Comparison and Study of Physicochemical, Nitrogen, Microbial, Antioxidant, Bioactive, and Sensory Properties of Mahyaveh Prepared in Optimal Conditions with Traditional Mahyaveh

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ABSTRACT: *Mahyaveh is the name of a traditional Iranian fish sauce made from the fermentation of small and low-consumption fish, anchovies, and sardines, and contains essential amino acids, vitamins, salts, metal ions, and desirable fatty acids such as Docosahexaenoic acid and Eicosapentaenoic acid. Production conditions and fermentation time can be effective in improving nutritional value and improving its health capabilities. The general purpose of this study was to compare and evaluate the physicochemical, nitrogen, microbial, antioxidant, bioactive, and sensory peptides of Mahyaveh prepared in optimal conditions with traditional Mahyaveh. The results showed that the amount of fat, dry matter, ash, fiber, and carbohydrates of Mahyaveh prepared under optimal conditions was significantly (p≤0.05) higher than traditional Mahyaveh. The amount of moisture and protein in traditional Mahyaveh was significantly (p≤0.05) higher than Mahyaveh prepared in optimal conditions. The amount of total nitrogen, formalin nitrogen, amino nitrogen, proteolysis of essential amino acids, total phenol, and sensory evaluation score in Mahyaveh prepared under optimal conditions was significantly (p≤0.05) higher than the traditional Mahyaveh sample. The amount of trimethylamine, volatile nitrogen, IC50, and microbial load of Mahyaveh prepared under optimal conditions was significantly (p≤0.05) lower than traditional Mahyaveh. The results of this study showed that by optimizing the production conditions of Mahyaveh, fish sauces with higher nutritional value, greater safety, and more desirable sensory properties can be produced than traditional Mahyaveh.*

KEYWORDS: *Mahyaveh; Bioactive peptides; Antioxidant properties; Microbial properties; Sensory evaluation.*

INTRODUCTION

Preparing fish sauce is one of the oldest methods of preserving and processing fish [40]. 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 [18]. In Iran, a kind of local fish sauce is produced which is called Mahyaveh, Maveh, Mahveh, or Suragh. Mahyaveh is traditionally produced by the natives in the southern provinces of Iran, including the cities of Fars and Hormozgan, and is generally made from sardines or

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(Sordinella sp) Hashina in the local language, and anchovies or Indian moto, salt, mustard (Brassica juncea), and water [59]. According to people in the southern region, eating Mahyaveh, which also contains mustard, prevents Pace skin disease [51]. The quality of fish sauce depends on factors such as fish species, type of salt, fish-to-salt ratio, additives used, and fermentation conditions [29]. 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 [42]. Due to the high concentration of salt in fermented products, the growth of disease microorganisms is controlled and the fish sauce tastes and smells good. However, salt-loving microorganisms can also grow in fish sauce [21]. 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 [45].

Total nitrogen in fish sauce is a combination of protein and non-protein nitrogen compounds. According to the Thai fish sauce standard, the formaldehyde nitrogen amount should be at least 40% of the total nitrogen amount [11]. Ammonia nitrogen, or volatile nitrogen compounds, which are part of the non-protein nitrogen compounds in fish sauce, are the result of breaking down liquid proteins and peptides into free amino acids and volatile nitrogen [4].

Hydrolyzed proteins are low molecular weight compounds known as bioactive peptides. Bioactive peptides are fragments of proteins with specific amino acid sequences that have no biological effects when present in the parent protein. These peptides exhibit various biological effects when released from the parent chain by hydrolysis or fermentation [31, 26]. These compounds are easily absorbed after entering the body and play an important biological role at the cellular level [55]. Different peptides with different amino acid sizes and sequences may exhibit different antioxidant activities [35]. Hydrolyzed proteins contain peptides that are electron donors and can react with free radicals to convert them into more stable compounds, resulting in the inhibition of the oxidation chain [27]. The function of peptides as antioxidants is not entirely clear, however, the presence of amino acids such as histidine, cysteine, valine, proline, phenylalanine, tyrosine, and tryptophan plays an important role in increasing the antioxidant activity of peptides and hydrolyzed fish protein [17]. Therefore, fish sauce is not just a flavoring and contains 0.6 to 1.2 percent nitrogen and has a good amount of essential amino acids in the body, and is used as a substitute for salt and flavoring in homes and food industries. However, the researchers' approach to this product has changed and in addition to creating a taste, this product is used as a carrier to transfer essential amino acids, salts, and metal ions (such as iodine, iron, and zinc) into the body [53]. *Fukami et al.*, (2002) [15] examined the aromatic substances in fish sauce and stated that this product has a different flavor with the unpleasant smell of fish and its smell and taste are special for fish sauce.

Despite the popularity of this product in the southern and coastal regions of Iran, there is no industrial factory to produce this product, and this product is traditionally prepared and there is no standard for it [60, 51]. In the food industry, the acceptance of a product by consumers guarantees the production of that product and its continued presence in the consuming market. Therefore, evaluation of physicochemical, nitrogen, microbial properties, sensory evaluation, antioxidant compounds (phenyl 2, 2-diphenyl-1-picrylhydrazyl), bioactive peptides (measurement of total amino acid content, determination of protein profile) in the production of Mahyaveh with higher valuable nutrients, greater safety, and more desirable sensory properties play a key role, and there is limited information about the final overall properties of Mahyaveh and quality of these compounds. Also, due to the increasing consumption of seafood, this fermented product is important to study the factors affecting the quality of Mahyaveh sauce. The general purpose of this study was to compare and evaluate the physicochemical, nitrogen, microbial, antioxidant, bioactive, and sensory peptides of Mahyaveh prepared in optimal conditions with traditional Mahyaveh.

