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Mahvieh, an Amazing Nutraceutical Bridge between Traditional and Modern Diet

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Abstract

Probiotic foods are products comprising of sufficient quantities of live microorganisms. The most important class of probiotic bacteria are Lactobacillus and Bifidobacteria, found in huge amount in dairy products, including kefir and yogurt. Probiotic microorganisms are most probable discovered in fermented fish foods as well. In this study, for the first time, we introduced a fermented fish food, which is customarily manufactured in southern Iran called Mahvieh. This fermenting product prepared from a kind of fish named motto (in native language) or sardines. This work aimed to specify total content of mineral elements, fat and protein in this food stuff and identify the potential probiotic microorganisms. In this regard, various samples were gathered from various area of the southern Iran (Narman, Varavi, Lamard); each was independently analyzed and mineral elements such as nitrogen, hydrogen, carbon and sulphur, as well as total lipid, fatty acids and protein content were established. The microorganisms in this seafood were isolated and identified by morphological and biochemical approaches along with probiotic features. The ultimate bacterial species were then isolated on the basis of these experiments and ultimately were recognized as normal microbial flora in this fermented seafood product. The findings of this research point out that the dominantly is belonged to the Lactobacillus genus in all samples, and the provided Mahvieh can be considered as a nutritional fermented seafood containing good probiotic bacteria.

Keywords: Probiotic bacteria, *Lactobacillus, Bacillus*, Total protein and lipid.

1. Introduction

Recently, fermented foods once again have obtained attentions as part of global diets that highlight artisanal processes because of their highly potential for promoting health. Over the past decade, many studies have recommended that fermented foods should be added to national dietary habits. Traditional fish products are popular and palatable foods in different regions of world especially in southeast and southern west Asia (1). Jeokotal, Shidal, Momoni, Feseekh, Mehyavah and Nukazuke are some examples for fish based dishes, which consume in South Korea, India, Ghana, Egypt, Iran and Japan, respectively (2, 3). In these countries, fish products were served as rich sources of easily digestible proteins as well as polyunsaturated fatty acids, vitamins and minerals (4). Since the fish is a perishable commodity and cold chain management for it is a costly and hardly available process, traditional procedures such as smoking, salting, drying, fermentation and the combination of these processes are fascinating techniques for long-term fresh fish preservation especially in warm and tropical regions (5).

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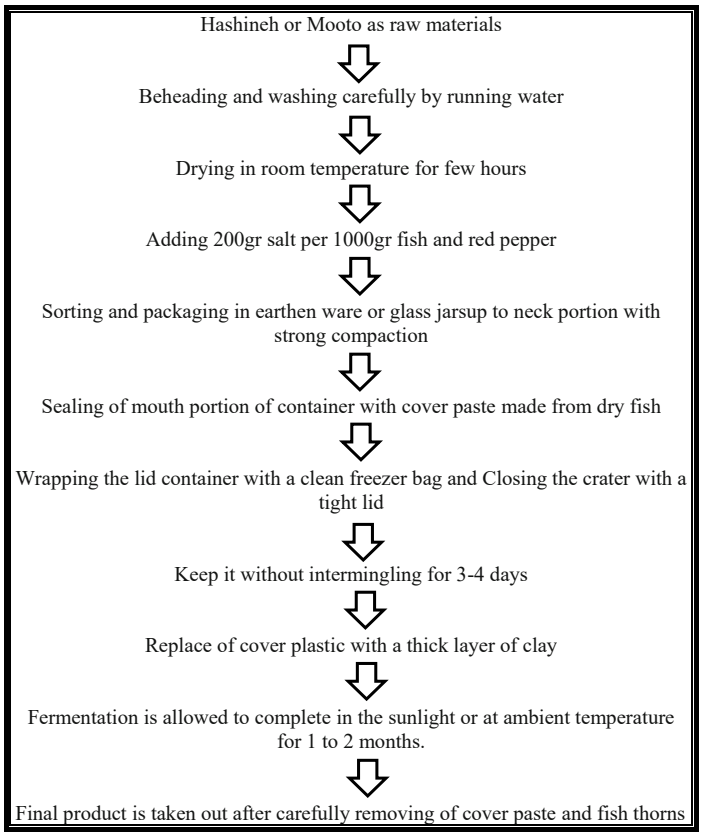
In addition, because of their popularly acceptable taste, fermented fish products are taken not only as side dishes and seasoning, but also as flavoring agent for many dishes, appetizer and sometimes as a main course (6, 7).

