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Low salt and biogenic amines fermented fish sauce (Mahyaveh) as potential functional food and ingredient

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ABSTRACT

The response surface methodology was used to evaluate the influence of salt content (5–20%), fermentation temperature (25–50 °C), and fermentation time (20–80 days) on the chemical characteristics and histamine level of Iranian fish sauce (Mahyaveh) prepared from Anchovy fish. The results showed that salt had a significant quadratic effect on pH, total nitrogen, salt, and histamine content. The total nitrogen, pH, and histamine of fish sauce decreased with increasing salt concentration. Salt content had a significant linear effect on amino nitrogen. The amount of amino nitrogen decreased with increasing salt. Time had a positive quadratic effect on histamine and total nitrogen levels in fish sauce, whereas it had a positive linear effect on amino nitrogen level. In contrast, temperature showed a significant effect on the total nitrogen, histamine, salt, and pH of fish sauce ($p < 0.05$). As the temperature increased, total histamine decreased whereas nitrogen concentration increased. The predicted optimal conditions as determined by Response Surface Methodology were salt content of 12.5%, fermentation temperature of 39.15 °C, and fermentation time of 39.79 days. Therefore, high quality Iranian fish sauce can be produced with low salt and histamine content as a potential functional food and ingredient.

1. Introduction

In East and Southeast Asia, fish sauce is prepared and consumed as traditional fermented condiment (Russo et al., 2020). In Iran, fish sauce (Mahyaveh) is widely consumed in the Southern areas, particularly in Fars and Hormozgan provinces. It's produced by salt fermentation of fish in the ratio of 2:1 (fish:salt) for 25–30 days at room temperature (Zarei et al., 2012). fish sauce is a source of all essential amino acids, some bioactive peptides, vitamins and trace elements (Gao et al., 2019; Kim et al., 2019). However, the high salt and histamine content of fish sauce are considered as major disadvantages. Since high-salt is harmful for human health, a low-salt fish sauce needs to be produced (Habermeyer et al., 2015). Biogenic amines (BAs) are nitrogenic low molecular weight organic compounds that include cadaverine, histamine, putrescine, tryptamine, and tyramine. BAs are produced by microbial decarboxylation or amination of certain free amino acids and can be found in fermented products (Zotou et al., 2003). According to the European Food Safety Authority (EFSA), histamine is the most significant BA in terms of toxicity because high amounts of this BA can cause heart palpitations, hypertension, and headaches in some people (Becker et al., 2001). Histamine in fish is generated during the decomposition or spoilage processes, thus it can be employed as a freshness or spoilage indicator (Kim et al., 2000; Ruiz-Capillas & Herrero, 2019). As Zarei et al. (2012) stated, histamine is the most prevalent BA in Iranian fish sauce, with levels as high as 2662 mg/kg. The quantities of histamine observed in the fish sauce were 1220 ppm (Muhammad et al., 2009). The European Union (EU) and Codex Alimentarius have set regulation limitations of 400 mg/kg for histamine content in fish sauce (World Health Organization, 2004).

Fish sauce fermentation is a complicated biochemical process that involves the interplay of several bacterial species and results in histamine production (Schirone et al., 2013). As a consequence, histamine synthesis can be lowered by controlling microbial growth or declining microbe decarboxylase activity (Mah et al., 2009; Shim et al., 2022). Histamine production is influenced by temperature, time, salt and pH (Kim et al., 2003; Klomklao et al., 2006). During the fermentation of fish sauce, Wang et al. (2018) observed an increase in histamine level. In salt-fermented soybean paste, higher salt concentration (12%) had a

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Table 1
Variables and levels used in Box–Behnken design.

Trial	Variable codes			Actual values		
	Salt (%)	Time (day)	Temperature (°C)	Salt (%)	Time (day)	Temperature (°C)
1	0	0	0	12.5	50	37.5
2	0	−1	+1	12.5	20	50
3	0	0	0	12.5	50	37.5
4	0	+1	−1	12.5	80	25
5	0	0	0	12.5	50	37.5
6	0	−1	−1	12.5	20	25
7	+1	0	+1	20	50	50
8	0	+1	+1	12.5	80	50
9	−1	−1	0	5	20	37.5
10	−1	0	−1	5	50	25
11	0	0	0	12.5	50	37.5
12	+1	−1	0	20	20	37.5
13	+1	+1	0	20	80	37.5
14	−1	+1	0	5	80	37.5
15	−1	0	+1	5	50	50
16	0	0	0	12.5	50	37.5
17	+1	0	−1	20	50	25

lower biogenic amines level compared to lower salt conditions (6% and 8% salt) (Kim et al., 2005). Therefore, production parameters, i.e. salt, time and temperature, could be modeled to reduce the biogenic amines in fish sauce (Gao et al., 2020). In this regard, a study has been carried out to minimize biogenic amines in fermented sausage using *E. faecalis EF37* optimum modeling (Gardini et al., 2008).