EXPERIMENTAL SECTION

Materials

To prepare the optimal Mahyaveh sauce, sardine was prepared in April 2020 by (Bandar Fish Company in Bandar Abbas, Iran) and was frozen at -18 °C and transferred to the Agricultural Laboratory of the University of Tehran. Table salt was purchased from Shimiaz Company, Iran, and the Mustard powder was purchased from G.S. Dunn Company, Canada. Traditional Mahyaveh was purchased from traditional manufacturers in the south

of Iran. The chemicals required in this study include silver nitrate, phenolphthalein reagent, sodium, sodium hydroxide, potassium chromate reagent, formaldehyde solution, hydrochloric acid, boric acid, magnesium oxide, methyl red reagent, picric acid, sodium sulfate, potassium hydroxide, Trichloroacetic acid, Ortho -phthalic aldehyde, Sodium dihydrogen phosphate, Acetonitrile, Folin-Ciocalteu reagent, Sodium bicarbonate, Gel Polyacrylamide, Comaxi Blue, were purchased from Merck company of Germany. Culture medium includes: Plate Count Agar, Dextrose Tryptone Agar, Mannitol Salt Agar, Violet Red Bile Agar, and Yeast Extract Agar were purchased from Quelab, Canada. Ascorbic acid, methanol, acetic acid, and, gallic acid were prepared from the (Mojalal, Iran) company, and a solution of 0.2 M DPPH, was produced by Sigma Company, Sigma, USA.

Preparing fish sauce Mahyaveh produced in optimal conditions and traditional

According to the results of *Kavian et al*., (2020) [23] the optimal conditions for the production of Mahyaveh with the lowest microbial load and the highest amount of nitrogen compounds, and the highest sensory score of sardines were 29% table salt concentration and 120 days of fermentation time. Therefore, to produce the optimal Mahyaveh, sardines fish along with viscera were washed with water and crashed to a size of 6 cm. Whole fish (including viscera) was mixed with water at a ratio of 1/1 and table salt with 29% concentration was added to the sample and then fish, water, and table salt were mixed in a blender (Ika model, Germany). They were transferred to wide-mouthed pottery with a capacity of 700 mL and the containers were closed with three-layer plastic films. These containers were kept in a greenhouse for 120 days at 37°C. After the fermentation time, the sample was smoothed with a sterile cleansing cloth. Then, the filtered extract was mixed with 10% mustard and kept at room temperature for 10 to 15 days. Then, the experiments were performed on the produced Mahyaveh in optimal conditions and compared to traditional Mahyaveh.

The traditional production method of Mahyaveh based on the manufacturer's description was as follows:

First, the heads of the sardine fish are cut. Then, the fish are washed along with viscus and poured into pottery or wide-mouthed glass containers with salt and warm water, and the containers containing a mixture of fish, water, and salt (20%) are exposed to the environment temperature and preferably to sunlight for 30 days. Then the mixture of salt and fish and squeezed and crushed and passed through filters made of stainless steel with large holes, and finally the created brown liquid is mixed with mustard and other spices.

The image of optimal and traditional Mahyaveh is shown in Fig. 1.

Physicochemical properties of Mahyaveh produced in optimal conditions and Traditional

The protein content was measured according to the AOAC 984.13 method by the Micro Kjeldahl device during three stages of digestion, distillation, and titration [2]. The fat content was measured according to AOAC 963.13 method by the Soxhlet device [2]. The humidity test was measured according to AOAC 925. 45B method [2]. The ash test was measured according to AOAC 920.153 method [2]. The Crude fiber measurement test was measured according to AOAC 991.43 method [1]. Total carbohydrate was determined by subtracting the sum of the ash, fat, fiber, humidity, and protein contents from 100 [39].

Tests of Mahyaveh nitrogen properties produced under optimal conditions and traditionally

Total nitrogen

The total nitrogen was measured according to AOAC940. 25 method by Micro Kjeldahl device during three stages of digestion, distillation, and titration and was calculated based on g/L in the sample [1].

Total formalin nitrogen

The formalin nitrogen content of fish sauce samples was measured as an indicator of protein hydrolysis according to the international standard of Thai fish sauce. For this purpose, a pH meter electrode was placed in a beaker containing 10 mL of fish sauce diluted 10 times. In the next step, the titration of the sample was applied with a 0.1 M solution of sodium hydroxide to a pH of 7. Then 10 mL of formaldehyde solution 38% was added to the sample and titration was continued until the pH was 9. Finally, formalin nitrogen was calculated according to Eq. (1) based on g/L in the sample [11].

$$\text{Formalin nitrogen (g/L)} = \text{(of sodium hydroxide at pH equal to -7 mL of sodium hydroxide at pH equal to 9)} \times 14 \times 0.1 \quad (1)$$

Traditional Mahyaveh

Optimal Mahyaveh

Fig 1: The image of optimal and traditional Mahyaveh.

Volatile nitrogen

Volatile nitrogen compounds were measured by direct distillation in boric acid by a Kjeldahl device. In this way, 10 g of fish sauce sample along with 3 g of magnesium oxide and 100 mL of distilled water were poured into the distillation flask of the Kjeldahl device and in 10 mL/min the vapors of compounds of volatile nitrogen were collected at a rate of 10 minutes distillation in Erlenmeyer containing 100 mL of 4% boric acid with a few drops of methyl red reagent. Finally, the contents of Erlenmeyer with 0.1 normal hydrochloric acids until the reappearance of the pink color of the methyl red reagent was titrated and the number of volatile nitrogen compounds in the samples was calculated with Equation 2 base on g/l in the sample [7].

Volatile nitrogen compounds (g/L) = (2)
Volume of acid consumption × the normality of acid × 14 × 10

Amino nitrogen

To measure the amino nitrogen content of the samples, the international standard method of Thailand in 1983 was used. Amino nitrogen in terms of formalin nitrogen and ammonia nitrogen (volatile nitrogen compounds) was calculated by Equation 3 [11].

Amino nitrogen = (formalin nitrogen - (3)
Volatile nitrogen compounds)

Trimethylamine

Trimethylamine was measured based on AOAC (2000) [1] according to the Deyr method or the same as acid picrate staining. After preparing fish extract in 75% (w/w) aqueous solution of trichloroacetic acid, 1 mL of the extract was transferred to a glass tube and made up to 4 mL with distilled water. Then 1 mL of formaldehyde 20%, 3 mL of 25% potassium hydroxide (w/w), and 10 mL of toluene were added to the tube, and the contents of the tube were mixed continuously by hand 40-60 times. Then 7-9 mL of the supernatant (toluene with trimethylamine) was poured into a dry tube containing 0.1 g of dry sodium sulfate until the water in the toluene was dry. Finally, 5 mL of dried toluene was poured into another dry tube with a lid, and add 0.5mL of Picric acid was added to the toluene and read after mixing the solution at 410 nm. Toluene was used to zero the spectrophotometer.

