# Optimization Production Conditions on the Amount of Nitrogen Compounds in Iranian Fish Sauce (Mahyaveh)

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**ABSTRACT:** *Mahyaveh is a traditional Iranian fish sauce produced by fermentation and hydrolysis. Fish sauce is considered a rich source of protein and contains essential amino acids. The type of fish, salt concentration, and fermentation time are effective on the nitrogen properties of Iranian fish sauce and its quality. Therefore, the aim of this research is to study the type of fish, salt concentration, and fermentation time on the nitrogen properties and increase the bioactive compounds of Iranian fish sauce (Mahyaveh). The results are shown that the increase in time (30 to 120 days), and salt concentration (35 to 15%) has a significant effect (P≤0.05) on the increase of nitrogen properties, fermentation time (30 to 120 days) and salt concentration (15 to 35%), but decrease on trimethylamine. Multiple optimizations to achieve the maximum nitrogen properties and minimum trimethylamine in the Mahyaveh with 95.56% desirability are obtained at 120 days of fermentation, a salt concentration of 18.73% with the use of sardines. By optimizing the conditions of production, Mahyaveh can be produced with nutritional value and higher quality.*

**KEYWORDS:** *Mahyaveh; Fish sauce; Nitrogenous properties; Trimethylamine.*

## INTRODUCTION

Fermentation and drying are the oldest methods to extend food shelf life, including meat preservation. The fermentation of meat products has traditionally depended on indigenous microflora [9]. Proteolysis is one of the most important biochemical reactions that occur during the fermentation process [26].

Fish sauce is the water or juice in the flesh of fish that is extracted during the process of prolonged salting and fermentation, which is called names in different continents, budu in Malaysia, patis in the Philippines, nampla in Thailand, pissala in France, yeesui in Hong Kong, nuoc-nam in South Asia. etc [14]. In Iran, also a type of local fish sauce is produced, called Mahyaveh, Mahweh, 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

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sardines are called in the local language Sardinella sp, Anchovy, or Indian moto, in addition to salt, Mustard (*Brassica juncea*), and water [27]. According to people in the southern region, eating Mahyaveh containing mustard prevents Pacey's skin disease. Fish sauce is not just a flavoring but contains 0.6 to 1.2 % nitrogen and a suitable amount of essential amino acids for the body. In addition, this sauce contains desirable fatty acids such as docosahexaenoic acid and eicosapentaenoic acid [19].

Hydrolyzed proteins are compounds with a low molecular weight that are known as bioactive peptides. Bioactive peptides are pieces of protein with a specific sequence of amino acids having no biological effects when they are in the parent protein. These peptides are shown different biological effects from themselves when released from the mother chain by the effect of hydrolysis or during fermentation [13,16]. These compounds are easily absorbed after entering the body and play an important biological role at the cellular level [24].

Total nitrogen in fish sauce is actually a combination of protein and non-protein nitrogen compounds, including free amino acids, nucleotides, peptides, ammonia compounds, urea, and trimethylamine oxide. These compounds play a major role in creating the specific flavor of fishery products [11, 25]. Formaldehyde nitrogen is used as a common indicator of the degree of protein hydrolysis [8]. According to the Thai fish sauce standard, the amount of formaldehyde nitrogen should be at least 40% of the total nitrogen amount [8]. The amount of amino nitrogen indicates the amount of the first amino acid groups in fish sauce. The increase in the concentration of amino acid nitrogen depends on the rate of decomposition and breaking of polypeptides [23]. Ammonia nitrogen is also one of the most important parameters for fish quality assessment. 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 [5]. Trimethylamine, which is a volatile and pungent amine, is the main cause of the specific smell of marine products. The reason for the formation of trimethylamine is the reduction of the inactive and odorless compound of trimethylamine oxide to the volatile compound of trimethylamine and dimethylamine, having a strong odor similar to ammonia. Rotting bacteria plays a major role in this conversion. The amount of trimethylamine in fresh fish is reported to be less than 8 mL/100g [2]. In the traditional method of producing Mahyaveh, first, the head of the fishes are cut. Then, the fishes are washed along with viscus and poured into pottery or wide-mouthed glass containers with salt and warm water, and the containers contain a mixture of fishes, water, and salt (15-30%) exposed to environment temperature and preferably to sunlight for 25 to 30 days. Then the mixture of salt and fishes 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 [3, 27].