Mahvieh, salted-fermented fish, is one of the traditional and popular fermented fish products in South of Iran, particularly in Lamerd, Bushehr and Parsian, which faces the middle of the Persian Gulf. This fermented product typically composed of fresh Sardinella melanura (Hashineh in local language) or Stolephorus indicus (Mooto in local language) fishes that impregnated with salt in water (8). The appearance of Mahvieh is pasty and the shape of the fish remains almost fully disintegrated (9). The color of best quality food is redish brown that gradually becomes brownish to brownish grey on continuous exposure to air. The tangy odor is the characteristic smell of Mahvieh and shows the product is ready to take. Flowchart 1 demonstrates traditional method for preparation of Mahvieh.

Besides providing a wealthy source of proteins and natural primary metabolites, such fermented foods possess some other biological effects, especially providing probiotic microorganisms, which have anti-inflammatory potential and promote GI tract health and protect the body against colitis and epithelial cell damage (10, 11). Fermented foods should be considered to have similar health benefits as those conferred by probiotic species. This concept is consistent with the understanding that core beneficial effects of probiotics can be attributed to a species, rather than to specific strains of a species (12). Also, peptides and amino acids founds in fermented products exhibit interesting biological effects, including antibacterial, antioxidatant, antithrombotic activities as well as blood pressure- and lipid-lowering effects (13).

Besides chemical composition and dominant microorganisms, no information regarding the lipid profile and bioactivity of this fermented fish products, Mahvieh, have been reported. Also, there was no report on detailed properties of dominant microorganisms. Therefore, the aim of present study was to provide information about chemi-



Flowchart 1. A diagram for Mahvieh production.

Figure 1. *Sardinella melanura* which were caught from Persian Gulf.

cal, microbial and nutrient profile of Mahvieh, a fermented product from Hashineh or Mooto fish.

2. Materials and Methods

2.1. Sample preparation

The Samples of Mahvieh were collected from north regions Persian Gulf including Lamerd, Narman, Varavi and Boushehr. These samples (n=11) were obtained as part of the marine biotechnology research studies of Biotechnology Research Center at Shiraz University of Medical Sciences. Fresh fishes including fresh Sardinella melanura or Stolephorus indicus (Figure. 1) were purchased from local sellers and frozen at -4 °C as soon as possible after sorting and counting. They were then transported to local Mahvieh suppliers.

2.2. Total protein assay

Total protein measurement for supernatant of "Mahvieh" was carried out according to Biuret assay. Briefly, the supernatant samples were treated with an equal volume of 1% potassium hydroxide followed by a few drops of aqueous copper (II) sulfate. A peptide of a chain length of at least 3 amino acids is necessary for a significant, measurable color shift with these reagents. At the present of protein, the color of solution was turned to purple. The intensity of color was detected by UV spectrophotometer at 565 nm which indicate amount of protein.

2.3. Total lipid assay

Colorimetric method was applied to measure lipid content of "Mahvieh" solution. For this purpose, 5 gr homogenized solution of each sample was freeze-dried and dissolved in a chloroform- methanol solution (2:1). After 30 s of sonication followed by 5 min centrifugation at 3000 rpm, the resultant was dried in room temperature for 2 weeks. Then, 100 µg of samples and standards were placed in a heating block set at 90 °C then 100 µl of concentrated sulfuric acid (98%) was added to each tube, fully vortexed, and then heated for 20 min. Samples were then removed from the heat block and poured in an ice pack for 2 min before adding 50µl of vanillin reagent (200 µg of vanillin and 17% phosphoric acid) and then vortexed vigorously. The pink color is allowed to develop for 5 min and then 200 µl of standards and samples were read at 540 nm. In this assay, olive oil was used as standard and the concentration of samples was measured by the resultant standard curve.

