

ORIGINAL ARTICLE

Microbial and Chemical Properties of Mahyaveh: A Traditional Iranian Fish Sauce in Zarrin Dasht City, Iran

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ABSTRACT

Background: Mahyaveh is a fermented sauce made from fish. This study was conducted to investigate microbial and chemical properties of Mahyaveh in Zarrin Dasht city of Fars province, southern Iran.

Methods: Twenty-three samples were randomly collected in sterile containers and transferred to the laboratory. Acidophilic mesophilic bacteria, lactic acid bacteria, *Coagulase Positive Staphylococci*, molds and yeasts were enumerated using plate count agar, MRS agar, Baird-parker agar and Dichloran-rose bengal chloramphenicol (DRBC) agar, respectively. AOAC method was used to measure the pH and acidity of the samples.

Results: The total number of mesophilic acidophilic bacteria, lactic acid bacteria, *Coagulase Positive Staphylococci* and mold and yeast were 6.23 ± 0.91 , 5.78 ± 1.02 , 3.52 ± 1.08 , and 2.27 ± 0.71 log CFU/gr, respectively. Comparing results with microbial standards in similar products, it was found that the number of *Coagulase Positive Staphylococci* in 58.33% of samples was higher than the standard. The pH mean of samples was 6.09 ± 0.84 and the acidity was 0.9 ± 0.29 (% lactic acid).

Conclusion: Since Mahyaveh is widely used in Southern provinces, standardization in this field, monitoring and hygienic control over production centers and the hygienic control of products in the markets seem necessary.

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Introduction

Most of the fermented fish products are commonly prepared and consumed in different parts of the world; such as Yuw in China, Jeotkal in Korea,

Nukazuke in Japan, and Bakasang in Indonesia. Fermented fish products are rich in protein which is commonly used in the diet of many Southeast Asian countries, as condiment in cooking, flavors

in many desserts, and occasionally as a main dish (1, 2). Mahyaveh is a kind of sauce which is also known as Mahveh and Mahyaveh. It is one of the native dishes of provinces like Fars, Hormozgan and some southern regions of Iran. The materials used to prepare Mahyaveh are often selected according to the availability of raw materials, consumer tastes, and the climatic conditions of the region. Therefore, there is a wide variety in the methods of production and composition of the ingredients in this fermented product (1).

Sardines are the main part of Mahyaveh, as well as salt and condiments as other ingredients (1, 2). Presence of high amount of salt (more than 20%) in this product is the most important factor in prevention of microbial contamination of meat. In addition, maintaining the hygienic standards in preparing and maintaining of Mahyaveh is very important. According to the fact that spoilage bacteria do not have the ability to tolerate 6 to 8 percent or more of salt, this product, due to high salt content, prevents the growth of such bacteria. At the same time, halophilic bacteria can grow only in the presence of high salt concentrations. In some cases, these bacteria can tolerate concentrations of 12 to 13 percent of salt, so the probability of growth of this type of bacteria in this high-salt product is high (3).

Among the bacteria that can be grown in this product are *Bacillus*, *Alcaligenes*, *Aeromonas* and *Vibrio*, and among the probable moulds are *Aspergillus* and *Penicillium*. *Candida* and *Saccharomyces* can be mentioned as probable yeasts (4). Moreover, it is also important to pay attention to the presence of biogenic amines in fermented fish sauce. Biogenic amines such as histamine, putrescin, tyramine and tryptamine are the nitrogen compounds that are existed in a wide range of foods and beverages. Among the microorganisms that generate these compounds, *Enterobacteriaceae*, micrococcus and *Lactobacillus* species can be mentioned. Consumption of foods containing significant amounts of these compounds can lead to food poisoning (5).

Several studies, including Rosma *et al.* (2009) (6), Majumdar *et al.* (2010) (7) and Schroder *et al.* (2011) (8), have investigated the physicochemical characteristics and microbial contamination in fish sauces produced in various regions such as southeast Asian countries. The aim of this study was to investigate the microbial and chemical properties of Mahyaveh supplied in Zarrin Dasht city of Fars province.

Materials and Methods

In the present cross-sectional study, 23 samples

of Mahyaveh were collected randomly in sterile containers from different retail points in Zarrin Dasht city of Fars province and transferred to the laboratory. In order to prepare the samples for microbial cultures, 10 g of each sample were mixed with 90 ml of sterile normal saline solution. After mixing, decimal dilution series were prepared. For total count of lactic acid bacteria, MRS Agar (Merck, Germany) were used. After samples inoculation, the plates was placed in an incubator at 40°C for 72 hours under anaerobic conditions using gas pack A (Merck, Germany) (2). The general identification of the acidophilic mesophilic bacteria was carried out using plate count agar, then the plates were placed in an incubator at 30°C for 48 hours (9).

