

Optimization of production conditions of Mahyaveh, a traditional Iranian fish sauce, with low microbial load

Fatemeh Kavian, Leila Nateghi*

Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

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ABSTRACT

Background and Objectives: Mahyaveh is a traditional Iranian fish sauce produced by fermentation and hydrolysis. The main production of Mahyaveh has been traditionally and scientific research and industrial measures has not been done on it with the aim to achieve a production with less microbial load. Therefore, the aim of this research was to study the type of fish, salt concentration and fermentation time on the bacterial population of Iranian fish sauce (Mahyaveh).

Materials and Methods: For this purpose, the effects of fish type (tuna, anchovy and sardine), salt concentration (15%, 25% and 35%) and fermentation time (30, 75 and 120 days) on the total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* were investigated. 15 treatments were designed according to the Box-Behnken Response Surface Methodology.

Results: Simultaneous optimization to achieve the minimum total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* in the Mahyaveh sauce was obtained with 99.49% desirability at the time of 103.63 days of fermentation with the third type of fish (Sardine) and at a salt concentration of 29.38%.

Conclusion: By optimizing the conditions of producing Mahyaveh sauce, fish sauce can be produced with the least amount of microbial load with more health safety.

Keywords: Mahyaveh; Total microbial count; *Micrococcus*; *Enterobacteriaceae*; *Bacillus*

INTRODUCTION

Fermented food is an edible product that is produced by the function of microorganisms in a natural way or by adding pure or combined cultivation of microorganisms. The term "fish fermentation products" refers to products derived from freshwater and marine fish, shrimp and crustaceans, which are fermented by the action of aquatic enzymes and bacterial enzymes along with salt and thus prevent spoilage (1). Fish sauce is a fermented product of fish. This product is a thick brown fish that has been used by the people of East Asia since ancient times to nowadays

as an additive and flavoring the food (2). Fish sauce is considered a rich source of protein and contains essential amino acids like lysine. Lots of vitamins and minerals are found in fish sauce and this product is a rich source of B12 vitamins, sodium, calcium, magnesium, iron, manganese, and phosphorus (3). It has various names depending on the country from which it is produced, such as nampla (Thailand), bakasang (Indonesia), yu-lu (China), patis (Philippines), ngapi (Burma), shotshuru (Japan), aekjeot (Korea), and budu (Malaysia) (4). In Iran, a type of local fish sauce is produced, which is called Mahyaveh, Mahweh or Suragh. Mahyaveh is traditionally produced by the

*Corresponding author: Leila Nateghi, Ph.D, Department of Food Science and Technology, Faculty of Agriculture, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran. Tel: +98-9125878775 Fax: +98-2136724767 Email: leylanatheghi@iauaramin.ac.ir

natives in the southern provinces of Iran, including the cities of Fars and Hormozgan, and is generally made from sardines called in the local language *Sardinella* sp, Anchovy, or Indian moto, salt, Mustard (*Juncea Brassica*) and water (5).

The quality of fish sauce depends on factors such as the type of fish, the type of salt, the ratio of fish to salt, the additives used and the fermentation conditions (6). The salt used in the preparation of the sauce not only acts as a preservative but also reduces the active water and the moisture content of the final product, which is unsuitable for the growth of microorganisms (7). Due to the high concentration of salt in fermented products, the growth of pathogenic microorganisms is controlled and the fish sauce has an acceptable taste and aroma. However, salt-loving microorganisms can also grow in fish sauce (8). In 2004, Thapa et al. examined the microbial population of a traditional fish fermentation product called Ngari and Hentar and reported that lactic acid bacteria were 4 to 2.7, cylindrical bacteria producing endospore was 3.3 to 4.6, yeast was 1 to 3.5 and aerobic mesophilic content was 4.3 to 7.3 units' colony formation in grams. The number of *Bacillus cereus*, *Staphylococcus aureus*, and *Enterobacteriaceae* were the highest. The existence of these bacteria in fish sauce was due to contamination during processing (9). Md Zoqratt and Gan, (2021) has been reported that microbial diversity has significant effect on organoleptic, quality and nutritional properties of Malaysian fermented anchovy sauce (Budu) (10).