2.4. Fatty acids and fatty alcohol composition of Mahvieh

Mahvieh were lyophilized using a freeze drier (Christ, 1-4 LD plus, Germany) by a two steps lyophilizing protocol. First, they were frozen at -70 °C for 24 h. In the primary step, the freeze-dryer was evacuated, the temperature was adjusted to -45 °C and pressure was set to 0.07 mbar for about 30 h and then, for secondary step, the pressure lowered to 0.04 mbar for 18 h.

The lyophilized dry samples were suspended in 10 ml of chloroform and refluxed for 24 h at 60 °C. Then, the mixture were filtered and 20 ml of ethanol 96% was added to the filtrates before vaccum drying. The residue were weighted and suspended in a mixture methanol:chloroform:sulfuric acid (10:1:1 v/v/v) under nitrogen, then incubated at 90 °C for 3 h using an oil bath, followed

by cooling in ice bath. A total of 2 ml water was added to mixture and vortexed to separate the organic phase. Then, this phase were dried over sodium sulfate and and stored at -20 °C until GC/MS qualitative analysis.

2.5. GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out by using Agilent7890 gas chromatograph with a mass detector (Agilent technologies model 5975 C). The gas chromatograph was equipped with a HP-5MS capillary column (phenyl methyl siloxan, 30 m ×0.25 mm i.d., Agilent technologies). The oven temperature was programmed from 60 °C (0 min) to 250 °C at the rate of 5 °C/min and then held for 10 min at 250 °C. Helium was selected as the carrier gas and flow rate was adjusted at 1 mL/min. The mass spectrometer (Agilent technologies 5975 C) was operating in EI mode at 70 eV. The interface temperature was 280 °C; mass range was 30-600 m/z. The constituents were identified by comparison of Kováts retention indices (KI), referring to compounds known from literature database and also by comparing their mass spectra with the Wiley library or with the published mass spectra. Relative percentage amounts were calculated from the total area under the peaks by the software of the apparatus.

2.6. Elemental analysis

The analysis of elements present in Mahvieh was done by Simultaneous Thermal Analyzer (STA) apparatus. In this technique, CHNS analysis is accomplished by combustion analysis. The sample was burned in an excess of oxygen, and various traps collected the combustion products-carbon dioxide, water, and nitric oxide. The masses of these combustion products used to calculate the composition of the unknown sample.

2.7. Isolation and screening of microorganisms

In this study, one gram of each sample was suspended in 9 mL of sterile normal saline (9 g/L NaCl), serially diluted to 10^{-7} and cultured on MRS (Merck) and TSA (Merck) agar plate. MRS plates was used for microaerophilic bacteria and transferred to microaerophilic jar. Anaerobic condition was created by anoxomat apparatus. After 48 h incubation at 37 °C and further sub culturing to obtain pure culture, Particular colonies were isolated and colonies were measured in diameter. Particular colonies were identified based on morphological features like size, color, elevation, shape and margin.

2.8. Biochemical identification tests

Various biochemical and physiological characterizations of the amylase-producing isolates were determined by using Bergey's Manual of Determinative Bacteriology. Biochemical tests such as Gram staining, catalase, spore production test, sugar fermentation tests (D-lactose, D-galactose, cellobiose, D-glucose, D-salicine, maltose, D-mannitol, D-mannose, D-sucrose and D-melibiose),Voges-Proskaeur and oxidase test, oxygen requirement and motility were also studied. Colony morphology such as form, margin, elevation, color and diameter (mm) after 24 h were observed by light microscopy and finally the genus of the selected strain was determined.

3. Results

3.1. Total lipid and protein content

Total lipid content in the specimens was obtained by the formula obtained from the standard olive oil charts in milligrams and was finally reported as percentage of primary dry weight, as shown in Table 1. The results showed that total lipid content was 203±25 mg/g dry weight of specimens.

The result of total protein content in the specimens is given in Table 1. The results showed that total protein content in Mahvieh was 527±19 mg/g dry weight of specimens.

3.2. Fatty acid and fatty alcohol composition of

Table 1. Total protein and lipid content of Mahvieh.

Specimen	Total lipid content (%)	Total prtein content (%)
A	11.7±0.7	43±3
B	11.05±1.8	38±6
C	10.80±0.1	74±2

Table 2. Fatty acid and fatty alcohol content of Mahvieh.