Coagulase Positive Staphylococci were counted in the Baird-parker agar (Merck, Germany) and then the plates were incubated at 37°C for 24 hours (2). Coagulase test for colonies growth on Baird-Parker agar was performed according to the Iranian National Standard number 1810 (9). Identification of mold and yeast was carried out using Dichloran-Rose Bengal chloramphenicol (DRBC) agar (Merck, Germany) and the plates were incubated at 25°C for 3 to 7 days (2). Since there is no standard for Mahyaveh in Iran, the results were compared with Food and Agriculture Organization (FAO) standard for fish sauce which limit standard for the count of acidophilic mesophilic bacteria was $<10^5$ log CFU/gr and of *Coagulase Positive Staphylococci* was $<10^3$ log CFU/gr (10).

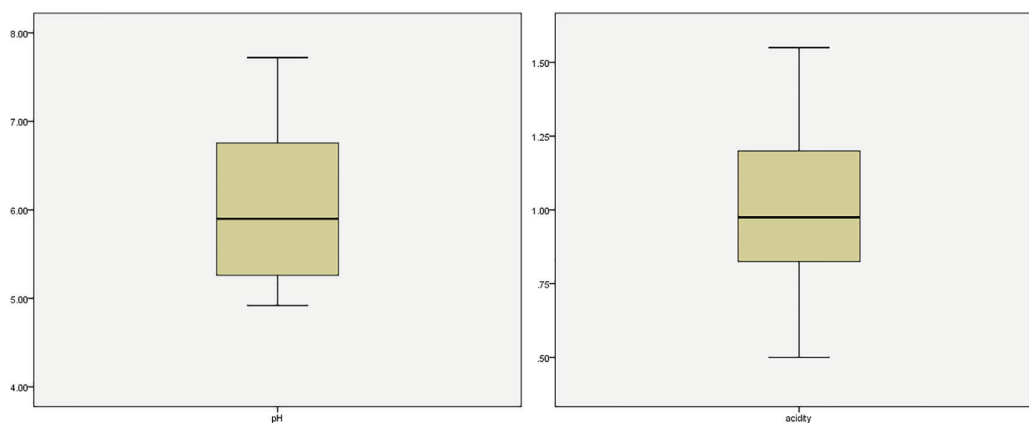
To measure the pH of the samples, a digital pH meter (Methrohm AG, Herisau, Switzerland) was used. After calibration of the device by standard buffers 4 and 7, the pH of the samples was measured using 10 ml of the sample, and according to the Codex, the limit standard of pH was 5- 6.5 (11). The acidity of the samples was measured according to AOAC, 1998. The result was reported as lactic acid percentage. Finally, the data of this study were analyzed by SPSS software (version 16, Chicago, IL, USA). In order to describe the results, descriptive statistics such as frequency and percentages, mean and standard deviation were calculated.

Results

Table 1 presented the minimum, maximum, mean, and standard deviation of the total count of mesophilic acidophilic bacteria, *Coagulase Positive Staphylococci*, mould and yeast, and lactic acid bacteria in Mahyaveh samples. The pH and acidity of the samples are also shown in Figure 1. Table 2 indicates the levels of mesophilic acidophilic bacteria and *Coagulase Positive Staphylococci* and

Table 1: Microbial properties of Mahyaveh samples

Total count	Min	Max	Mean±SD (log CFU/gr)
Mesophilic acidophilic bacteria	4.41	7.99	6.23±0.91
<i>Coagulase Positive Staphylococci</i>	2	5.64	3.52±1.08
Mould and yeast	1.00	3.40	2.27±0.71
Lactic acid bacteria	4.15	7.50	5.78±1.02

**Fig. 1:** Average pH and acidity of the Mahyaveh samples.**Table 2:** Comparison of total counts of mesophilic acidophilic bacteria, *Coagulase Positive Staphylococci* and pH of Mahyaveh samples with standards.

Parameters	Percentage more than the permitted limit	Permitted limit
Mesophilic acidophilic bacteria (log CFU/gr) *	95.45	<10 ⁵
<i>Coagulase Positive Staphylococci</i> (log CFU/gr)*	58.33	<10 ³
pH**	30.43	5-6.5

*Based on document repository FAO corporate; **Standard codex alimentarius commission

the pH of the Mahyaveh samples were compared with standard values.

Discussion

Fish sauce is a good source of protein, amino acids, vitamins and minerals. It is widely used in South Asian countries. Also, this fermented product is produced and consumed in some parts of the South of Iran. Therefore, this study was carried out to evaluate the microbial and chemical properties of traditionally produced Mahyaveh in Zarrin Dasht city of Fars province, southern Iran. In the present study, the mean of pH in 23 samples was 6.09±0.84, which is within the permitted pH range for fish sauce by Codex. The fermentation process by lactic acid bacteria led to reduction of pH of this product.