Given that, the type of fish, salt concentration and fermentation time affect the bacterial population of Iranian fish sauce and its quality but to date the Mahyaveh has been produced traditionally and scientific research and industrial measures on it with the aim to achieve microbial load has not been done. Therefore, the general aim of this research is to investigate the type of fish, salt concentration and fermentation time on the bacterial population of Iranian fish sauce (Mahyaveh).

MATERIALS AND METHODS

In order to prepare Mahyaveh sauce, tuna, anchovies and sardines were supplied in April 2017 (from Bandar Fish Company in Bandar Abbas, Iran) and frozen at -18°C and transferred to the Agricultural Laboratory of the University of Tehran. Cultivation

media included: Plate Count Agar, Dextrose Tryptone Agar, Mannitol Salt Agar and Violet Red Bile Agar were purchased from Quelab Company, Canada. Table salt was purchased from Shimiaz Company, Iran. Mustard powder was produced by G.S.Dunn Company, Canada.

Preparation of Iranian fish sauce. First (1: tuna, 2: anchovies, and 3: sardines) were washed with water, along with viscous, and cut to a size of 6 cm. The whole fish (along with viscera) was mixed with water in a ratio of 1 to 1, and the required amount of table salt was added to the treatments according to Table 1. Then, fish, water and table salt were mixed in a mixer, Model ika, Germany and transferred to dishes with a capacity of 700 ml, and the lids were closed with three-layer plastic films. These utensils were kept in incubator in time intervals of 30, 75 and 120 days at 37°C. After passing the fermentation time of each treatment, the samples were passed through a sterile cleaning cloth. Then, the filtered extract was mixed with 10% mustard and the samples were kept at environment temperature for 10 to 15 days, and then microbial experiments were performed on the resulting extracts.

Table 1. Different samples of Mahyaveh prepared by fish type, salt concentration and different fermentation time by Response Surface Methodology (Box-Behnken)

Treatment	Fish type *	Salt concentration (%ww ⁻¹)	Time (day)
1	1	15	75
2	1	25	120
3	2	25	75
4	2	25	75
5	2	25	75
6	3	15	75
7	1	25	30
8	3	25	120
9	3	35	75
10	2	15	30
11	2	15	120
12	1	35	75
13	3	25	30
14	2	35	30
15	2	35	120

* Fish Code: 1: Tuna, 2: Anchovy, 3: Sardines

Microbial tests of Iranian fish sauce samples: total count population of microbial, *Micrococcus*, *Bacilli* and *Enterobacteriaceae*. For all microbial tests the utensils were first sterilized by Ultra Violet lamps, and then all surfaces sterilized with alcohol. Also, all the used dishes in the test were sterilized by oven and autoclave (11). To evaluate the total *Micrococcus*, *Bacillus*, and *Enterobacteriaceae* microbial count, respectively the plate count agar (PCA), Mannitol salt agar (MSA), Dextrose tryptone agar, and Violet red bile agar were used (12). For the total count of bacteria, the method of pour plate and double cultivation method were used (13). First, the necessary dilutions of the samples were made in 0.9% salt solutions in distilled water. Then, 1 ml of each dilution was poured into a pure sterile plate and about 9 ml of sterile liquid cultivation environment with a temperature of 40 to 45°C, was poured on the desired dilution and moved to spread on all surfaces like the form of number 8. The cultivation environment of PCA plates were heated at 30°C for 48 hours. After two days, the bacteria were counted by colony counter. In some plates, cream-colored colonies resembling yeast were observed.

In order to count the Micrococci, Enterobacteriaceae and bacilli bacteria the pour plate method and double cultivation were used (12). First, the necessary dilutions of the samples were made in 0.9% salt solutions in distilled water. Then, 1 ml of each dilution was poured into a pure sterile plate and about 9 ml of sterile liquid cultivation media with a temperature of 40 to 45°C was poured on the desired dilution and moved to spread on all surfaces like the form of number 8. After closing the cultivation media, they were placed in upside down incubator. MSA plates (mannitol salt agar) were incubated at 30°C for 48 h and some plates showed amethyst colonies. *Enterobacteriaceae* were incubated for 24 h at 37°C and *Bacillus* plates were incubated for 48 h at 35°C. In some plates, the colors of their colonies were pink and yellow, respectively. After two days, to count the plates, they divided into four parts and one part was counted and then multiplied by four.