The amount of trimethylamine in the samples using the standard diagram of trimethylamine and the equation obtained from equation 4 based on g/100g in the sample.

Trimethylamine (g/100g) = (4)
(mg/mL sample) trimethylamine × 8 × 5

Microbial tests of Mahyaveh produced in optimal conditions and traditional Mahyaveh

For all microbial tests, first Ultra Violet lamp and then all surfaces were sterilized with alcohol. Also, all the containers used in the test were sterilized by oven and autoclave [5]. To evaluate the microbial total count of Micrococcus, Bacillus, and Enterobacteriaceae were used respectively by plate count agar-PCA, Mannitol Salt Agar (MSA), Dextrose tryptone agar, and Violet red bile agar [3]. To count the total number of bacteria according to the method (*Sedaghati et al*., 2021) [47], and pour plate method and dual culture were used. First, the necessary dilutions were taken from the samples in 0.9% salt solutions in distilled water. Then 1 mL of each dilution was poured into an empty sterile plate at any time and about 9 mL of sterile liquid culture medium at 40 to 45 °C

was poured onto the desired dilution to be spread on all surfaces and was moved in the shape of the English 8 number. Culture medium PCA plates (plate count agar) were incubated at 30 °C for 48 hours. After two days, the bacteria were counted by the colony counter.

Number of colonies per mL of sample (Cfu/mL) = counted the number of colonies × reverse of dilution factor (5)

To count the bacteria of Micrococcus, Enterobacteriaceae, and Bacillus according to the method (*Anihoulvi et al*., 2007) [3] pour plate method and dual culture were used. The first necessary dilutions were taken from the samples in 0.9% salt solutions in distilled water. Then 1 mL of each dilution was poured into an empty sterile plate and about 9 mL of sterile liquid culture medium with a temperature of 40 to 45 ° C was poured on the desired dilution to be spread on all surfaces and was moved into the shape of the English 8 number. After closing the culture medium, they were placed upside down in the incubator. MSA plates (mannitol salt agar) were incubated at 30 ° C for 48 h and some plates showed purple colonies. Enterobacteriaceae were incubated for 24 h at 37 ° C and Bacillus plates were incubated for 48 h at 35 ° C [47]. In some plates, the colors of their colonies were pink and yellow, respectively. To count the halophilic bacteria from the plate count agar (PCA) medium with 10% NaCl, the plates were kept at 37 ° C for 24 hours and then counted [25]. Finally, Potato Dextrose Agar (PDA) was used to cultivate mold and yeast, which were incubated for 5 days at 30°C. The results were expressed as colony-forming units per gram of product (log CFU g^{-1}) [10].

Antioxidant activity tests of Mahyaveh produced in optimal conditions and traditional Mahyaveh

Comparison of total phenol content of Optimal Mahyaveh and traditional Mahyaveh

The total phenol content of the total Mahyaveh was determined according to the method of *Blainski et al*., (2013) [6]. So that 900 microliters of the extract in 10% of Folin-Ciocalteu reagent were placed at room temperature for 5 minutes. A solution of 7.5% (volumetric /volumetric) of sodium bicarbonate (600 µL) was added to the mixture and incubated at 50 °C for 10 minutes and the absorption of 3 mL of the mixture was measured at 765 nm. The gallic acid in concentrations of 0.1, 0.2, 0.4, and 0.5 µg/mL was used as standard and the results were reported as a concentration equivalent to gallic acid.

Evaluation of free radical control activity of DPPH in Mahyaveh produced in optimal conditions and traditional Mahyaveh

The ability of Mahyaveh to give hydrogen or electron atom was measured by decolorizing the DPPH ethanolic solution. 2,2 - diphenyl - 1- Picrylhydrazyl is a stable radical compound with a purple color that is converted to yellow diphenyl picryl hydrazine by reduction with electron or hydrogen donor elements (antioxidant compounds). For this purpose, 5 µL of the prepared extract was mixed with 1000 µL of DPPH 0.135 mIM methanol solution. The control samples went through all the extraction steps without adding the sample. The samples were then shaken for one minute and 30 min were placed in the dark the sample's absorption was read by the spectrophotometer L 800 Aqualytic model, Germany at 517 nm, and the percentage of free radical control was calculated using Eq. (6) [56].

$$100\times \text{DPPH} = \frac{\text{Percentage of sample absorption} - \text{Percentage of control absorption}}{\text{Percentage of control absorption}}$$

For better evaluation of the antioxidant activity of the extract, IC50 factor was used, which indicates the percentage of the extract that can neutralize 50% of DPPH free radicals. The extract concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage plotted against different extract concentrations (0.1, 0.3, 0.5, 0.7, 0.9, 1.1 mg/mL).

Comparison of the total amount of amino acids of Mahyaveh produced in optimal conditions and traditional Mahyaveh

To determine the amino acids using liquid chromatography by high-efficiency reverse phase method was done by the ultraviolet-visible detector. To extract all amino acids, the Pico-Tag method was used [44]. The tested sauce was divided into four weights based on its protein percentage. The hydrolysis was then started by adding 10 mL of hydrochloric acid to the sample and stirring thoroughly, the mixture was exposed to nitrogen gas for 1 minute and then the sample was incubated for 24 hours at 110 ° C. After cooling, the sample was poured into a volumetric flask and reached a volume of 50 mL with deionized water and then filtered with Whatman No. 1 paper. To remove high molecular weight proteins and fats

from the sample, before amino acid analysis, the sample was passed through a C18 cartridge and then passed through a 0.2 μm cellulose syringe filter. Extracted amino acid samples automatically with Ortho-Phthalic-Aldehyde (OPA) (Agilent), Technologies, Germany, catalog number: 5061-3335 were derived by robotic sampling programming. The method of derivatives was under the printed method of Agilent and was noted by [36]. Then the derivatives equivalent of 0.5 μL from each sample were injected into a column ((AAA) Zorbax Eclipse - Amino Acid Analysis)), (4.6 mm × 15.5 μm), (Agilent Technologies, Waldbronn, Germany), at 40 °C with detection in λ =338 nm. Mobile phase A, Sodium dihydrogen phosphate 40 mL whose pH was set to 7.8 with sodium hydroxide, while mobile phase B included acetonitrile, methanol, and water. Isolation with a flow rate of 2 mL /min with a gradient program for 1.9 min in B continued at 0% and for 16.3 min the detergent B increased to 53%. Then the washing operation in B 100% was performed until the washing equilibrium was reached at B 0%. The total analysis time was 26 min. Amino acids were determined according to storage time and comparison with standard solutions. The curve below the diagram was calculated according to the area under the curve of amino acids for each amino acid and covered a wide range of concentrations. The concentration of each amino acid was expressed as g/100g protein.