Types of fatty acids	Percent in Mahvieh extraction (%)± SD	
	fatty acid	fatty alcohol
Sum of saturated fatty acids	11.3±1.1	49.6±0.01
Sum of monosturated saturated fatty acids	73.6±1.2	46.9±0.08
Sum of polyunsaturated fatty acid	13.3±0.7	2.8±0.02
Other	1.8±0.02	4.2±0.04

Mahvieh

The results of GC-MS analysis showed that the most important fatty acids group founded in Mahvieh were monounsaturated fatty acids (Table 2). Monounsaturated fatty acids in Mahvieh samples amounted to about 71.7-79.1% of total fatty acids. There also were a negligible amount of saturated fatty acids. Total saturated fatty acids of all Mahvieh samples ranged from 7.85-15.8% of total fatty acids (Table 2). In addition, the amount of polyunsaturated fatty acids were at similar levels to saturated fatty acids in Mahvieh lipids which ranged from 8.1-19.7% of total fatty acids.

3.3. Elemental Analysis of Mahvieh

By using the elemental combustion analysis, the percentages of carbon, hydrogen, nitrogen and sulphur in the specimens were measured, as tabulated in Table 3. As shown in Table 3, carbon, nitrogen and sulphur content account for 42%, 11% and 4% of total elements existing in the product, respectively.

3.4. Screening and Isolation of Microorganisms

Table 3. The results of elemental analysis of Mahvieh.

Basic elements	Weighted percent (%)±SD
Carbon	41.9±1.2%
Hydrogen	6.04±0.2%
Nitrogen	11.02±0.6%
Sulphur	4.24±0.09%

From each specimen, a serial dilutions from 10 to 10^{-5} were made. After several subcultures, gram staining was performed to obtain a single clone. According to the results, gram positive rod-shape bacteria were isolated. Each of the isolated microorganisms was assigned an English letter from A to R. The bacteria were subjected to further biochemical analyses to identify the strains. The biochemical experiments including catalase, spore staining, carbohydrate fermentation tests were used for better identification.

3.5. Identifying the Selected Strains

3.5.1 Catalase assay results

In order to perform catalase assay, new cultures of bacteria were made in MRS and TSA media and H_2O_2 solution (6%) was made. These bacteria were placed adjacent to this solution; the presence of air bubbles and production of oxygen means that the assay is positive. The results of catalase assay are given in Tables 4.

3.5.2. Spore staining results

The bacteria were cultured in TSA medium for 5 to 6 days. The results of malachite green staining of bacterial spores showed that spores were round or oval (Table 5). According to the results, bacteria A, C, G, J, K, and Q did not have the ability to produce spores.

Table 4. Catalase assay results in MRS of bacteria.

A	B	C	D	E	F	G	H	I	J	K	C	M	N	O	Q	R
-	-	-	-	+	-	-	+	+	-	+	+	+	+	+	+	+

Table 5. Spore stain results (+: spore production).

A	B	C	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
-	+	-	+	+	-	+	+	-	-	+	+	+	+	+	-	+

Table 6. Results of carbohydrate fermentation by bacteria.

carbohydrate / Isolate	Glucose	Lactose	Maltose	Sucrose
A	-	-	-	-
B	-	-	-	-
C	+	-	+	+
D	-	-	-	-
E	+	-	+	+
F	-	-	-	-
G	+	+	+	+
H	+	+	+	+
I	-	-	-	-
J	-	-	-	-
K	+	-	+	+

+: ability of the bacterium in fermenting the carbohydrate.

3.5.3. Carbohydrate fermentation profile

The results of carbohydrate fermentation are summarized in Table 6. It should be noted that the change in red colour associated with phenol, which yellow colour is a sign of fermentation of each carbohydrate. According to the results, 60% of bacteria (bacteria K, A, C) were not able to ferment lactose.

4. Discussion

Fermented fish products help considerably to intake of protein for a great number of the World's populations. In some countries, like as Kampuchea, Thailand, Malaysia, Philippines, Cambodia, Indonesia in South East Asia as well as South of Iran in Middle East, these foodstuffs are a main fraction of the diet (14). Mahvieh is one of the main fermented fish product that consumed widely in south of Iran. This product contains salt (20%), which uses as a preservation. In some cases, this method may be applied with other approaches, like as sun-drying that expedite the process, however offer other alterations. This procedure is usually regarded as "salt fermentation", which is the preliminary stage of dehydration caused by the osmotic effect (15). This decreases the humidity content to a point which may stop spoilage processes. Moreover, salt demonstrate its own anti-bacterial activity (2). In the fish fermentation process of Mahvieh, enzymes generate the textural alteration and contribute in manufacturing some of the taste. Conversely, bacteria are included in the improvement of flavor and aroma.

