sample in 10 ml Cooked Meat Broth (Difco, Germany, 226730), cultured duplicate in Baird Parker Agar (Merck, Germany, 105406) containing sterile Egg Yolk Tellurite Emulsion (Merck, Germany, 103785) as selective media and incubated at 37 °C for 24 h. Yeast Extract Agar and Sabouraud Dextrose Agar with chloramphenicol (Merck, Germany, 118425) media were used for enumeration of mold and yeast at 25 °C for 3-5 days by surface plate method. The total Enterobacteriaceae was determined by double layer pour plate method on Crystal-Violet Neutral-Red Bile Glucose Agar culture (Merck, Germany, 110275) which was incubated at 37 °C for 24 h. Eosin Methylene-Blue Lactose Sucrose Agar (Merck, Germany, 101347) culture was used to determine *E. coli*, at 37°C for 48 h, then 4-5 green metallic shine colony were taken for further biochemical tests such as nitrate reduction, simmon's citrate, indol, methyl red, voges–proskauer test (Eaton and Franson, 2005).

Chemical analysis

IUPAC (International Union of Pure and Applied Chemistry) method was used to measure acidity and peroxide values. To measure acidity value in Sohan, 25 ml diethyl ether (Merck, Germany, 100926) was mixed with 25 ml ethanol. Then, it was titrated with 0.1 M sodium hydroxide (Merck, Germay, 106467) in the presence of 1 ml of 1% (v/v) phenolphthalein in ethanol solution (pH 8.2-9.8, Merck, Germany), final stage of titration persist a pink color. Sodium hydroxide volume consumed was proportional to acidity. In order to measure peroxide value, 1-4 g Sohan samples were dissolved in 10 ml chloroform (Merck, Germany 102395), then 15 ml of 100% (v/v) glacial acetic acid (Merck, Germany, 100066) and 1 ml of freshly prepared saturated aqueous potassium iodide solution (Merck, Germany, 105044) were added to the solution and put in a dark place for 5 min. Then, 75 ml water was added. The mixture was titrated with 0.01 M sodium thiosulfate solution (Merck, Germany, 106512) using 1% (w/v) soluble starch solution (Merck, Germany, 101253) as an indicator (IUPAC, 1997). Final stage of titration was dark blue color. Sodium thiosulfate volume consumed was proportional to peroxide content.

Statistical analysis

Using SPSS software (version 16.0), statistical analysis was carried out by analysis of variance (ANOVA) and Tukey test. $P<0.01$ were considered as significant. All experiments were carried out in duplicate.

Results

Microbial and fungal analysis of Sohan samples have been presented in Table 1 and Table 2, respectively. This result indicated that the number of Enterobacteriaceae, *S. aureus*, *E. coli*, mold and yeast were higher than national standard limit in 51%, 19%, 10%, 55% and 40% of samples, respectively. It should be noted that the national accepted limit for mentioned items are 10^2, 10^3, 0, 10^2 and 10^2 CFU/g, respectively (ISIRI, 2007).

The bacterial and fungal contamination level were significantly ($p<0.01$) higher during the warm months (July, August, and September). However, chemical analysis of the samples had no statistical difference among various months.

The results of chemical analysis of Sohan samples are shown in Table 3. All Sohan samples had peroxide values and acidity in acceptable levels (≤5 mEq/Kg and ≤1% respectively) described by IUPAC (1997).

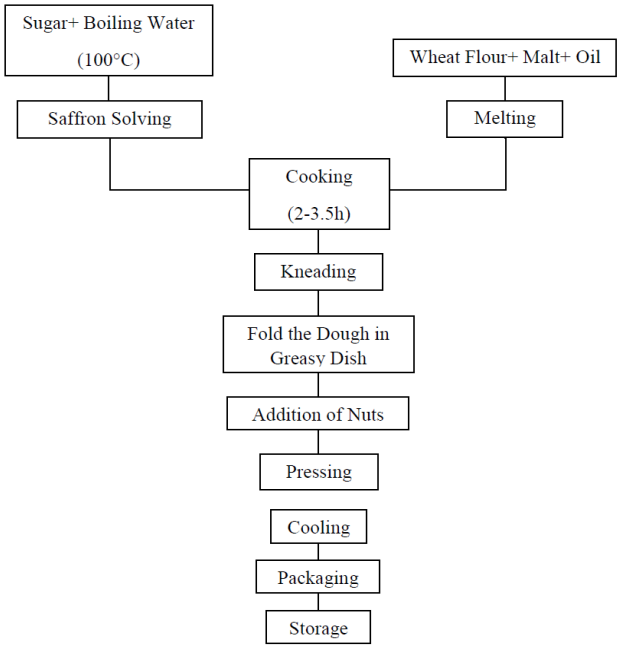

Fig. 1: A flow sheet of Iranian traditional Sohan manufacturing

Table 1: Bacterial analysis of Sohan samples obtained from Qom retail markets of Iran during July to December 2013