Despite the popularity of this product in the southern and coastal regions of Iran, there are no industrial factories for the production of this product and Mahyaveh is mainly produced traditionally. Mahyaveh fermentation is regarded as an indigenous process, which involves less technological input and requires a long fermentation period to ensure the solubilization of fish mixture. Furthermore, no controlled measure is applied during this spontaneous process, which is usually associated with the inconsistency of product quality and low production yield. Given that, fermentation conditions affect the number of nitrogen compounds in Mahyaveh sauce and the number of nitrogen compounds produced in Mahyaveh affects the tastes and smells of sauce, so by optimizing the production conditions of Mahyaveh sauce. It is possible to achieve products with desirable quality properties. Optimization of Mahyaveh production with the aim of achieving nitrogen properties has been done for the first time in Iran and in the world and the results of this study can be used as an effective solution for Mahyaveh production with higher nutritional properties. The aim of this research is to investigate the type of fish, salt concentration, and fermentation time of the nitrogen compounds of Iranian fish sauce (Mahyaveh).

## EXPERIMENTAL SECTION

### *Materials*

Tuna, anchovies, and sardines are supplied in order to prepare Mahyaveh sauce, in April 2019 (from Bandar Fish Company in Bandar Abbas, Iran) and frozen at -18 °C and transferred to the Agricultural Laboratory of the University of Tehran. Salt is purchased from Shimiaz Company, Iran. Mustard powder is produced by G.S. Dunn

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

| Treatment | Fish type* | Salt concentration (%w/w) | 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*

Company, Canada. Silver nitrate, phenolphthalein reagent, soda, sodium hydroxide, potassium chromate reagent, formaldehyde solution, hydrochloric acid, boric acid, magnesium oxide, methyl red reagent, picric acid, sodium sulfate, and potassium hydroxide are supplied from Merck Company, Germany.

### *Preparation of Iranian fish sauce*

First, the fishes (1: tuna, 2: anchovies, and 3: sardines) are washed with water, along with viscus, and cut to a size of 6 cm. The whole fish (along with viscera) is mixed with water in a ratio of 1 to 1, and the required amount of salt is added to the treatments according to Table 1. Then, fish, water, and table salt are mixed in a mixer, Model Ika, Germany, and transferred to wide-mouthed clay pottery with a capacity of 700 mL, and the lids are closed with three-layer plastic films. These utensils are kept in an incubator in time intervals of 30, 75, and 120 days at 37 °C. After passing the fermentation time of each treatment, the samples are passed through a sterile cleaning cloth. Then, the filtered extract is mixed with 10% mustard and the samples are kept at the environment temperature for 10 to 15 days, and then physicochemical experiments are then performed on the extracts produced.

### *Physicochemical tests of Iranian fish sauce samples*

#### *Total nitrogen*

This test is measured according to the AOAC 940.25 method by a Kjeldahl device during three stages of digestion, distillation, and titration, and calculated based on g/L in the sample [1].

#### *Total formaldehyde nitrogen*

The formaldehyde nitrogen content of fish sauce samples is measured as a protein hydrolysis index according to the international standard of Thai fish sauce. For this purpose, a pH meter electrode is placed inside a beaker containing 10 mL of fish sauce diluted 10 times. In the next step, the sample titration is performed with 0.1 M sodium hydroxide solution to reach a pH of 7. Then 10 mL of 38% formaldehyde solution is added to the sample and titration is continued until the pH reached 9. Finally, the formaldehyde nitrogen is calculated on the basis of g/L in the sample through equation 1 [8].

Formaldehyde nitrogen (g/L) = (1)
(Milliliter of Consumed Salt at pH 7 - Milliliter Consumed Salt at pH 9) × 0.14 ×0.1

#### *Volatile nitrogen*

Volatile nitrogen compounds are measured by direct distillation in boric acid and by a Kjeldahl device. In such a way, 10 g of fish sauce sample with 3 g of magnesium oxide and 100 mL of distilled water are poured into the distillation flask of the Kjeldahl device. At 10 mL/min, vapors of volatile nitrogen compound at the speed of distillation time of 10 minutes are collected in Erlenmeyer containing 100 mL of 4% boric acid along with a few drops of methyl red reagent. Finally, Elenmeyer's contents are titrated with 0.1 normal chloride acid until the pink color of the methyl red reagent appeared and the amount of volatile nitrogen compounds in the samples calculated using equation 2 and on the basis of g/L of the sample [7].