Statistical analysis. In this research, the Box-Behnken Response Surface Methodology was used to design the treatments and optimize the extraction conditions and achieve the Mahyaveh with the lowest microbial load. The extraction conditions were three independent factors of fish type (tuna, an-

chovy, sardine) and salt concentration (15, 25, 35%) and fermentation time (30, 75, 120 days). So 15 treatments were designed. The results of the tests were analyzed by Minitab 16 software using Box-Behnken Response Surface Method.

RESULTS

Microbial evaluation of Mahyaveh fish sauce. Table 2, shows a comparison between the total count of microbes, *Micrococcus*, *Enterobacteriaceae*, and *Bacillus* in the tested Mahyaveh sauce prepared under different conditions. According to the results, there was no significant difference between the tested and predicted microbial evaluation results ($P > 0.05$). According to the results of Table 2, it was observed that the different conditions of production of Mahyaveh sauce had a significant effect on the total count of microbes, *Micrococcus*, *Enterobacteriaceae* and *Bacillus*. The results showed that by the passing of fermentation time (from 30 to 120 days), increasing the salt concentration (from 15 to 35%) had a significant effect ($P \leq 0.05$) on reducing the population of microbes of Mahyaveh sauce. The type of fish did not have a significant effect on reducing the microbial population of Mahyaveh sauce ($P > 0.05$).

The highest total microbial count (5.041 Log cfu ml⁻¹), *Micrococcus* (5.146 Log cfu ml⁻¹), *Enterobacteriaceae* (4.398 Log cfu ml⁻¹) and *Bacillus* (4.633 Log cfu ml⁻¹) were observed in the sample of Mahyaveh sauce (T10) containing 15% ww⁻¹ salt, 30 days' fermentation time and fish type 2 (Anchovy). The lowest total microbial counts (1.845 Log cfu ml⁻¹), *Micrococcus* (0.911 Log cfu ml⁻¹), *Enterobacteriaceae* (0.840 Log cfu ml⁻¹) and *Bacillus* (0.752 Log cfu ml⁻¹) were observed in the Mahyaveh sauce sample (T15) containing 35% ww⁻¹ salt, 120 days' fermentation time and fish type 2 (Anchovy).

Analysis of variance of response surface model of microbial total count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus*. Table 3 shows the results of the analysis of variance response surface model (RSM) of microbial total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus*. According to the results of linear effects, salt concentration variables and fermentation time and the square effect of salt concentration on total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* of Mahy-

Table 2. Comparison between total count, *micrococcus*, *enterobacteriaceae*, and *bacillus* of tested and predicted Mahyaveh under different conditions.

Treatment	Total Count (log cfu ml ⁻¹)		<i>Micrococcus</i> (log cfu ml ⁻¹)		<i>Enterobacteriaceae</i> (log cfu ml ⁻¹)		<i>bacillus</i> (log cfu ml ⁻¹)	
	Tested	Predicted	Tested	Predicted	Tested	Predicted	Tested	Predicted
1	4.519	4.353	4.991	4.566	2.041	2.294	4.491	4.178
2	2.079	1.810	1.000	0.890	1.000	0.861	1.000	0.791
3	2.146	2.175	18.34	0.956	1.000	1.000	1.477	1.360
4	2.204	2.175	1.602	1.670	1.000	1.000	1.000	1.360
5	2.176	2.175	1.200	1.670	1.000	1.000	1.602	1.360
6	4.732	4.528	1.778	4.566	2.204	2.352	4.602	4.163
7	2.322	2.553	2.255	2.411	2.255	2.290	2.204	2.288
8	2.000	1.910	1.000	0.390	1.000	0.965	1.000	0.917
9	4.114	2.279	1.477	1.612	1.301	1.049	1.301	1.615
10	5.041	4.976	5.146	5.306	4.398	4.111	4.633	4.864
11	2.653	3.088	2.362	3.111	2.322	2.209	2.322	2.845
12	2.041	2.246	1.000	0.897	1.000	1.287	1.000	1.439
13	2.531	2.801	2.342	2.411	2.301	2.440	2.114	2.323
14	2.230	1.796	2.255	2.352	2.176	2.289	2.176	1.653
15	1.845	1.770	0.911	0.871	0.840	0.852	0.752	0.770

Table 3. Results of analysis of variance of response surface model of microbial total count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* Mahyaveh sauce