Date of sampling	Sample (n)	*S. aureus* (CFU/g)		Enterobacteriaceae (CFU/g)	
		Min-Max	Mean ±SE	Min-Max	Mean ±SE
July	17	$0\text{-}6.00\times10^{3}$	$21.12\times10^{2}\pm84.6$	$0\text{-}1.40\times10^{4}$	$5.01\times10^{3}\pm1321.5$
August	25	$0\text{-}1.00\times10^{4}$	$24.35\times10^{2}\pm824.2$	$0\text{-}1.60\times10^{3}$	$6.44\times10^{2}\pm215.2$
September	20	$0\text{-}1.00\times10^{4}$	$31.03\times10^{3}\pm1223.7$	$0\text{-}5.00\times10^{2}$	$2.28\times10^{2}\pm98.1$
October	16	$0\text{-}2.00\times10^{3}$	$0.78\times10^{2}\pm125.5$	$0\text{-}5.00\times10^{2}$	$2.71\times10^{2}\pm96.8$
November	9	$0\text{-}1.00\times10^{2}$	$3.42\times10 \pm14.3$	$0\text{-}2.00\times10^{3}$	$8.44\times10^{2}\pm345.5$
December	13	$0\text{-}1.00\times10^{2}$	$2.48\times10 \pm11.4$	$0\text{-}4.00\times10^{2}$	$15.38\times10\pm65.2$
Total	100	$0\text{–}1\times10^{4}$	$5.38\times10^{2}\pm593.5$	$0\text{-}1.4\times10^{4}$	$2.14\times10^{2}\pm623.6$

Table 2: Fungal analysis of Sohan samples obtained from Qom retail markets of Iran during July to December 2013

Date of sampling	Sample (n)	Mold (CFU/g)		Yeast (CFU/g)	
		Min- Max	Mean ± SE	Min- Max	Mean ± SE
July	17	$0\text{-}2.00\times10^{3}$	$9.95\times10^{2}\pm457.4$	$0\text{-}1.20\times10^{3}$	$5.61\times10^{2}\pm214$
August	25	$0\text{-}5.00\times10^{2}$	$3.36\times10^{2}\pm114$	$0\text{-}1.30\times10^{3}$	$6.40\times10^{2}\pm249.1$
September	20	$0\text{-}1.30\times10^{3}$	$6.10\times10^{2}\pm219.2$	$0\text{-}6.00\times10^{2}$	$3.45\times10^{2}\pm129.8$
October	16	$0\text{-}5.00\times10^{2}$	$2.33\times10^{2}\pm78.1$	$0\text{-}7.00\times10^{2}$	$3.31\times10^{2}\pm145.6$
November	9	$0\text{-}8.00\times10^{2}$	$4.22\times10^{2}\pm103.8$	$0\text{-}5.00\times10^{2}$	$26.55\times10\pm87.5$
December	13	$0\text{-}4.00\times10^{2}$	$19.23\times10\pm89.6$	$0\text{-}5.00\times10^{2}$	$24.69\times10\pm125.9$
Total	100	$0\text{-}2\times10^{3}$	$1.56\times10^{2}\pm221.5$	$0\text{-}1.3\times10^{3}$	$2.51\times10^{2}\pm164.9$

Table 3: Chemical analysis of Sohan samples obtained from Qom retail markets of Iran during July to December 2013

Date of sampling	Sample (n)	Peroxide (mEq/Kg)		Acidity (%)	
		Min- Max	Mean ±SE	Min- Max	Mean ± SE
July	17	0.70-4.50	2.29 ±0.3	$0\text{-}1.20\times10^{3}$	$5.61\times10^{2}\pm214$
August	25	0.95-4.70	2.86 ±0.2	$0\text{-}1.30\times10^{3}$	$6.40\times10^{2}\pm249.1$
September	20	0.98-4.82	2.93 ±0.3	$0\text{-}6.00\times10^{2}$	$3.45\times10^{2}\pm129.8$
October	16	0.83-4.60	2.75 ±0.3	$0\text{-}7.00\times10^{2}$	$3.31\times10^{2}\pm145.6$
November	9	0.57-4.10	2.12 ±0.4	$0\text{-}5.00\times10^{2}$	$26.55\times10\pm87.5$
December	13	0.57-4.10	2.04 ±0.3	$0\text{-}5.00\times10^{2}$	$24.69\times10\pm125.9$
Total	100	0.57-4.82	2.59 ±1.2	$0\text{-}1.3\times10^{3}$	$2.51\times10^{2}\pm164.9$