Determination of protein molecular weight using sodium gel electrophoresis dodecyl sulfate-polyacrylamide

To determine the molecular weight of the protein, 100 mg of Mahyaveh was added to 13.5% polyacrylamide gel (w/v) and the polyacrylamide gel was placed at 100 volts for 60 minutes. To view the protein bands, the gels were stained for 30 minutes using 0.1% Coomassie Blue in 40% methanol: 10% acetic acid: 50% of H2O solution and then dried with 10% methanol: 10% acetic acid: 80% H_2O solution [9].

Sensory evaluation tests of Mahyaveh produced in optimal conditions and traditional Mahyaveh

The sensory test was performed under the method of *Moayedi* and *Mousavi Nasab* (2013) [34] and by the 5-point Hedonic method and by 10 semi-trained evaluators. The samples were transferred to the evaluation room after being placed in disposable containers and coded. The order of the samples for each evaluator was randomly placed. In the intervals between each evaluation, some water was drunk by the evaluator. These people consumed Mahyaveh sauce prepared in optimal conditions and Mahyaveh sauce prepared from the market and then judged the color, smell, taste, and general acceptance and from 1 to 5 points (excellent, very good, good, average, and Bad) rated the samples.

Data analysis method

All tests were performed on Mahyaveh produced in optimal conditions and traditional Mahyaveh conditions with three replications. The test results were analyzed using a one-way analysis of variance (Duncan) at a 95% confidence level by Minitab 16 software.

RESULTS AND DISCUSSION

The results of the physicochemical properties of optimal Mahyaveh and traditional Mahyaveh

The results of the physicochemical properties of the optimal Mahyaveh and traditional Mahyaveh were reported in Table (1). According to the results, the amount of fat (0.76%), dry matter (28.37%), ash (6.05%), fiber (1.27%), and carbohydrates (0.23%) in Mahyaveh prepared in optimal conditions was significantly ($p \leq 0.05$) higher than traditional Mahyaveh sample. The amount of moisture (71.20%) and protein (20.23%) of traditional Mahyaveh was significantly ($p \leq 0.05$) higher than optimal Mahyaveh. Variations in protein levels may be related to the amount of proteolysis in the Mahyaveh samples. The results of the physicochemical properties of the protein, fat, and moisture, of sardine fish, which were done in part one of the studies, respectively were 72.62, 10.41. 69.88.

Consistent with the results, *Oji Fard* and *Bishirzadeh* (2018) [38] stated that the amount of fat and protein in fish sauce decreased compared to the fish itself after 180 days of fermentation. *Park et al*. (2001) [40] have reported the amounts of moisture, protein, and ash produced from the whiting fish sauce of the Pacific Ocean, 61.40-79.20% and 9.0-13.70% and 18.20-25.80% respectively.

Results of nitrogen properties of Mahyaveh produced under optimal conditions and traditional Mahyaveh

The results of nitrogen properties of optimal Mahyaveh and prepared of the market are shown in Table 2. According to the results, the amount of total nitrogen (33.56 g/L), formalin nitrogen (18.30 g/L), amino nitrogen (17.01 g/L), Mahyaveh prepared under optimal conditions

Table 1: Comparison of physicochemical properties of Mahyaveh prepared under optimal conditions and traditional Mahyaveh.

Sample	Protein (%)	Fat (%)	Moisture (%)	Dry matter (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
Optimal Mahyaveh	19. 373 ± 0.184 [b]	0. 760 ± 0.055[a]	71. 203 ± 0.354 [b]	28. 373 ±0.330[a]	6. 053 ± 0.157[a]	1. 270± 0.065[a]	0. 023 ± 0.005[a]
traditional Mahyaveh	20. 233 ± 0.106[a]	0. 650 ± 0.036[b]	73. 430 ± 0.101[a]	26.603 ± 0.060[b]	5. 226 ± 0.095[b]	1. 126± 0.015[b]	0. 014 ± 0.004[b]

The results are shown as the mean ± standard deviation
Different small letters indicate a significant difference in each column

were significantly higher than ($p \leq 0.05$) the traditional sample. Also, the amount of trimethylamine (14.78 g/100g) and volatile nitrogen (0.19 g/L) Mahyaveh prepared under optimal conditions were significantly ($p \leq 0.05$) lower than traditional Mahyaveh.