Source	Microbial total count (log cfu ml ⁻¹)		<i>Micrococcus</i> (log cfu ml ⁻¹)		<i>Enterobacteriaceae</i> (log cfu ml ⁻¹)		<i>Bacillus</i> (log cfu ml ⁻¹)	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Constant	11.26	0.008	8.61	0.014	16.72	0.003*	8.75	0.014*
Linear effects	23.79	0.002*	19.29	0.004*	33.22	0.001*	19.71	0.003*
Type of fish (a)	0.14	0.724	0.12	0.741	0.40	0.553	0.04	0.846
Salt concentration (b)	61.11	0.001*	43.89	0.001*	46.82	0.001*	45.39	0.001*
fermentation time (c)	10.13	0.024*	13.85	0.014*	52.43	0.001*	13.69	0.014*
Square effects	7.79	0.025*	6.01	0.041*	16.08	0.005*	6.18	0.039*
Type of fish× Type of fish (a ²)	1.29	0.307	0.92	0.383	0.45	0.531	0.86	0.396
Salt concentration × Salt concentration (b ²)	21.12	0.006*	17.06	0.009*	24.86	0.004*	17.89	0.008*
Fermentation time × Fermentation time (c ²)	0.73	0.431	0.14	0.720	25.02	0.004*	0.03	0.875
Interaction effect	2.21	0.205	0.53	0.682	0.86	0.519	0.36	0.785
Type of fish× Salt concentration (a×b)	0.03	0.865	0.11	0.753	0.817	0.0048	0.03	0.871
Type of fish× Fermentation time (a×c)	0.13	0.729	0.00	0.948	0.174	0.2023	0.01	0.938
Fermentation time × Salt concentration (b×c)	6.46	0.052	1.47	0.279	0.939	0.0005	1.05	0.353

aveh sauce were significant ($P \leq 0.05$). It should be noted that the square effect of fermentation time was also significant on the changes of the amount of *Enterobacteriaceae* in Mahyaveh sauce ($P \leq 0.05$). The linear and square effects of fish species on total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* of Mahyaveh sauce were not significant (P

>0.05). Interaction effects of all studied variables (fish type, Salt concentration and fermentation time) on the changes of microbial load of Mahyaveh sauce were not significant ($P > 0.05$).

Regression model of microbial population regression of Mahyaveh sauce. The multi- sentence

Table 4. Regression model of total microbial count, *micrococcus*, *enterobacteriaceae* and *bacillus* of Mahyaveh sauce

Source	Model	R ²	R ² -adj
Microbial total count (log cfu ml ⁻¹)	Y= 2.17545 +0.05206 a -1.08933 b -0.44351 c +0.23340 a ² +0.94272 b ² -0.17562 c ² -0.03533 ab -0.07211 ac+0.50071 bc	95.30	86.84
<i>Micrococcus</i> count (log cfu ml ⁻¹)	Y= 1.46007+0.07778 a -1.47699 b -0.82967 c +0.31398 a ² +1.35534 b ² -0.12463 c ² +0.10478 ab -0.02179 ac+0.38228 bc	93.94	83.02
<i>Enterobacteriaceae</i> count (log cfu ml ⁻¹)	Y= 1.00000+ 0.06369 a -0.68607 b -0.72601 c -0.09918 a ² +0.73581 b ² -0.73825 c ² - 0.03458 ab -0.01144 ac+ 0.22491 bc	96.78	91.00
<i>Bacillus</i> count (log cfu ml ⁻¹)	Y= 1.35973+0.04019 a -1.32150 b -0.72568 c +0.26773 a ² +1.22116 b ² -0.04794 c ² +0.04758 ab +0.02254 ac+0.28379 bc	94.03	83.28

a: type of fish, b: salt concentration, c: fermentation time

model of microbial population regression of Mahyaveh sauce is shown in Table 4. The coefficient of explanation of the regression model of total count (R²) was 95.30% and its modified coefficient of explanation (R²-adj) was 86.84%. The coefficient of explanation of the regression model of *Micrococcus* (R²) was 93.94% and the modified coefficient of explanation (R²-adj) was 83.02%. The coefficient explanation of the regression model of *Enterobacteriaceae* (R²) was 96.78% and its modified coefficient of explanation (R²-adj) was 91.00% and the coefficient explanation of the regression model of *Bacillus* (R²-adj). (R²), 94.33% and its modified coefficient of explanation (R²-adj) was 83.28%. These results indicated a good fit of the model related to the experimental data.