Volatile nitrogen compounds (g/L) = (2)
Volume of acid consumed × Normal acidity × 14 ×10

***Table 2: Comparison between total nitrogen amount, formaldehyde nitrogen, volatile nitrogen, amino nitrogen and trimethylamine of tested or predicted Mahyaveh sauce under different conditions.***

| Treatment | Total nitrogen (g/L) | | Formaldehyde nitrogen (g/L) | | Volatile nitrogen (g/L) | | Amino nitrogen (g/L) | | Trimethylamine (mL/ 100 g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Tested | Predicted | Tested | Predicted | Tested | Predicted | Tested | Predicted | Tested | Predicted |
| 1 | 27.067 | 27.533 | 13.393 | 13.700 | 0.980 | 0.949 | 12.413 | 12.751 | 25.600 | 26.275 |
| 2 | 31.733 | 31.325 | 14.782 | 14.853 | 1.008 | 0.992 | 13.744 | 13.861 | 12.200 | 11.300 |
| 3 | 26.133 | 26.444 | 12.658 | 12.958 | 0.728 | 0.705 | 11.930 | 12.253 | 20.800 | 21.933 |
| 4 | 27.067 | 26.444 | 13.312 | 12.958 | 0.700 | 0.705 | 12.612 | 12.253 | 19.800 | 21.933 |
| 5 | 26.133 | 26.444 | 12.903 | 12.958 | 0.686 | 0.705 | 12.217 | 12.253 | 25.200 | 21.933 |
| 6 | 27.067 | 27.650 | 13.761 | 14.639 | 0.952 | 0.938 | 12.809 | 13.701 | 26.600 | 28.325 |
| 7 | 17.733 | 18.258 | 8.902 | 9.402 | 0.434 | 0.467 | 8.468 | 8.935 | 27.000 | 28.950 |
| 8 | 33.133 | 32.608 | 17.558 | 17.058 | 1.050 | 1.017 | 16.508 | 16.041 | 15.600 | 13.650 |
| 9 | 23.800 | 23.333 | 12.209 | 11.903 | 0.476 | 0.508 | 11.733 | 11.395 | 18.600 | 17.925 |
| 10 | 21.933 | 20.942 | 11.433 | 10.627 | 0.630 | 0.628 | 10.803 | 9.999 | 43.400 | 40.775 |
| 11 | 34.067 | 34.008 | 18.008 | 17.630 | 1.148 | 1.195 | 16.860 | 16.435 | 17.000 | 17.225 |
| 12 | 22.400 | 21.817 | 11.842 | 10.964 | 0.462 | 0.476 | 11.380 | 10.488 | 17.200 | 15.475 |
| 13 | 18.200 | 18.608 | 9.147 | 9.075 | 0.448 | 0.464 | 8.699 | 8.611 | 30.200 | 31.100 |
| 14 | 15.400 | 15.458 | 7.799 | 8.177 | 0.252 | 0.205 | 7.547 | 7.972 | 24.400 | 24.175 |
| 15 | 28.467 | 29.458 | 13.802 | 14.608 | 0.714 | 0.716 | 13.088 | 13.892 | 10.000 | 12.625 |

***Amino nitrogen***

The Thai International Standard Method in 1983 is used to measure the number of amino nitrogen samples. Amino nitrogen is calculated by the following Eq. (3) based on formaldehyde nitrogen and ammonia nitrogen (volatile nitrogen compounds) [8].

Amino nitrogen (g/L) = (3)
(formaldehyde nitrogen - volatile nitrogen compounds)

***Trimethylamine***

Trimethylamine is measured based on AOAC [1] according to the Dyer method or the same coloring as the Picrate method. After supplying the extract of fish in a water solution of 7.5% (%w/w) trichloroacetic acid, 1 mL of the extract is transferred to a glass tube and reached to the volume of 4 mL with distilled water. Then 1 mL of 20% formaldehyde, 3 mL of 25% potassium hydroxide (%w/w), and 10 mL of toluene are added to the tube and the contents of the tube are mixed continuously by hand 40-60 times. Then 7-9 mL of the surface phase (toluene with trimethylamine) is poured into a dry tube containing 0.1 g of dry sodium sulfate to dry the water in the toluene. Finally, 5 mL of dried toluene is poured into another dry tube with a lid, 0.5 mL of picric acid is added to it, and after mixing, the solution is read at a wavelength of 410 nm. Toluene is used to reset the spectrophotometer. The amount of trimethylamine in the samples is reported by using of trimethylamine standard diagram and the equation obtained from it and Equation (4) according to mL/100g of the samples.

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

***Statistical Analysis***

In this research, the Box-Behnken Response Surface Methodology is used to design the treatments, optimize the extraction conditions and achieve the Mahyaveh with the highest amount of total nitrogen, formaldehyde nitrogen, amino nitrogen, and the lowest amount of trimethylamine. The extraction conditions are three independent factors of fish types (tuna, anchovy, sardine), salt concentration (15%, 25%, 35%), and fermentation time (30, 75, 120 days).

Therefore, 15 treatments are designed. The results of the tests are analyzed by Minitab 16 software using Response Surface Method (RSM).