To the best of our knowledge, there are relatively few reports on the optimization of Iranian fish sauce production. the current research is one of the few attempts to: (a) investigate the impact of Iranian fish sauce production parameters, such as salt, fermentation time, and temperature, on chemical characteristics and histamine in fish sauce; (b) determine the optimum parameters for functional Iranian fish sauce preparation; (c) Verify the precision of the Box-Behnken design to examine the antagonistic and/or synergistic impacts of production variables on the quality of fish sauce.

2. Materials and methods

2.1. Fish sauce production

The chemical characteristics and histamine level of dried anchovies fish sauce were studied concerning salt, fermentation time and fermentation temperature as variables. The response surface methodology was used to find out the optimum amount of examined variables to produce a functional fish sauce. Brine solution with different levels of salt (NaCl) (5–20%) was prepared and added to dried anchovy fish) 6.66 - 1.33:1 fish:salt w/w). The mixtures were then packed in glass bottles with a capacity of 200 mL and sealed with a plastic cap. Fermentation starts at different temperatures (25–50 °C) and lasts for different periods of time (20–80 days). The variables used in the preparation of fish sauce are listed in Table 1. At the end point of fermentation process, the entire mixture was filtered through the gauze pad, and the obtained liquid was then filtered using Whatman No. 1. The obtained fish sauce was used for further analysis.

2.2. Determination of pH

The pH of fish sauce was determined directly using a digital pH meter (AZ86502, Taiwan).

2.3. Determination of salt content

The salt content of samples was determined using the AOAC method (2000). Samples (20 mL) were diluted with 180 mL of distilled water. One mL of the diluted sample was combined with 10 mL of concentrated HNO_3 (nitric acid) and 10 mL of 0.1 N $AgNO_3$ (silver nitrate). The mixture was slowly heated on a hot plate until all solids except $AgCl_2$ (silver chloride) were dissolved (usually 10 min) and the mixture was cooled with running water. Fifty mL of distilled water and 5 mL of ferric alum indicator ($FeNH_4(SO_4)_2{\cdot}12H_2O$) were added. The solution was titrated with standardized 0.1 N KSCN (potassium thiocyanate) until solution became permanent light brown. The % salt in the samples was calculated using the following formula:

$$\text{Salt}\ (\%) = \frac{(VA \times NA) - (VB \times NB) \times 0.058 \times 100}{W} \quad \text{(Equation 1)}$$

V_A = volume of $AgNO_3$ (mL), V_B = volume of KSCN (mL), N_A = concentration of $AgNO_3$ (N), N_B = concentration of KSCN (N), and W = weight of sample (g).

2.4. Measurements of total nitrogen

The total nitrogen content of fish sauce samples was measured using the Kjeldahl method (AOAC, 2000). Total nitrogen content was calculated as follows:

$$\text{Total nitrogen content}\ (g\,/\,l) = \frac{(VA - VB)N \times F \times 1000}{S} \quad \text{(Equation 2)}$$

V_A and V_B= Volume standard acid required for sample and blank, N = normality of standardized acid, F = milliequivalent weight to nitrogen (14 mg/mmol), S= Volume of sample digested (mL).

2.5. Amino nitrogen

The formalin titration method was used to determine amino nitrogen with some modifications (Cai & Yuan, 1982, pp. 8–9). Briefly, the fish sauce (10 mL) was mixed with 25 mL of water and adjusted to pH 8 with 1 N NaOH and 2 mL of 36% (w/v) formalin solution (CH_2O) was then added. The pH of the mixture was adjusted to 9.2 using 0.1 N NaOH. The amino nitrogen concentration of the samples was obtained using the following formula:

Amino nitrogen content (g/l)= (A-B) × C × 0.014 × D × 1000/10(Equation 3)

here A is the titrated volume of 0.1 N NaOH consumed by the sample (mL), B is the titrated volume of 0.1 N NaOH consumed by the blank (mL), 0.014 represents the molar mass of the N (g/mmol), C is the concentration of NaOH solution, D is the dilution factor, 1000/10 (to convert to liters).