The manufacturing processes for Mahvieh include salt and fish being packed in glass jar and allow remaining for a long period, which resulted in the cellular liquid extraction to forms the "pickle". The proteolytic enzymes in the fish are released inside the cells and attack the interior muscle and membranes (16). The resultant solubilized protein exudes and in turn is attacked by the enzymes as they are released, to produce Mahvieh.

The results showed that total protein content was 53% in the Mahvieh. Therefore, nitrogen content in this product should be theoretically about 9-11%. Measurements show that average nitrogen content is 11%, which is consistent with the theoretical protein ratio. According to the results, it seems that this fermented fish product is a good source of protein, unsaturated fatty acids and other minerals and it can be preserved for long time. Many changes occur during Mahvieh manufacture. Knowing of the alterations is crucial if the fabrication rates is to be quicker or if fewer valued species are to be used. It is assumed that all kind of Mahvieh or similar products endure the similar alterations; however, it can be actually differs duo to the fish species as well as variation of microorganisms that will be vary in relation to the environment (17). However, the chief fraction of the production of all fish products including Mahvieh is the conversion of insoluble fish protein into a

'suspended' or soluble form (18). The mediators are the enzymes existence in the fish, while the microbial enzymes may contribute (19, 20).

The major products are polypeptides and amino acids, which significantly assist to get flavor, relatively. Moreover, ammonia and other low molecular mass nitrogenous compounds are side products as well. According to Beddows et al., the polypeptides and free amino acid content elevated progressively until it got into a maximum after 45 days and remained at this level (21). When fermentation was finished, about 84 % of the total nitrogen was organic. In this regard, the free amino acid content reached to 49%, then the polypeptide got into 14% of the total organic nitrogen along with ammonia was reported to reach 17%. The decay process of the fish flesh was finished after 4-5 months, while the ammonia concentration tends to elevate.

In this study, MRS and TSA media were used to isolate the bacteria. After incubation at 37 °C for 48 h, single colonies were isolated from the media. By comparing their morphological and biochemical results with the table prepared by Schink (22) and Bergey (23), probiotic strains *L. acidophilus*, *L. fermentum*, *L. casei*, and *Bacillus amyloliquefaciens* were identified. The predecessors may consider this type of fish product as a way to store and supply seafood in the absence of this type of food. However, since the concentration of salt in this food product is about 12.5%, on average, it seems to be a constraint for people with cardiovascular problems. However, the present of high salt concentration stop the growth of pathogenic bacteria including Vibrio parahaemolyticus, Aeromonas etc (24). On the one hand, this product has a high nitrogen content which may endanger human health. Parts of this nitrogen is likely to be converted into biological amines by the microorganisms (9). Toxicologically, high intake of these biological amines is harmful to human health (25). On the other hand, there are reports on the presence of histamine amino acid in the Mayawah (9); if the Mahvieh also contains high amounts of histamine, it should be considered nutritionally. In short, despite the high nutritional value of Mahvieh as well as probiotics present in it, intake of this product in low amounts is recommended. However, it should be noted that overconsumption of this product due to its high levels of nitrogen and salt can cause poisoning in humans.

5. Conclusion

In general, this study is a report on introduction, characterization and identification of microbial diversity in the fermented food Mahvieh. In terms of nutritional value, this product contains high amounts of protein as well as lipid and some basic elements necessary for the body; however, overconsumption needs to be reviewed, particularly for cardiovascular patients. The results of this study indicate the presence of specific strains of *Lactobacillus* and *Bacillus*. The *Lactobacillus genus* is the dominant genus in all samples, which suggests that Mahvieh can be considered as a product containing probiotic bacteria. On the other hand, bacteria can be isolated and used at the industrial level to produce probiotic therapeutic products.

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Conflict of Interest

None declared.

6. References

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