Low pH, organic acids (mainly lactic acid) and salt are the most important preservatives in fermented fish products. In the study carried out by Zarei *et al.*, the pH of Mahyaveh samples from 4 different parts in Fars and Hormozgan provinces was 6.34 (1). Also, the pH of similar fermented fish products such as Mackerel-nukazuke (Japanese fermented fish), Momoni (the fermented fish sauce prepared in Ghana), and Bakasang (Indonesian fermented fish

sauce) were in the range of 5.26-6.37, 6.47-6.56 and 5.9-6.3, respectively due to the growth and activity of lactic acid bacteria (12-14).

In our study, the mean of lactic acid bacteria in the samples was 5/78±1.02 log CFU/gr which was higher than the levels of lactic acid bacteria in a similar study by Zarei *et al.*, in which the levels of lactic acid bacteria were reported as 4.13 CFU/gr (1). In other fish fermented products such as Momoni and Bakasang, the levels of lactic acid bacteria were reported as 4.8-6.15 log CFU/gr and 4.61-4.79 log CFU/gr (13, 14). Lactic acid bacteria are known as the dominant microorganisms in many fermented fish products, and as some of them are probiotic bacteria, they can have beneficial effects for both humans and animals.

On the other hand, due to the effect of these bacteria on reduction of pH, their presence is one of the preservative and preventative factors of the product's degradation. Moreover, several studies have shown that some species of lactic acid bacteria such as *L. plantarum* and *L. casei*, due to their negative decarboxylase activity, decreased the accumulation of histamine, putrescin, tyramine and tryptamine in some types of fish sauce, including silver carp.

In addition, some *Lactobacillus* species can reduce the production of biogenic amines in these products due to bacteriocin compounds (5). Therefore, the presence of significant amounts of these bacteria in the fermented fish sauce can be considered as one of the beneficial features of this food product.

The total number of *Coagulase Positive Staphylococci* and mold and yeast in Mahyaveh samples were 3.52 ± 1.08 log CFU/gr and 2.27 ± 0.71 log CFU/gr, respectively. This showed that the *Coagulase Positive Staphylococci* in 58.33% of the samples was higher than the standard limit. Similarly, Taheri *et al.* (1393) in a study on Mahyaveh found that *Staphylococci* are the dominant microorganisms in this product. *Staphylococci* are often not seen in fish in hot areas, so these microorganisms can enter the product by used salt. Also, the population of yeasts gradually increases during storage of the product (15).

In a study by Thapa *et al.* (2004) on two traditionally fermented fish products, Negari and Hentak, the levels of lactic acid bacteria were 4 to 7.2, yeast 1 to 3.5, and aerobic mesophilic bacteria 4.3 to 3.7 log CFU/gr (16). In fact, many non-halophilic bacteria are present at the beginning of fermentation, but since this sauce has high salt concentrations, these bacteria lose their growth potential, and in the next stages of fermentation, they are replaced by halophilic bacteria (2). One of these bacteria is *Coagulase Positive Staphylococci*, which grows at a salt concentration of 7.5-15%. Since, in some cases, Mahyaveh is consumed with bread and without any additional processing; its contamination is a risk for the hygiene and safety of the food and consumers. Therefore, it appears that the salt and poor hygienic status during preparation of this product and the transferring of disease-causing microorganisms from contaminated containers or environment and the inappropriate storage temperature of these products can also be an important factor in the prevalence of large number of these microorganisms in foods (17). Using the inappropriate methods for cleaning the equipment cause the formation of biofilms on the food contact surfaces. Therefore, it is very important to undertake the appropriate cleaning and sanitizing process (18).

In this study, the average total count of mesophilic acidophilic bacteria in 95.45% of the products was higher than the standard limit, which requires paying more attention to food safety in the production process of this product. Today, many researchers have isolated lactic acid bacteria such as *Tetragenococcus* from fermented fish products, so that they can be used as starter for processing of fermented products (19). Since the quality and

physicochemical characteristics of such products are influenced by its microbial population, so the using starter can improve the quality and physicochemical properties of final product. In addition, this method reduces the risk of contamination of the product and increases the health of consumer.

Conclusion

Due to the presence of a large number of lactic acid bacteria in Mahyaveh and insufficient knowledge about the characteristics of the genus and species involved in the fermentation process of this product, further studies are necessary to provide useful information. In addition, high microbial load of Mahyaveh samples showed poor quality control and hygienic practices during production and storage of the product. So, prevention of cross contamination, effective cleaning and sanitizing programs and training good manufacturing practices are appropriate strategies to produce safer product. Moreover, the development of national standards for the fermented fish product, and also the production of starter in order to be used in the traditionally Mahyaveh production process are useful in this field.

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Conflict of Interest

None declared.

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