2.6. Histamine determination

The method of Kim et al. (2011) was adopted to quantify histamine content of fish sauce samples with some modification. Ten mL of 0.4 M perchloric acid ($HClO_4$) containing 125 μL of 1,7-diaminoheptane ($C_7H_{18}N_2$) was used as an internal standard to homogenize each 5 g sample. The homogenate was centrifuged for 5 min at 8000×*g* at 4 °C. Aliquot (100 μL) of the extracted sample or standard solution was combined with 300 μL of sodium carbonate solution (Na_2CO_3), 60 μL of 2 M Sodium hydroxide (NaOH) and 400 μL of dansyl chloride solution ($C_{12}H_{12}ClNO_2S$). The solution was then incubated at 40 °C for 45 min. The remaining dansyl chloride ($C_{12}H_{12}ClNO_2S$) was removed by adding 30 μL of ammonium hydroxide (NH_4OH). Peaks were detected with a UV detector at 254 nm after separation on a C18 column (5 μm, 250 mm × 4.6 mm). The gradient elution procedure was used with a mixture of water (H_2O) as solvent A and acetonitrile (CH_3CN) as solvent B. The gradient elution program was as follows: 0 min, A:B (40:60%); 11 min, A:B (25:75%); 11.1min, A:B (5:95%); 20 min, A:B (5:95%); 20.1 min, A:B (40:60%) and 30 min, A:B (40:60%). The flow rate, injection volume, and column temperature were 1 mL/min, 20 μL, and 25 ± 2 °C,

Table 2
Analysis of variance for the predicted quadratic polynomial models for properties of fish sauce.

Source	DF	Total nitrogen		Amino nitrogen		pH		NaCl		Histamine	
		coefficients	Sum of sequares	coefficients	Sum of sequares	coefficients	Sum of sequares	coefficients	Sum of sequares	coefficients	Sum of sequares
Model	9	22.77***	332.53	10.97***	149.20	6.38***	8.70	12.53***	496.29	313.57***	1.032E+06
Linear											
A	1	−4.11***	134.89	−3.95***	124.58	−0.8912***	6.35	7.86***	494.87	−241.41***	4.662E+05
B	1	3.18***	81.03	1.59***	20.22	ns	0.1711	ns	0.1012	163.99***	2.151E+05
C	1	1.44*	16.50	ns	4.40	−0.2600*	0.5408	0.2250**	0.4050	−42.87*	14701.84
Quadratic											
A^2	1	−2.71**	30.81			0.5172**	1.13	0.3852***	0.6249	255.31***	2.745E+05
B^2	1	−2.71**	30.98			ns	0.1257	ns	0.0205	−108.17*	49269.95
C^2	1	−2.53**	26.95			ns	0.2533	ns	0.1081	64.07*	17281.90
Interaction											
AB	1	ns	0.6972			ns	0.1406	ns	0.0012	ns	165.77
AC	1	ns	0.2116			ns	0.0169	ns	0.0992	ns	723.88
BC	1	ns	0.0380			ns	0.0484	ns	0.0462	ns	320.77
Lack of Fit	3	ns	6.70	ns	9.83	ns	0.1256	ns	0.0787	ns	4753.05
Pure Error	4		3.74		4.41		0.2819		0.0571		6870.70
Cor Total	16		342.97		163.44		9.11		496.42		1.043E+06
C.V		6.42		9.54		3.76		1.09		9.87	
R^2		0.9696		0.9129		0.9553		0.9997		0.9889	
Adjusted R^2		0.9304		0.8928		0.8978		0.9994		0.9745	

A: Salt; B: Time; C: Temperature.
ns: no significant effect at level <0.05.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
CV: coefficient of variation.

respectively.

2.7. Statistical analysis

A Box-Behnken design (BBD) was constructed using the Design Expert software Version 12 (Stat-Ease Corporation, Minneapolis, MN, USA) and was used for estimating the effect of independent variables on the amino nitrogen, total nitrogen, salt and histamine content. This research evaluated three production variables: salt content, fermentation time, and fermentation temperature (Table 1). The design includes 17 sets of test conditions for each manufacturing technique, with three levels assigned to each factor at high, central, and low levels, as well as four repeated center points. The maximum and minimum treatment levels were determined by preliminary screening tests and based on literature reports and instrumental factors. All experiments were carried out in three parallel tests. To verify the validity of the models, the experimental and predicted values were compared using a paired *t*-test with Minitab 15 (Minitab Inc., State College, PA, USA) software.

3. Results and discussions

3.1. Model fitting

Salt (A), Time (B) and Temperature (C) were the production variables. According to Table 1, the combined effects design included 17 trials. Table 2 shows the ANOVA data for the effects of factors on Amino nitrogen, Histamine, NaCl, Total nitrogen, and pH, as well as the corresponding coefficients of multiple determinations (R^2). The regression models had acceptable coefficients of determination and were very significant ($R^2 = 0.9129$–0.9997).