According to the Thai national standard, the total nitrogen content of fish sauce is divided into three degrees, a first degree of more than 20 grams per liter, the second degree between 15 to 20 grams per liter, and third-degree less than 15 grams per liter[11]. The amount of total nitrogen in Mahyaveh sauce produced in optimal conditions was (33.56 g/L) and Mahyaveh prepared in the market was (23.36 g/L), and both were classified as first-grade fish sauce and the amount of formalin nitrogen and amino nitrogen of both fish sauces in this study was at least 40% of the total nitrogen content, which according to the Thai fish sauce standard should be at least 40% of the total nitrogen content [11]. The amount of volatile nitrogen in both fish sauces was found to be within the acceptable range, which according to the standard acceptable limit for volatile nitrogen ranged from 0.141 to 3,389 mg/g, which is found in most East Asian fish sauce products [8]. *Saisithi et al*. (2006) [46] Stated that Thai commercial sauces contain trimethylamine and can be separated by diethyl ether solution. *Tsai et al.,* (2006) [54] Reported that the amount of trimethylamine in Thai commercial fish sauce is about 269 mg per 100 g, which has a significant difference from the amount of trimethylamine obtained with the optimal Mahyaveh which its amount was reported as 14.78 and even the Mahyaveh market which was 23.80 mg100 g^{-1}. In confirmation of the results of this study, *Moayedi* and *Mousavi Nasab*, (2013) [34] examined the changes in nitrogen compounds during the fermentation process of Mahyaveh for 54 days at 37 °C during six stages of fermentation. The results showed that with increasing fermentation time, the amount of total nitrogen, formaldehyde nitrogen, amino acid nitrogen, and volatile nitrogen increased in all stages. *Shakib* and *Mousavi Nasab* (2013) [50] examined the preparation of dried rainbow sardine sauce (Dussumieria acuta) on four studied treatments that studied the effect of the mechanical process of grinding dried fish, adding salt at two levels of 80 and 100% by weight of dried fish and also adding citric acid at the level of 2%. The results showed that the amount of total nitrogen increased with fermentation time. And the amount of trimethylamine in all treatments was significantly reduced during the fermentation process. An increase in total nitrogen indicates an increase in protein hydrolysis and an increase in the nutritional value of fish sauce and a decrease in trimethylamine indicates a decrease in spoilage bacteria during the fermentation process of fish sauce. Kavian *et al.* (2022) [24], Evaluated 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 nitrogen amount, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine investigated. 15 treatments were designed according to the Box-Behnken Response Surface Methodology. The results showed that increasing fermentation time (30 to 120 days) and decreasing salt concentration (35 to 15%), total nitrogen amount, formaldehyde nitrogen, volatile nitrogen, and amino nitrogen are significantly increased, and increasing fermentation time (30 to 120 days) and increasing salt concentration (15 to 35%), trimethylamine decreased and the type of fish has no significant effect on the nitrogen properties of the tested treatments.

Results of microbial properties of Mahyaveh produced under optimal conditions and traditional Mahyaveh

The results of microbial properties of Mahyaveh produced under optimal conditions and traditional are shown in Table (3). According to the results of the total count (1.8 Log $cfuml^{-1}$), micrococcus (0.96 Log $cfuml^{-1}$), Enterobacteriaceae (0.69 Log $cfuml^{-1}$), bacillus (0.98 Log $cfuml^{-1}$),

Table 2: Nitrogen properties of Mahyaveh prepared under optimal conditions and traditional Mahyaveh.

Sample	Total nitrogen g/L	Formalin nitrogen g/L	Amino nitrogen g/L	Trimethylamine $g100g^{-1}$	Ventilate nitrogen g/L
Optimal sample	33. 565 ±0.066 [a]	18. 307 ±0.061[a]	17. 011 ±0.165[a]	14. 781± 0.050[b]	0. 191 ± 0.002[b]
Traditional sample	23. 361 ± 0.264[b]	12. 196 ± 0.029[b]	12. 381 ± 0. 236[b]	23. 801 ± 0.165 [a]	0. 857 ± 0.023[a]

The results are shown as the mean ± standard deviation.
Different small letters indicate a significant difference in each column

Table 3: Microbial properties of Mahyaveh prepared under optimal conditions and traditional Mahyaveh.

Sample	Total count Log $cfuml^{-1}$	Micrococcus Log $cfuml^{-1}$	Enterobacteriaceae Log $cfuml^{-1}$	Bacillus Log $cfuml^{-1}$	fungi and yeast Log $cfuml^{-1}$	Halophilic bacteria Log $cfuml^{-1}$
Optimal sample	1. 800 ± 0.028[b]	0. 966 ±0.021[b]	0. 693 ± 0.010[b]	0. 985 ± 0.005[b]	0. 365± 0.021 [b]	0. 400 ± 0.042[b]
Traditional sample	3. 863 ± 0.017 [a]	3. 741 ± 0.014 [a]	1. 975 ± 0.009[a]	4. 686 ± 0.078[a]	0. 605 ± 0.035[a]	0. 695 ± 0.021[a]

The results are shown as the mean ± standard deviation
Different small letters indicate a significant difference in each column.

fungi and yeast (0. 36 Log $cfuml^{-1}$) and halophilic bacteria (0.4 Log $cfuml^{-1}$) Mahyaveh prepared in optimal conditions were significantly ($p\leq0.05$) lower than traditional Mahyaveh sample. One of the key factors limiting the use of fish is microbial and autolytic spoilage during processing and storage [50]. The origin of all the microorganisms observed in fish sauce is related to the type of fish, salt, time, and other additives used in fermentation. Besides, differences in the method of fermentation of fish sauce (such as aerobic or anaerobic process, fermentation temperature, pH, and fermentation time) are effective in changing the type of microorganisms in fermented products [57]. In preparing the sauce, the salt not only acts as a preservative but also reduces the water activity and moisture content of the final product, which is unsuitable for microbial growth [43], *Taheri et al*. (2014) [50] reported that 70% of the total population of fish sauce bacteria was related to micrococcus and staphylococcus. *Mooraki* and *Sedaghati* (2019) [33] stated that the high increase in histamine in Mahyaveh fish sauce is related to the high level of decarboxylase activity of halophilic, micrococcus, and Enterobacteriaceae. *Lorenza et al*., (2018) [30] reported that bacillus spores are everywhere and are a major cause of contamination of the raw materials of many fermented foods and their products. *Faisal et al.* (2013) [13] reported that the total number of aerobic bacteria in fish sauce decreased from 5.99×10^6 Log $cfuml^{-1}$ to 1.53×10^4 Log $cfuml^{-1}$ through 9 months. They reported the identified predominant bacteria in the fish sauce including micrococcus, bacillus, lactobacillus, and pseudomonas. *Gassem*, (2019) [16] also confirmed the predominance of gram-positive bacteria in the fermented fish product. *Kakati* and *Goswami*, (2013) [22] also observed a small amount of yeast and fungi (1.2-1.7 Log $cfuml^{-1}$) in their traditional fermented fish samples. Therefore, microbial examination of Mahyaveh sauce can be an indicator of product safety for consumption [51].