Single optimization of total microbial count, *Micrococcus*, *Enterobacteriaceae*, and *Bacillus* of Mahyaveh Sauce. Fig. 1, shows single optimization conditions for the total count, *Micrococcus*, *Enterobacteriaceae*, and *Bacillus* of the Mahyaveh sauce.

Fig. 1a shows the individual optimization conditions for the total count of the Mahyaveh sauce. According to this Figure, it is predicted that the minimum total count of Mahyaveh sauce (1.4644 Log cfu ml⁻¹) with 100% desirability, belongs to the time of 120 days, type of fish 2 (Anchovy) and salt concentration of 28.1313%. Optimal conditions for reducing production of total count of microbes was practically applied in the laboratory and the amount of 1.445 Log cfu ml⁻¹ was achieved, which there was no significant difference between the predicted and practical total count.

Fig. 1b shows the conditions of single optimization for *Micrococcus* count of Mahyaveh sauce. According to this figure, it is predicted that the minimum

Micrococcus counts of Mahyaveh sauce (0.2849 Log cfu ml⁻¹) with 100% desirability belongs to 120 days, fish type 2 (Anchovy) and salt concentration of 29.1414%. Optimal conditions for reducing the production of the amount of *micrococcus* counts was practically applied in the laboratory and its amount was 0.2889 Log cfu ml⁻¹, which there was no significant difference between the predicted and practical *Micrococcus* count.

Fig. 1c shows the single optimization conditions for the preparation of *Enterobacteriaceae*. According to this Figure, it is predicted that the minimum amount of *Enterobacteriaceae* count from Mahyaveh sauce (0.5341 Log cfu ml⁻¹) with 100% desirability belongs to the time of 93.6364 days, fish type 1 (tuna) and salt concentration of 29.3434%. Optimal conditions for reducing the production of the amount of *Enterobacteriaceae* count were practically applied in the laboratory and its amount was 0.5489 Log cfu ml⁻¹, which there was no significant difference between the predicted and practical *Enterobacteriaceae* count.

Fig. 1d shows the single optimization conditions for *Bacillus* count. According to this Figure, it is predicted the minimum amount of *Bacillus* count from Mahyaveh sauce (0.3658 Log cfu ml⁻¹) with 100% desirability belongs to the time of 120 days, fish type 2 (Anchovy) and salt concentration of 29.3434%. Optimal conditions for reducing production of the amount of *Bacillus* count were practically applied in the laboratory and the amount was 0.3975 Log cfu ml⁻¹, which there was no significant difference between the predicted and practical *Bacillus* count.

Multiple optimization, total microbial count, *Micrococcus*, *Enterobacteriaceae* and *Bacillus*