## RESULTS AND DISCUSSION

### *Evaluation of nitrogen properties of Mahyaveh fish sauce*

In this research, the nitrogen properties of Mahyaveh sauce including the amount of total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine are evaluated during the fermentation period and compared with the nitrogen properties of Mahyaveh sauce (Table 2). According to the results of Table 2, there are no significant differences between the results of the evaluation of the tested and predicted nitrogen properties ($P$ >0.05).

The highest amount of total nitrogen (34.067 g/L), formaldehyde nitrogen (18.008 g/L), volatile nitrogen (1.148 g/L), amino nitrogen (16.860 g/L) are observed in the sample of Mahyaveh sauce ($T_{11}$) containing 15 %w/w salt, 120 days' fermentation time and the fish type 2 (Anchovy). The lowest amount of total nitrogen (15.400 g/L), formaldehyde nitrogen (7.799 g/L), volatile nitrogen (0.252 g/L), and amino nitrogen (7.547 g/L) are observed in the sample of Mahyaveh sauce ($T_{14}$) containing 35 % w/w salt, 30 days' fermentation time and the fish type 2 (Anchovy). The results are shown that increasing the fermentation time (from 30 days to 120 days) and decreasing the salt concentration (from 35% to 15%) has a significant effect ($P \leq 0.05$) on an increase in total nitrogen, formaldehyde nitrogen, volatile nitrogen, and amino nitrogen of Mahyaveh sauce. The type of fish doesn't have a significant effect on increasing the nitrogenous properties of the Mahyaveh sauce ($P$ >0.05).

In confirmation of the results of this study, *Moayedi* and *Mousavi Nasab* [17] examined the changes in nitrogen compounds during the fermentation process of Mahyaveh for 54 days at 37 °C during six fermentation stages. The results are shown that with increasing fermentation time, the total nitrogen compounds, formaldehyde nitrogen, amino acid nitrogen, and volatile nitrogen increased at all stages. Total nitrogen contents from the pacific whiting fish sauce increased from 6.40 to 15.7 g/L during the first 10 days of fermentation [15].

The amount of total nitrogen in fish sauce is the most important physical and chemical parameter that determines the quality and price of the final product. According to Thailand's national standard, the total nitrogen amount of fish sauce is divided into three grades including first grade (more than 20 g/L), second grade (between 15 and 20 g/L), and third grade (less than 15 g/L) [8].

According to the results of Table 2, the range of the amount of total nitrogen in the produced Mahyaveh sauce in this study is varied between (15.400 g/L) to (34.067 g/L) and classified as first-class fish sauce. The pH range of Mahyaveh sauces is ranged from 5.25 to 6.64 and their brix varied from 17 to 29. *Shakib* and *Mousavi Nasab* [21] examined the amount of total nitrogen content of sardine fish sauce during the three-month fermentation process. The results of these researchers show that by increasing fermentation time, the number of total nitrogen compounds significantly increased, and after ninety days of fermentation, the amount of these compounds reached 224 mg in 100 g of fish sauce sample.

Formaldehyde nitrogen is an important indicator for classifying the quality of fish sauce in China [6]. By increasing fermentation time, formaldehyde nitrogen reported based on g/L, is increased in almost all stages. The production conditions of Yu-lu, a traditional Chinese fermented fish sauce, are simulated in a laboratory with 30% salt concentration for 180 days at a temperature of 30 °C. Then the temperature is increased up to 50 °C for 7 days. The results are shown that formaldehyde nitrogen at intervals of 30, 60, 90, 120, 150, 180 days is continuously increased, but increased significantly by increasing temperature to 50 °C for the period of 7 days on the 187th day [11].

Another important parameter for evaluating the quality of a fish sauce is volatile nitrogen, which includes compounds such as trimethylamine, dimethylamine, ammonia, and other volatile nitrogen compounds. Increased ammonia nitrogen amount can be due to fish enzymes that are active during fermentation [5]. According to research by *Xu et al.*[25], the amount of volatile nitrogen compounds from fish sauce samples prepared from Squid fish waste is increased at all stages of fermentation, and by the end of the 30th day, the amount of these volatile nitrogen compounds reached 300 mL/100g. Researchers, such as *Moeini* and *Sobhanipour* [18], who researched the benefits of salt fish, reported an increase in the amount of volatile nitrogen (TVN) in the product. They believe that the reason for the increase in TVN in this product is the broken down of amino acids by heat

into small molecules, as well as the existence of non-protein nitrogen.

The amount of amino nitrogen, which represents the first group of amino acids in fish sauce, according to the standard of Thai fish sauce, should be at least 40% of the total nitrogen [8]. According to the results of Table 2, the amount of amino nitrogen of all the Mahyaveh sauces studied in this study is at least 40% of the total nitrogen amount. *Jesebel* and *Erlinda* [10] examined the fermented tuna sauce with different salt concentrations of 10%, 17.5%, and 25% for 7 days at environment temperature and observed that its amount of amino nitrogen, total nitrogen, and volatile nitrogen increased by increasing fermentation time.