Results of antioxidant and phenol properties of Mahyaveh produced under optimal conditions and traditional Mahyaveh

The results of antioxidant and phenol properties of Mahyaveh prepared in optimal conditions and traditional are shown in Table (5). According to the results of phenol content (1.18 mg/mL), Mahyaveh prepared under optimal conditions was significantly ($p\leq0.05$) higher than the traditional Mahyaveh sample. The Dpph antioxidant value (Ic50=12.17 mg/mL) of Mahyaveh prepared in optimal conditions was significantly ($p\leq0.05$) lower than the traditional sample. Therefore, the antioxidant compounds in Mahyaveh sauce, which are phenolic and flavonoid compounds, were higher in optimal Mahyaveh due to more proteolysis than traditional Mahyaveh, which was due to the more bioactive peptide compounds in optimal Mahyaveh than the traditional sample.

Takano et al., (2012) [52] reported phenolic compounds in the fish sauce are Guaiacol, 3-Ethyl-phenol, and 4-Ethyl-2-methoxy-phenol. *Choksawangkarn et al.* (2018) [9] reported that the phenolic compounds in a fish sauce are derived from tyrosine residues in proteins/peptides or free tyrosine. Tyrosine can have strong antioxidant properties due to its phenol side chain (*Matsui et al.*, 2018).

Table 4: Antioxidant properties of Mahyaveh prepared in optimal conditions and traditional Mahyaveh.

Sample	Phenol (gallic acid) ($MgmL^{-1}$)	Antioxidant IC 50 ($MgmL^{-1}$)
Optimal sample	1. 184 ± 0.105 [a]	12. 176 ± 0.047[b]
Traditional sample	0. 347 ± 0.005 [b]	16. 070 ± 0.071[a]

The results are shown as the mean ± standard deviation
Different small letters indicate a significant difference in each column

Esmaeili Kharyeki et al. (2018) [12] Found that Skipjack fish has the highest catch among tuna worldwide. Its head contains about 65% protein. Many hydrolyzed proteins and various peptides from marine sources have high antioxidant power. Therefore, in this study, the antioxidant activity of a protein-hydrolyzed mixture with DPPH radical inhibition activity was measured at 15, 60, 120, and 240 days. The results showed that DPPH radical inhibition activity increased with increasing hydrolysis time and there was a significant difference in all samples obtained from different times ($p< 0.05$) and the highest rate of radical inhibition of DPPH was obtained at a concentration of 2 $mgmL^{-1}$ in 4 hours and more than 70%. Finally, they stated that the protein hydrolysis in Skipjack tuna's head has high antioxidant activity and can be used in food to increase oxidative stability. *Je et al*., (2009) [20] Investigated the activity of DPPH radical inhibition in the hydrolyzed protein of tuna spine at different concentrations and expressed the highest inhibitory rate (65%) was a concentration of 1.6 mg/mL.

Results of sensory properties of Mahyaveh produced in optimal conditions and traditional Mahyaveh

The results of the sensory properties of Mahyaveh prepared under optimal conditions and traditional Mahyaveh are shown in Table 4. Mahyaveh samples prepared under optimal conditions and traditional samples did not significantly differ from each other in terms of sensory evaluation (color, taste, general acceptance). The optimal Mahyaveh sample was slightly higher in taste and overall acceptance than the traditional sample, which is considered due to lower microbial load, lower levels of undesirable nitrogen compounds that produce bad smell compounds, and also due to the presence of more amino acids such as glutamic acid, Glycine, lysine, and alanine in optimal Mahyaveh because these amino acids play an important role in the taste of the sauce. In confirmation of the results of this study, *Moayedi* and *Mousavi Nasab*, (2013) [34] studied the evaluation of sensory factors such as color, smell, taste, and overall acceptance without adding spices to the fish sauce for 54 days in a greenhouse at 37 °C, in six stages of fermentation. The results showed that the fermentation process caused a significant increase in the score related to the taste of fish sauce during the fermentation process, which is related to the increase of flavored compounds resulting from the fermentation process. The lowest score was related to the smell in sensory scores, which was related to not using different spices such as mustard, thyme, nutmeg, turmeric, etc. Because adding spices at the end of fermentation creates a special aroma and reduces the unpleasant smell of fish sauce. Increasing the fermentation time improved the color of the product. Therefore, the first and second stages of fish sauce had the lowest score and the fifth and sixth stages had the highest score in terms of overall acceptance.

Quantification of amino acids in optimal Mahyaveh and traditional Mahyaveh by high-performance liquid chromatography

Table 6 shows the index of inhibition and the number of amino acids available in the traditional and optimal Mahyaveh. According to Table 6, the results showed that the amount of optimal Mahyaveh amino acids was significantly higher than traditional Mahyaveh amino acids ($p\leq0.05$). Both fish sauces contained more essential amino acids than non-essential amino acids. All essential and non-essential amino acids in the optimal Mahyaveh the sauce were higher than in the traditional Mahyaveh sauce. Also, the total amino acid prepared under optimal conditions was almost twice as much as the amino acid prepared traditionally. Optimal Mahyaveh sauce also contains high amounts of glutamic acid, glycine, lysine, and alanine. Therefore, it can be concluded that the amino acid compound of Mahyaveh sauce prepared under optimal conditions was more than the amino acids of traditional Mahyaveh sauce. To confirm the results, *Yazdanpanah* and *Mahasti* (2011) [59] produced sardine sauce with the formulation of 100g of sardines + 100 grams of mustard + 2 g of Omani lemon powder + 2 g of thyme powder and after 40 days of processing was tested for amino acid analysis. The results showed that sardines are rich sources

Table 5: Sensory properties (scores) of Mahyaveh prepared in optimal conditions and traditional Mahyaveh.

Sample	Overall acceptance	Taste (flavor and smell)	Color
Optimal sample	4. 750 ± 0.014[a]	4. 715 ± 0.190[a]	5. 000 ± 0.000[a]
Traditional sample	4. 615 ± 0.091[a]	4. 625 ± 0.318[a]	5. 000 ± 0.000[a]

The results are shown as the mean± standard deviation.
Different small letters indicate a significant difference in each column.

Table 6: Inhibition index and amount of amino acids in optimal and traditional Mahyaveh.