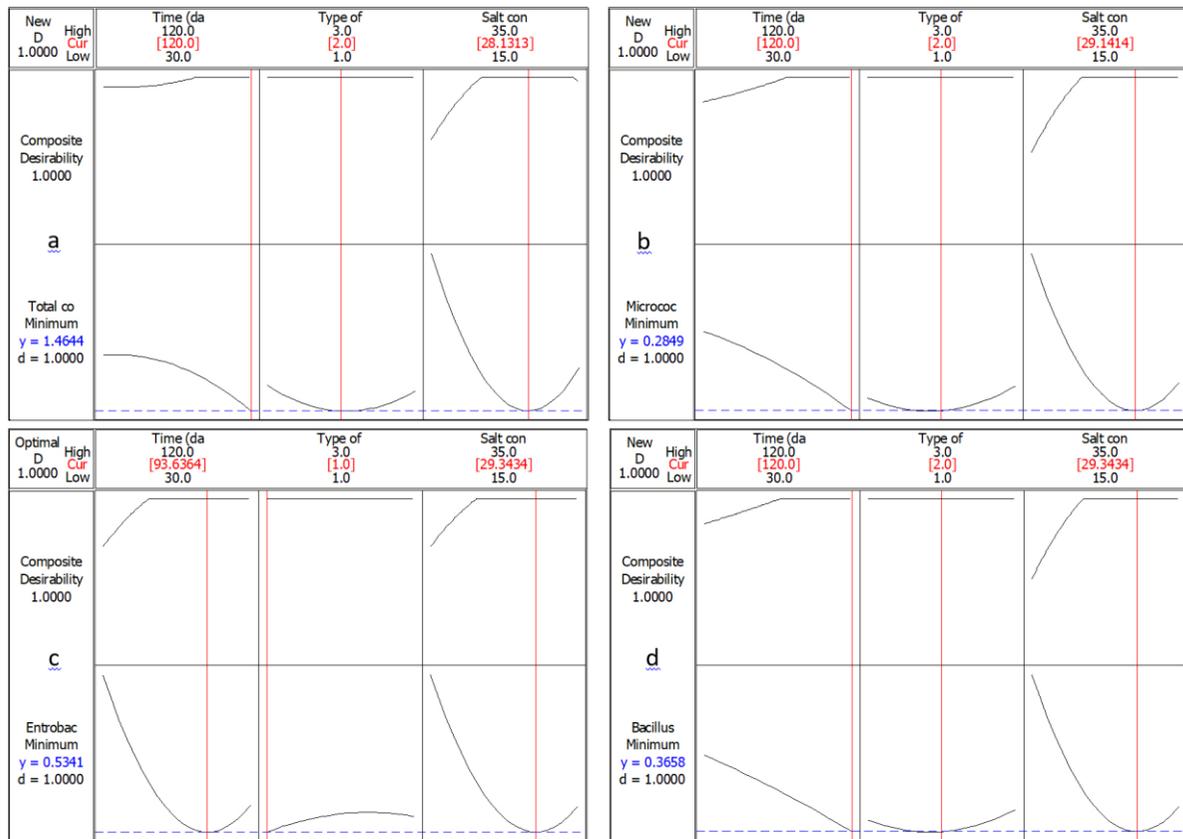


Fig. 1. Single optimization diagram for microbial properties of Mahyaveh sauce (a), total count (b), *Micrococcus* (c) *Enterobacteriaceae* (d) *Bacillus*

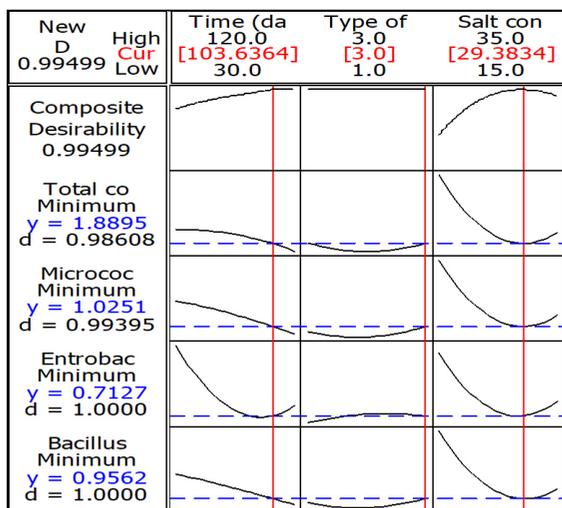


Fig. 2. Multiple optimization diagram, microbial count of Mahyaveh sauce.

sauce. Fig. 2. shows the optimal simultaneous conditions for the total microbial count, *Micrococcus*, *Enterobacteriaceae*, and *Bacillus*. As can be seen, it was predicted that the optimal conditions to achieve the minimum microbial population in Mahyaveh sauce were obtained with 99.49% desirability at the time of 103.63 days' fermentation with the third type of fish (sardine) and at a salt concentration of 29.38%. The predicted results were performed by confirmatory experiments in the laboratory and no significant difference was observed between the predicted and actual values.

DISCUSSION

Microbial examination of Mahyaveh sauce can be an indicator of product safety for consumption (5). The origin of all observed microorganisms in fish

sauce is related to the type of fish, salt, time and other additives used in fermentation. In addition, differences in the method of application of fermentation process of fish sauce (such as aerobic or anaerobic process, fermentation temperature, pH and fermentation time) are effective in changing the type of microorganism existed in fermentation products (14). Frazier and Westhoff. (1978) reported that *Micrococcus* is among the halophilic and halotolerant bacteria that can survive and grow until the end of the fermentation process in the live environment (15). Tepkasikul et al. (2022) reported that histamine degradation in fish sauce can be related to pH, temperature and salt concentration during fermentation. It is worth mentioning halophilic bacterium such as *Bacillus piscicola* FBU1786 has ability to degrading histamine and other biogenic amines (16). *Enterobacteriaceae* are absolute fermenters and also produce lactic acid (17). Zarei et al. (2012) indicated the amount of 3.41 Log cfu ml⁻¹ *Enterobacteriaceae* in the result of their studies on Mahyaveh (5).