Trimethylamine is one of the most important causes of the undesirable, spicy, and pungent smell of fish sauce. In fact, trimethylamine is an indicator of rotting fish microbial and its value is directly related to the number of rotting bacteria in fish. The permissible amount of trimethylamine in fresh fish is generally less than 8 mL/100g [2]. This research is shown that in the early stages of fermentation, the amount of trimethylamine is increased and passing time the fermentation decreased, which is significantly different from each other in all stages of fermentation. The results of Table 2 are shown that the highest amount of trimethylamine (43.400 mL/100g) observed in the sample of Mahyaveh sauce ($T_{10}$) containing 15% w/w salt, 30 days' fermentation time and type 2 fish (anchovies). The lowest amount of trimethylamine (10 mL/100g) is observed in a sample of Mahyaveh sauce ($T_{15}$) containing 35% w/w salts, 120 days, fermentation time, and fish type 2 (anchovy).

*Kilinc et al*.[12] are reported that trimethylamine of fish sauce samples made from sardines increased to the 27th day and then decreased due to a decrease in the number of bacteria by the end of the fermentation process. Many researchers, including *Shakib* and *Mousavi Nasab* [21] are studied the effect of dried rainbow sardine (Dussumieria acuta) fish sauce on the four treatments under investigation including the mechanical process of grinding dry fish, adding salt at two levels of 80% and 100% of dry fish weight, adding citric acid at a level of two percent. The results are shown that the total nitrogen amount increased by passing time and the amount of trimethylamine in all treatments decreased significantly during fermentation processes. 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 the amount of trimethylamine indicating a decrease in the bacteria that cause rotting during the fermentation process of fish sauce.

Aroma is a prime indicator of the quality of fish sauce [22]. Nitrogenous compounds from the supernatant liquor could have a significant effect on consumer acceptability. Other nitrogenous compounds are equally important in terms of flavor and nutritional values [20].

By analyzing the results, it is found that all nitrogen compounds increased in the samples over time. The process of fermenting fish sauce caused the decomposition of protein bonds and their breakdown into smaller peptides, amino acids, and the nutritional value of the product and finally caused a reduction of microbial load and the number of trimethylamine samples.

### *Analysis of the response level model of total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine Mahyaveh sauce*

Table 3 shows the results of the analysis of the response level model of total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine Mahyaveh sauce. According to the results of linear effects of salt concentration variables and fermentation time on total nitrogen amount, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine of Mahyaveh sauce are significant ($P \leq 0.05$).

Linear effects type of fish is not a significant effect on total nitrogen amount, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine of Mahyaveh sauce ($P > 0.05$). The square and interaction effects of all the studied variables (fish type, salt concentration, and fermentation time) on the changes of nitrogen properties and trimethylamine of Mahyaveh sauce were not significant ($P > 0.05$).

### *Regression model of nitrogen properties of Mahyaveh sauce*

The multi-sentence regression model of nitrogen properties of Mahyaveh sauce is shown in Table 4. The coefficient of determination ($R^2$) values of total nitrogen is 98.94% and its adjusted ($R^2$-adj) is 97.02%. The coefficient of determination ($R^2$) values of formaldehyde nitrogen is 96.42% and its adjusted ($R^2$-adj) is 89.97%. The coefficient of determination ($R^2$) values of volatile nitrogen was 98.96 % and its adjusted ($R^2$-adj) is 97.09 %.

***Table. 3: Results of analysis of variance of response surface model of total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine of Mahyaveh sauce***