Free amino acid	Sample Inhibition Index Standard (minutes)	Amino acids in traditional Mahyaveh (g 100g pro-1)	Amino acids in optimal Mahyaveh (g 100g pro-1)
Unessential amino acid			
Aspartic acid	2.759	3.54	5.22
Glutamic acid	5.046	8.15	15.34
Serein	7.552	1.76	5.21
Tyrosine	11.237	0.46	1.76
Proline	16.699	1.94	4.19
Alanine	10.176	7.13	10.12
Glycine	8.78 4	5.87	7.12
Essential amino acid			
Threonine	8.950	2.26	4.68
Arginine	9.545	2.85	8.16
Histidine	8.345	3.12	5.27
Valine	13.141	2.86	7.22
Methionine	13.335	1.65	3.45
Phenylalanine	14.491	1.54	4.13
Isoleucine	14.675	2.19	6.12
Lucien	15.290	7.87	11.18
Lysine	15.508	12.45	17.12
Total unessential amino acids		28.85	48.96
Total essential amino acids		65.64	116.29

of essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, and valine). Sardines naturally contain proteinase, which is present in their pancreas. According to *Simpsoon*, (2000) [49] the digestive system of fish is a source of various proteinases. Therefore, to speed up the process of dissolution and autolysis and conversion of fish proteins to amino acids presence of Trypsin and chymotrypsin enzymes can increase the rate of autolysis.

Nguyen et al., (2020) [37] measured the amino acids of fermented fish sauce by using Marinococcus halotolerant as a starting culture with 30% salt concentration and Anchovy fish for six months by high-performance liquid chromatography. The results showed that glutamic acid, aspartic acid, and lysine are the predominant amino acids. The free amino acids are precursors of flavor compounds as well as important flavor compounds [14].

Consistent with the obtained results, *Ojifard* and *Bashirzadeh* (2018) [38] compared the amino acid profiles of the total traditional fish sauce of Suragh with rose (A)

and without rose (B). The results showed that in both sauces the number of essential amino acids was more than unessential amino acids. The Suragh sauce was high in amino acids such as glutamic acid, glycine, lysine, and alanine. These amino acids play an important role in the taste of the sauce [19].

Chromatograms

Fig. 2 shows a standard sample of amino acid profiles, and Fig. 3 and Fig. 4 show a traditionally prepared sample and a sample prepared under optimal conditions by high-performance liquid chromatography to quantify amino acids. The amino acid profiles of Mahyaveh prepared under optimal conditions and traditional Mahyaveh are shown in Table 6.

Quantification of amino acids of optimal and traditional Mahyaveh by high-performance liquid chromatography

Results of Possible Molecular Weight of Proteins and Possible Identification of Bands in SDS-PAGE Model Mahyaveh sauce samples Prepared under Optimal Conditions and traditional Mahyaveh

Fig. 5 shows the model changes of peptide bands of the Mahyaveh sauce sample prepared under optimal conditions and traditional Mahyaveh sauce on SDS-PAGE electrophoresis compared to the standard protein marker. Six protein bands with a molecular weight of about 10 to 60 kDa were observed. The band densities related to the light myosin chain with a weight of about 10, 23, and 27 kDa, then tropomyosin with a molecular weight of 35 kDa, then actin with a molecular weight of 45 kDa, and finally proteinase with a molecular weight of about 60 kDa were observed. In general, it can be concluded that the reduction in the number of bands in fermentation can be due to the denaturation of proteins and their conversion into lower molecular weight peptides, soluble nitrogen compounds, amino acids, nucleotides, and other soluble nitrogen compounds such as aura, ammonia as the effect of the activity of internal enzymes in the fish body and the microbial flora enzymes in fish sauce. During fermentation, fish proteins are hydrolyzed under the action of proteases, the endogenic ones (mostly from the digestive tract), and those produced by halophilic bacteria [28]. However, in the first days of this process, when the bacterial community is not yet established, it is considered that this initial proteolysis (liquefaction) is mostly due to the internal fish enzymes. Nevertheless, recent studies on sardines and

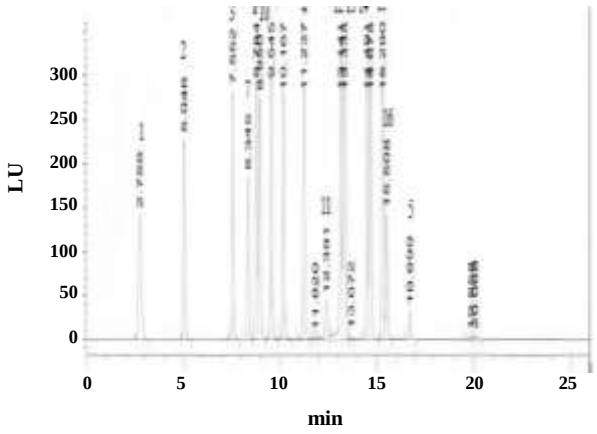

Fig. 2: standard sample chromatogram of amino acids profile by high-performance liquid chromatography.

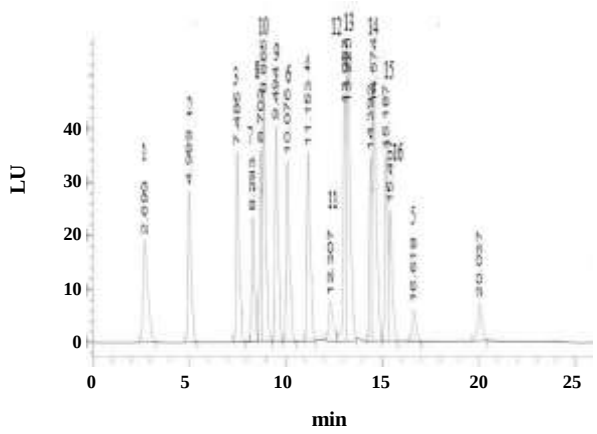

Fig. 3: Chromatogram of amino acids profile of traditional Mahyaveh by high-performance liquid chromatography.