In (2007), Anihouvi et al. examined the microbial flora of fermented fish called Cassava. They extracted 224 bacteria from it and reported that *Bacillus* and *Staphylococcus* were the dominant bacteria in the Cassava to the end of the fermentation stage (12). In 2004, Achinewhu et al. reported that *Bacillus licheniformis* and *Staphylococcus epidermis* were the dominant bacteria in fermented production of sardines (18). Lorenzo et al. (2018) reported that *Bacillus* spores are everywhere and are a major cause of contamination of many fermented foods and their products (19). Feng et al. (2021) also confirmed the predominance of Gram-positive bacteria in fish fermentation production (20). Therefore, in addition to the total microbial count, bacteria such as *Micrococcus*, *Bacilli*, and *Enterobacteriaceae* can be the predominant bacteria in fish sauces. The reason for the high total bacterial count in the early stages of fermentation could be due to the presence of natural and corrosive microorganisms in the fish. Because, in the early stages of production, salt has not yet penetrated well into the fish tissue, it has not yet had a deterrent effect on preventing the growth of microorganisms, and the total number of bacteria in the early stages of fermentation is high (5). A study conducted by Oetterer et al. (2003) on the amount of anchovy mesophilic fish that the process of fermentation was done after 30 days showed that the reduction in bacteria was due to a decrease in available

food and increased competition between bacteria specifically for halophilic bacteria the total number of live bacteria decreased during fish sauce fermentation (21). The effect of heat process, percentage of salt consumption, fermentation time and use of enzymes and edible microbes can play a significant role in the preparation time, taste, color and acceleration in fermentation of fish sauce (22). Sim et al. (2015) in a research on Malaysian fish sauce fermentation observed that after 4 months the growth of *Enterobacteriaceae* bacteria was continuously reduced (4). Regarding the reason for the decrease in microbial load by adding the amount of salt, it should be noted that Mahyaveh has a high concentration of salt, so it has a great effect on reducing the microbial count and the amount of Mahyaveh fermentation and thus increases the quality and safety of the product (5). Lopetcharat and Park, (2002) believed that the main reason of severe decline in aerobic and proteolytic bacteria in fish sauce is high concentration of salt and the resulting osmotic pressure (6).

CONCLUSION

This research was conducted with the aim to investigate the type of fish, salt concentration and fermentation time on the bacterial population of Iranian fish sauce (Mahyaveh). In this regard, the effect of fish type (tuna, anchovies and sardines), salt concentration (15, 25 and 35%) and fermentation time (30, 75 and 120 days) on total count of *Micrococcus*, *Enterobacteriaceae* and *Bacillus* were investigated. 15 treatments were designed according to the Box-Behnken Response Surface Methodology. The results showed that with increasing fermentation time and salt concentration, total count of *Micrococcus*, *Enterobacteriaceae* and *Bacillus* were significantly reduced. The type of fish had no significant effect on the microbial load of the tested treatments. Multiple optimal conditions to achieve the minimum total microbial, *Micrococcus*, *Enterobacteriaceae* and *Bacillus* count were obtained in Mahyaveh sauce, with 99.49% desirability at the 103.63 days of fermentation time, the third type of fish (sardine) and in salt concentration of 29.383%. The predicted results were performed by confirmatory experiments in the laboratory and no significant difference was observed between the predicted and actual values. By optimizing the production conditions of Mahy-

aveh sauce, it is possible to produce Mahyaveh sauce in a triumphant manner with the least amount of microbial load and desirable quality properties.