| Source | Total nitrogen ( g/L) | | Formaldehyde nitrogen ( g/L) | | Volatile nitrogen ( g/L) | | Amino nitrogen ( g/L) | | Trimethylamine ( mL/100 g) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | F-value | P-value | F-value | P-value | F-value | P-value | F-value | P-value | F-value | P-value |
| Constant | 51.64 | 0.000* | 14.96 | 0.004* | 52.82 | 0.000* | 12.07 | 0.007 | 10.76 | 0.009 |
| Linear effects | 152.93 | 0.000* | 44.09 | 0.001* | 157.43 | 0.000* | 35.45 | 0.001* | 30.51 | 0.001* |
| Type of fish (a) | 1.46 | 0.280 | 2.18 | 0.200 | 0.11 | 0.759 | 2.07 | 0.209 | 1.09 | 0.344 |
| Salt concentration (b) | 55.25 | 0.001* | 18.51 | 0.008* | 194.71 | 0.000* | 12.54 | 0.017 | 24.18 | 0.004* |
| Fermentation time (c) | 402.07 | 0.000* | 111.58 | 0.000* | 277.49 | 0.000* | 91.73 | 0.000* | 66.27 | 0.000* |
| Square effects | 1.67 | 0.288 | 0.09 | 0.960 | 0.81 | 0.540 | 0.11 | 0.951 | 0.46 | 0.719 |
| Type of fish ($a^2$) × Type of fish | 1.29 | 0.308 | 0.12 | 0.746 | 1.69 | 0.251 | 0.16 | 0.704 | 0.56 | 0.486 |
| Salt concentration × Salt concentration ($b^2$) | 2.58 | 0.169 | 0.00 | 0.994 | 0.58 | 0.482 | 0.00 | 0.966 | 0.63 | 0.464 |
| Fermentation time × Fermentation time ($c^2$) | 1.88 | 0.229 | 0.18 | 0.686 | 0.00 | 0.981 | 0.18 | 0.691 | 0.10 | 0.762 |
| Interaction effect | 0.34 | 0.799 | 0.69 | 0.594 | 0.23 | 0.874 | 0.65 | 0.614 | 1.29 | 0.373 |
| Type of fish(a×b) × Salt concentration | 0.54 | 0.496 | 0.00 | 1.000 | 0.58 | 0.666 | 0.00 | 0.983 | 0.00 | 0.950 |
| Type of fish(a×c) × Fermentation time | 0.24 | 0.646 | 1.98 | 0.218 | 0.09 | 0.772 | 1.88 | 0.228 | 0.00 | 0.975 |
| Fermentation time × Salt concentration (b×c) | 0.24 | 0.646 | 0.10 | 0.763 | 0.37 | 0.567 | 0.08 | 0.789 | 3.87 | 0.106 |
| Lack of fit | 4.56 | 0.185 | 11.71 | 0.080 | 6.96 | 0.128 | 1.3089 | - | 1.21 | 0.482 |

The coefficient of determination ($R^2$) values of amino nitrogen ($R^2$) is 95.60% and its adjusted ($R^2$-adj) is 87.68%, and the coefficient of determination ($R^2$) values of trimethylamine is 95.09% and its adjusted ($R^2$-adj) is 86.25%. These results are indicated a good fit of the model related to the experimental data.

### *Single Optimization of Nitrogen properties of Mahyaveh Sauce*

Fig. 1 shows the single optimization conditions for total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine of the Mahyaveh sauce. Fig. 1-a shows the single optimization conditions of total nitrogen of the Mahyaveh sauce. According to this Figure, it is predicted that the maximum amount of total nitrogen of Mahyaveh sauce (33.1528 g/L) with 100% desirability, belongs to the time of 120 days, type of fish 2 (anchovy) and salt concentration of 15%. Optimal conditions to increase the amount of total nitrogen production are practically applied in the laboratory and the amount of total nitrogen is %33.1333, which there is no significant difference from the predicted total nitrogen of %33.1528.

Fig. 1-b shows the conditions of individual optimization for formaldehyde nitrogen of Mahyaveh sauce. According to this Figure, it is predicted that the maximum amount of formaldehyde nitrogen of Mahyaveh sauce (18.5724 g/L) with 100% desirability belongs to 120 days, fish type 3 (sardine), and salt concentration of 15%. Optimal conditions to increase the amount of formaldehyde nitrogen production are practically applied in the laboratory and its amount was 18.0075%, which there was

***Table. 4: Multi-sentence regression model of total nitrogen, formaldehyde nitrogen, volatile nitrogen, amino nitrogen and trimethylamine of Mahyaveh sauce***

| Source | Model | $R^2$ | $R^2$-adj |
|---|---|---|---|
| Total nitrogen (g/L) | Y= 26.4444 + 0.4083 a-2.5083b+ 6.7667c-0.5639 $a^2$ -0.7972$b^2$-0.6806$c^2$ +0.3500ab+0.2333 ac +0.2333bc | 98.94 | 97.02 |
| Formaldehyde nitrogen (g/L) | Y= 12.9578+0.4696 a-1.3679 b+3.3585 c -0.1599 $a^2$ +0.0034 $b^2$ –0.2008 $c^2$ + 0.6329 ac-0.1429bc | 96.42 | 89.97 |
| Volatile nitrogen (g/L) | Y= 0.704667+ 0.005250a -0.225750b +0.269500c -0.030917$a^2$-0.018083$b^2$-0.$^{000583c2}$ +0.010500ab +0.007000ac-0.014000bc | 98.96 | 97.09 |
| Amino nitrogen (g/L) | Y= 12.2531+0.4643a -1.1422b +3.0890c -0.1908$a^2$+ 0.0215 $b^2$-0.2002$c^2$ -0.0105ab +0.6259ac-0.1289bc | 95.60 | 87.68 |
| Trimethylamine (mL/100 g) | Y= 21.9333+1.1250a-5.3000b -8.7750c -1.1917$a^2$+ 1.2583$b^2$-0.5083$c^2$+0.1000ab +0.0500 ac+3.0000bc | 95.09 | 86.25 |

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

no significant difference with the predicted formaldehyde nitrogen of 18.5724%.