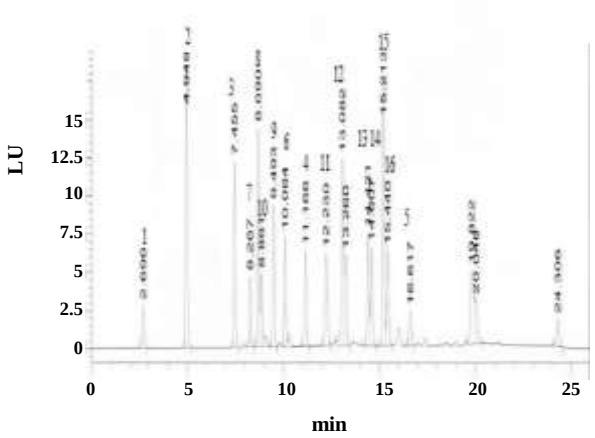

Fig 4:chromatogram of amino acids profile of Mahyaveh prepared under optimal conditions by high-performance liquid chromatography.

Table 7: The approximate molecular weight of proteins and possible identification of bands in the SDS – PAGE pattern of optimal Mahyaveh sauce sample.

Possible identification	Calculated molecular weight (kDa)	Band
light myosin chain	10	A
light myosin chain	23	B
light myosin chain	27	C
Tropomyosin	35	D
Actin	45	E
Proteinase	59	F

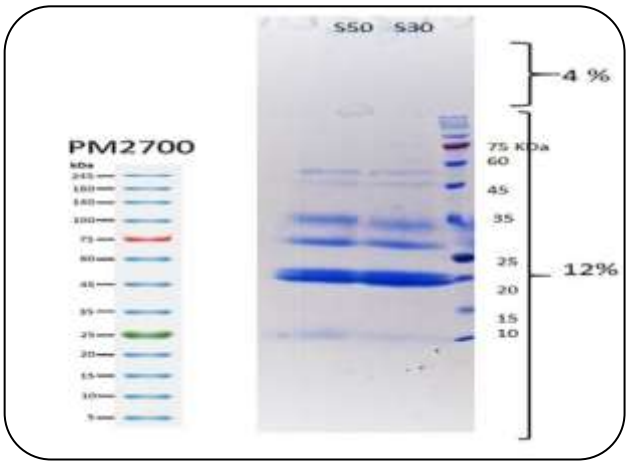

Fig. 5: comparison OF SDS – PAGE model of fish sauce sample prepared in optimal conditions and traditional Mahyaveh.

anchovy have demonstrated that the activity of these internal enzymes decreases with increased NaCl concentration [48]. Therefore, the results of electrophoresis somehow confirmed other changes in fish sauce, such as increasing nitrogen compounds including amino acid nitrogen, and increasing the concentration of amino acids in the fermentation process. Table 7 shows the approximate molecular weight of the proteins and the possible identification of the bands in the SDS-PAGE pattern of the fish sauce sample. The results of *Yin et al*., (2006) [58], electrophoresis model on lactic minced mackerel by bacterial lactic acid showed that in addition to microbial proteolytic enzymes, protease enzymes in the body of cathepsin fish secreted from lysozyme have reduced protein bands and has increased the production of soluble nitrogen compounds and amino acids so that after 72 hours of fermentation, no protein bands were observed in the mackerel fish electrophoresis pattern.

Also, in line with the obtained results, *Moayedi* and *Mousavi Nasab* (2013) [34] have studied changes in SDS- PAGE pattern of fish sauce peptide bands in different stages of fermentation on days (9, 18, 27, 36, 45, and 54). The results showed that with increasing fermentation time, the number and intensity of protein bands decreased and peptides with a molecular weight of 168 and 19.9 KDa (light myosin chain 17), 44.4 kDa (actin 18), and 60 KDa (proteinase) appeared.

Also, the results of the SDS-PAGE showed that due to more hydrolysis in the sample of optimal Mahyaveh sauce, the number and intensity of protein bands were reduced and as a result, it increased the polypeptide chain compared to the traditional Mahyaveh. In confirming this, for the quantification of these bands, the gel scanner software was used. All bands were scanned in this software, the traditional Mahyaveh and optimal Mahyaveh available peaks in bands 1, 2, 3, 4, 5, 6 showed 534, 387, 2159, 1670, 3846, 653, and 665, 477, 2201, 1734, 4339, 965 in the area, respectively. The results in Fig. 6 showed that the S30 sample (optimal Mahyaveh) had higher peaks than the S50 sample (traditional Mahyaveh).

CONCLUSIONS

The present study aimed to investigate and compare the physicochemical, nitrogenous, antioxidant, bioactive, and sensory properties of Mahyaveh produced under optimal conditions with traditional Mahyaveh. The results showed that the rate of proteolysis in the optimal Mahyaveh was higher than in the traditional Mahyaveh so more bioactive compounds such as phenolic compounds, total nitrogen, formalin nitrogen, amino nitrogen, and essential amino acids were produced in the optimal Mahyaveh. The antioxidant activity of Mahyaveh produced under optimal conditions was higher than traditional Mahyaveh. The safety of optimal Mahyaveh due to its lower the microbial load was less than the traditional one. The organoleptic properties of optimal Mahyaveh due to lower microbial load, lower levels of undesirable nitrogen compounds that produce bad smell compounds, and also

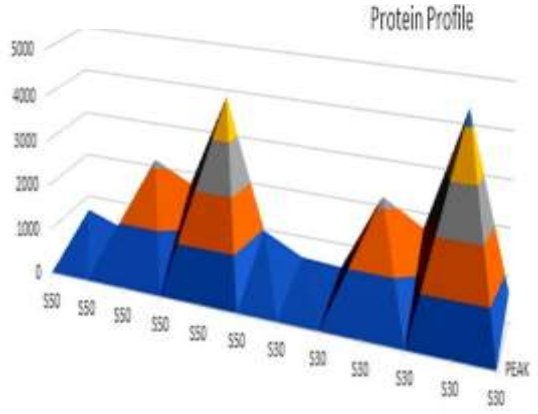

Fig. 6: Comparison of quantitative graphs of protein molecular weight of Gel Scanner in optimal Mahyaveh and traditional Mahyaveh.

due to the presence of more amino acids were higher than in traditional Mahyaveh. By optimizing the production conditions of Mahyaveh, can be produced fish sauce with higher quality, nutritional value, safety, and more desirable sensory properties in comparison with traditional Mahyaveh.

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