REFERENCES

- Dimidi E, Cox SR, Rossi M, Whelan K. Fermented foods: definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. *Nutrients* 2019; 11: 1806.
- Kilinc B, Cakli S, Tolasa S, Dincer T. Chemical, microbiological and sensory changes associated with fish sauce processing. *Eur Food Res Technol* 2006; 222: 604-613.
- Nazari M, Mooraki N, Sedaghati M. Chemical and microbial properties of a fermented fish sauce in the presence of *Lactobacillus plantarum* and *Paenibacillus polymyxa*. *Iran J Fish Sci* 2021; 20: 663-677.
- Sim KY, Chye FK, Anton A. Chemical composition and microbial dynamics of Budu fermentation, a traditional Malaysian fish sauce. *Acta Aliment* 2015; 44: 185-194.
- Zarei M, Najafzadeh H, Eskandari MH, Pashmforoush M, Enayati A, Gharibi D, et al. Chemical and microbial properties of Mahyaveh, a traditional Iranian fish sauce. *Food Control* 2012; 23: 511-514.
- Lopetcharat K, Park JW. Characteristics of fish sauce made from pacific whiting and surimi by-products during fermentation stage. *J Food Sci* 2002; 67: 511-516.
- Purnomo H, Suprayitno E. Physiochemical characteristics, sensory acceptability and microbial quality of Wadi Betok a traditional fermented fish from south Kalimantan, Indonesian. *Int Food Res J* 2013; 20: 933-939.
- Jiang JJ, Zeng QX, Zhu ZW, Zhang LY. Chemical and sensory changes associated Yu-lu fermentation process, a traditional Chinese fish sauce. *Food Chem* 2007; 104: 1629-1634.
- Thapa N, Pal J, Tamang JP. Microbial diversity in Ngari, Hentak and Tungtap, fermented fish products of North-East India. *World J Microbiol Biotechnol* 2004; 20:599-607.
- Md Zoqratt MZH, Gan HM. The inconsistent microbiota of *Budu*, the Malaysian fermented anchovy sauce, revealed through 16S amplicon sequencing. *Peer J* 2021; 9: e 12345.
- Benson HJ (2001). Microbiological applications: A laboratory manual in general microbiology. 8th ed . McGraw-Hill Science/Engineering/Math. New York. USA.
- Anihouvi VB, Sakyi-Dawson E, Ayernor GS, Hounhouigan JD, et al. Microbiological changes in naturally fermented cassava fish (*Pseudotolithus* sp) for lanhouin production. *Int J Food Microbiol* 2007; 116: 287-291.
- Kilinc B, Cakli S, Tolasa S. Chemical, microbiological and sensory changes associated with fish sauce processing. *Eur Food Res Technol* 2006; 222: 604-613.
- Du F, Zhang X, Gu H, Song J, Gao X. Dynamic changes in the bacterial community during the fermentation of traditional Chinese fish sauce (TCFS) and their correlation with TCFS quality. *Microorganisms* 2019; 7: 371.
- Frazier WC, Westhoff DC (1988). Food Microbiology. 4th ed. McGraw-Hill., New York, USA.
- Tepkasikul P, Santiyanont P, Booncharoen A, Abhisingha M, Mhuantong W, Chantarasakha K, et al. The functional starter and its genomic insight for histamine degradation in fish sauce. *Food Microbiol* 2022;104:103988.
- Zhao SH, Yang F, Wang Y, Fan X, Feng CH, Wang Y. Dynamics of fermentation parameters and bacterial community in high-moisture alfalfa silage with or without lactic acid bacteria. *Microorganisms* 2021; 9: 1225.
- Achinewhu SC, Amadi EN, Barimalaa IS, Eke J. Microbiology of naturally fermented fish (*Sardinella* sp). *J Aquat Food Prod Technol* 2004; 13: 47-53.
- Lorenzo JM, Munekata PE, Dominguez R, Pateiro M, Saraiva JA, Daniel F. Chapter-main groups of microorganisms of relevance for food safety and stability: general aspects and overall description. *Innov Technol Food Preserv* 2018; 53-107.
- Feng L, Tang N, Liu R, Gong M, Wang Z, Guo Y, et al. The relationship between flavor formation, lipid metabolism, and microorganisms in fermented fish products. *Food Funct* 2021; 12: 5685-5702.
- Oetterer M, Perujo SD, Gallo CR, de Arruda LF, Borghesi R, da Cruzm AMP. Monitoring the sardine (*Sardinella brasiliensis*) fermentation process to obtain anchovies. *Sci Agric* 2003; 60: 511-517.
- Yongsawatdigul J, Rodtong S, Raksakulthai N. Acceleration of Thai fish sauce fermentation using proteinases and bacterial starter cultures. *J Food Sci* 2007; 72: M382-390.