Fig. 1-c shows the individual optimization conditions for the volatile nitrogen of Mahyaveh sauce. According to this Figure, it is predicted that the minimum amount of volatile nitrogen Mahyaveh sauce (0.2048 g/L) with 100% desirability belongs to the time of 30 days, fish type 2 (anchovy) and salt concentration of 35%. Optimal conditions to reduce the amount of volatile nitrogen production are practically applied in the laboratory and the amount of volatile nitrogen is 0.252%, which there is no significant difference from the predicted volatile nitrogen amount of 0.2048%.

Fig. 1-d shows the individual optimization conditions for amino nitrogen of Mahyaveh sauce. According to this Figure, it is predicted that the maximum amount of amino nitrogen from Mahyaveh sauce (17.3444 g/L) with 100% desirability belongs to the time of 120 days, fish type 3 (sardine) and salt concentration of 15%. Optimal conditions to increase the amount of amino nitrogen production are practically applied in the laboratory and the amount of its amino nitrogen is 16.8595%, which there is no significant difference with the predicted amino nitrogen of 17.3444%.

Fig. 1-e shows the individual optimization conditions for trimethylamine of Mahyaveh sauce. According to this Figure, it is predicted that the minimum amount of trimethylamine from Mahyaveh sauce (10.1557 mL/100g) with 99% desirability belongs to the time of 120 days, fish type 1 (tuna) and salt concentration of 34.5960 %. Optimal conditions to decrease the amount of trimethylamine production are practically applied in the laboratory and the amount of its trimethylamine was 10%, which there is no significant difference from the prediction.

### *Multiple optimizations, total nitrogen amount, formaldehyde nitrogen, amino nitrogen, and trimethylamine of Mahyaveh sauce*

Fig. 2 shows the multiple optimization conditions for the total nitrogen amount, formaldehyde nitrogen, amino nitrogen, and trimethylamine which are the most important factors affecting the quality properties of Mahyaveh. As can be seen, it is predicted that the optimal conditions to achieve the maximum nitrogen properties and minimum trimethylamine in Mahyaveh sauce are obtained with 95.56% at the time of 120 days, fermentation with the third type of fish (sardines) and at a salt concentration of 18.73%. The predicted results are performed by confirmatory experiments in the laboratory and no significant difference is observed between the predicted and experimental values.

## CONCLUSIONS

This research is performed with the aim to investigate the type of fish, salt concentration, and fermentation time on the nitrogen properties 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 nitrogen amount, formaldehyde nitrogen, volatile nitrogen, amino nitrogen, and trimethylamine are investigated. 15 treatments are designed according to the Box-Behnken