Discussion

Sohan may be contaminated due to mishandling during preparation, packaging in factories and subsequently abuse time-temperature storage at supermarkets prior to consumption. *S. aureus* is the causative agent of the numerous food borne diseases outbreaks that may be related to a number of virulence factors such as the heat stable enterotoxins (Sandel and Mckillip, 2004). In this study, *S. aureus* range and mean±SE were found between <10 to 1×10^4 and $5.38\times10^2\pm593.5$ CFU/g, respectively. Previous studies indicated that the traditional confectionary products like Tahini Halva had lower *S. aureus* than Sohan (Kutzekidue, 1998; Sengun et al., 2005; Kahraman and Issa, 2010). In confectionary products, *S aureus* isolation in amounts lower than 10^3 CFU/g doesn't seem hazardous for consumer's health (Smoot and Clifford, 2001). In our investigation, 11 out of 100 (11%) Sohan samples had more than 10^3 CFU/g *S. aureus*. So, they can be classified as potential health hazard. Since, it has been proved that Sohan heat processing successfully diminishes *S. aureus*; it seems that post-processing contamination due to the presence of *S. aureus* on workers' skin, mouth and nose may cause Sohan contamination.

Enterobacteriaceae family is among the first proliferated bacteria in intestine and shed in feces of a wide range of animals and human since most strains have similar growth requirements and survival characteristics (Baylis et al., 2004). In this survey, the range and mean±SE of Enterobacteriaceae were <0.10-1.4×10^4 CFU/g and $2.14\times10^2\pm623.6$ CFU/g, respectively. According to Sengun et al. (2005), the amount of *Entrobacteriaceae* in 63 Tahini Halva samples was reported lower than 10 to 8.5×10^2 CFU/g. *E. coli* belonging to Enterobacteriaceae family is an important fecal indicator and usually isn't found <1 CFU/g by direct plating on EMB agar in confections (Smoot and Clifford, 2001). It can contaminate Sohan via personnel, containers, nuts and water that are consumed in factory. Oil content generally afford noticeable microbial survival, so *E. coli* survives in oil rich foods even for several months after manufacturing and can be recognized as a potential public health hazard.

Fungi are widely distributed in environment either in air, water, soil and dust. These are potential threats to the consumer's health via mycotoxin production and allergic reactions (Jarvis et al., 1983). In the current study, the range and Mean±SE of molds and yeasts were <10 to 2×10^3, ($1.56\times10^2\pm221.47$) CFU/g and <10 to 1.3×10^3, ($2.51\times10^2\pm164.93$) CFU/g, respectively, which were higher than another survey results found in 63 Halva samples in Izmir city, Turkey (Sengun et al., 2005). Kotzekidou (1998) reported that yeast count in Halva samples in Greece was 4.9×10^3 CFU/g which was higher than amounts revealed in our work. However, molds were not detected in any Halva samples in recent mentioned survey carried out in Greece. Contamination of Sohan with molds and yeasts can be derived from raw nuts, additives and packaging material. When the hot final Sohan products become packed in sealed containers, it may lead to condense moisture and localize molds and yeasts growth in moisture-proof containers. As indicated formerly, the main sources of Sohan contamination are unsanitary environments, equipment, personnel of different processing plants. These personnel can contaminate Sohan samples with mishandling during each stage of production such as processing of raw materials, putting in containers and packaging.

According to our results, most of unacceptable samples by regard to microbial and fungal parameters were found in warm months (July, August and September); this finding may be due to high temperature and humidity in these months. Therefore, it is necessary to apply more hygienic conditions for manufacturing and packaging of Sohan during warm months.

Oil oxidation affects severely the quality and shelf-life of foods and results in loss of flavor, color and nutrient value of these products. Two current tests for measuring of rancidity are acidity and peroxide value (Addis, 1986). Peroxide content is used as an indicator of early stages of oxidation in oils that generally believed to measure the effectiveness of storage conditions (Sumainah et al., 2000). In this research, peroxide values of all Sohan samples were in acceptable level (≤5 mEq/Kg; IUPAC, 1997) that may be due to the presence of antioxidants in consumed oils. Similarly, Namiki (1995) stated that Tahini is resistant to oxidative deterioration due to the presence of endogenous antioxidants (sesamol and sesaminol) and tocopherols. According to Kahraman and Issa (2010), the peroxide value of Tahini halva was higher than amounts presented in our findings. All foods containing lipids are susceptible to oxidation, especially when they are dehydrated, subjected to high temperature or cooked, and subsequently stored. The acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase, heat and light. As rancidity is usually accompanied by free fatty acid formation, it is often used as a general indication of oils edibility (Addis, 1986). In present survey, all Sohan samples acid value was in acceptable range (≤1%; IUPAC, 1997). Another investigation was showed higher amount for acid value (Eissa and Zohair, 2006). Differences in acid values among products from different sources may be due to a lack of standardization, storage conditions and using different types of product components (Ghaneian et al., 2013).

Conclusion

In this survey, all Sohan samples had peroxide values and acidity in acceptable range, but the large numbers of food

borne pathogens were found in Sohan samples especially in warm months. So, such contaminated Sohan could be a potential health hazard to consumers. Since, Sohan is a popular Iran souvenir, it is necessary to employ principals of Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) to minimize the risk of contamination with these pathogens.

Conflicts of interest

There is no conflict of interest.

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