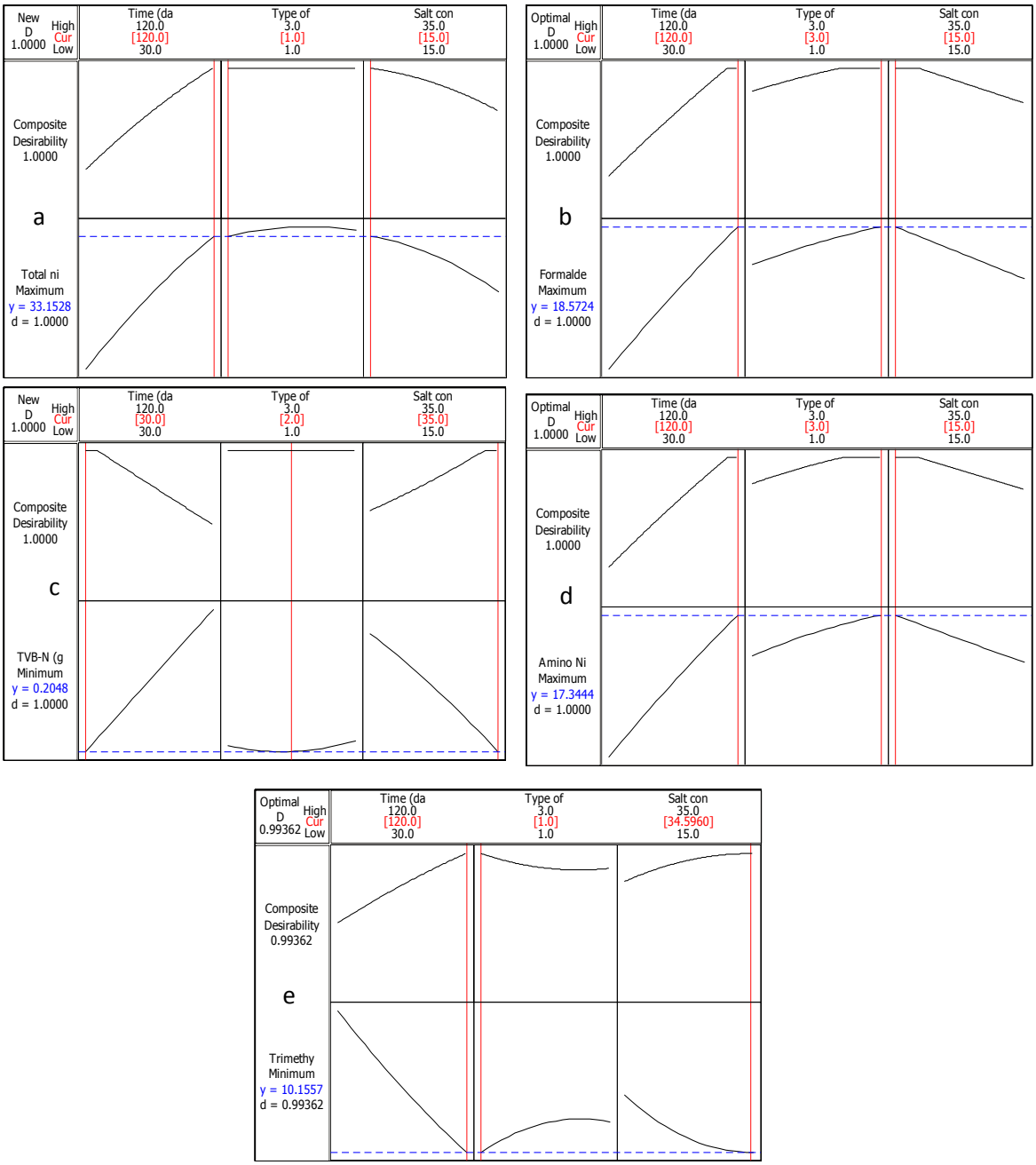


***Fig 1: Single optimization diagram of total nitrogen (a), formaldehyde nitrogen (b), volatile nitrogen (c) amino nitrogen (d), and trimethylamine (e) of Mahyaveh sauce***

Response Surface Methodology. The results are shown that by 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. The type of fish has no significant effect on the nitrogen properties of the tested treatments. Optimal conditions to achieve the maximum amount of total nitrogen,

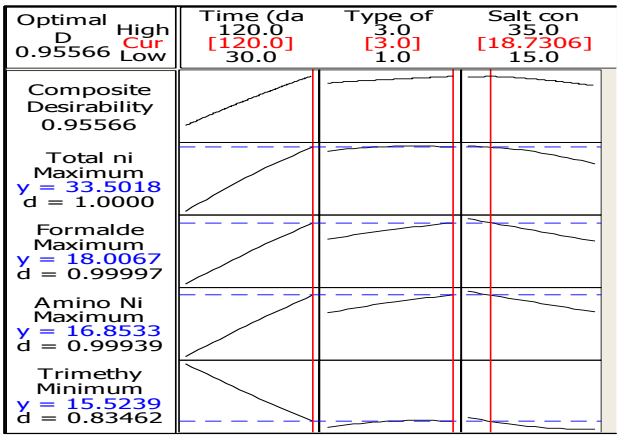


***Fig 2: Simultaneous optimization diagram, total nitrogen amount, formaldehyde nitrogen, amino nitrogen, and trimethylamine of Mahyaveh sauce***

formaldehyde nitrogen, and amino nitrogen and minimum amount of trimethylamine are obtained in Mahyaveh sauce, with 95.56% desirability at the 120 days of fermentation time, the third type of fish (sardines) and in salt concentration of 18.73%. The predicted results are performed by confirmatory experiments in the laboratory and no significant difference is observed between the predicted and experimental values. An increase in nitrogen compounds indicates an increase in protein hydrolysis and, as a result, an increase in the nutritional value of fish sauce. A decrease in trimethylamine indicates a decrease in spoilage bacteria during the fermentation process. Finally, by multiple optimizations of total nitrogen, formaldehyde nitrogen, amino nitrogen, and trimethylamine, it is possible to produce Mahyaveh sauce with higher quality properties and more nutritional value. Therefore, without more changes can be produced quality fish sauce that can be used by all people and is easy to absorb as a nutrient for humans.

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