As a result, it is preferred to utilize an adj R^2 to verify the model's suitability. The models' adjusted determination coefficient values (adj $R^2 = 0.8928$–0.9994) also demonstrated that the models were highly significant. Furthermore, the coefficient of variation (CV) indicates the degree of data dispersion. Variables' CV were within a permissible range (1.09–9.87). The lower the value of CV, the greater the repeatability because it is a measure of standard deviation expressed as a percentage of the mean. In general, a large CV shows that the variety in the average value is considerable and therefore a good response model cannot be developed (Daniel & Cross, 1995). Since the lack-of-fit test, which

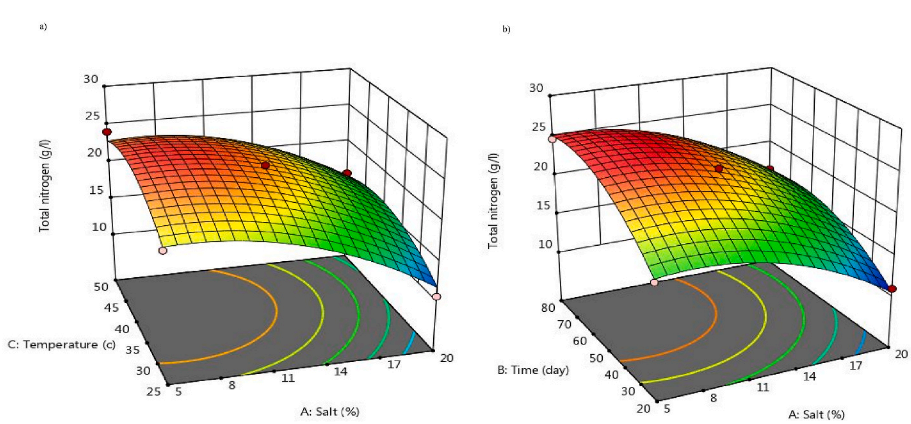

Fig. 1. Total nitrogen response surfaces for (a) salt content and temperature at 50 days, (b) salt content and time at 37.5 °C.

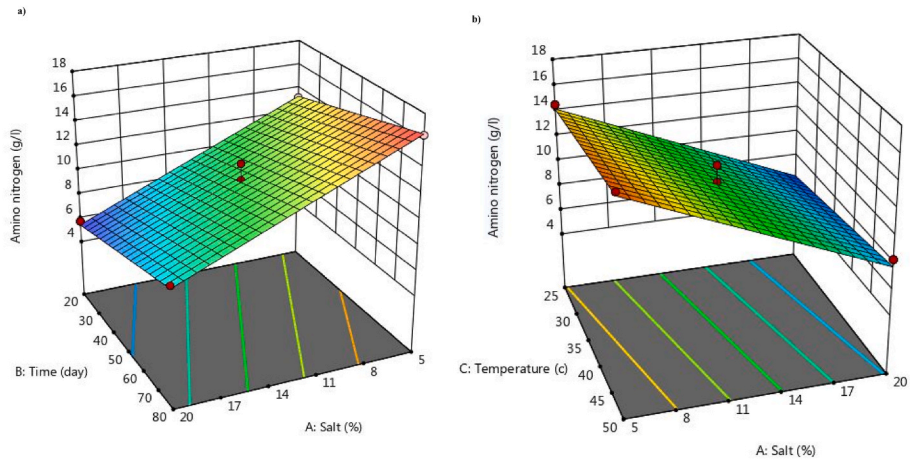

Fig. 2. Amino nitrogen response surfaces for (a) salt content and time at 37.5 °C, (b) salt content and temperature at 50 days.

evaluates the model's fitness, did not provide a significant F-value, the models' ability to forecast the Amino nitrogen, Histamine, NaCl, Total nitrogen, and pH is satisfactory.

3.2. Total nitrogen content

ANOVA data for the total nitrogen content were reported in Table 2. The model is significant with a p-value of 0.0002. As depicted in Table 2, salt concentration, fermentation time, and the temperature had a significant quadratic and linear effect on total nitrogen content ($p < 0.05$).

Three-dimensional response surface plots for the total nitrogen content of fish sauce samples are shown in Fig. 1. Salt concentration had a negative effect while time and temperature of fermentation had a positive effect on total nitrogen content. The total nitrogen content showed an overall decreasing trend as the salt content increased (Fig. 1 (a)). The total nitrogen content in fish sauce with 15% and 25% salt was reported to be 25 and 20 g/L, respectively, after 6 months of fermentation (Klomklao et al., 2006). Other studies have also found that when salt content rises, the level of total nitrogen decreases (Auttanak et al., 2022; Kim et al., 2012). The high salt content of fish sauce is a limiting factor for protein hydrolysis (Klomklao et al., 2004). According to the findings of Siringan et al. (2006), the residual autolytic activity of Indian anchovy at a concentration of 10% NaCl was around 90%. As a result, lowering the salt concentration accelerates protein hydrolysis and reduces the required time for fermentation (Klomklao et al., 2006) As shown in Fig. 1(a), the total nitrogen content of fish sauce increases as fermentation temperature increases. According to Yongsawatdigul et al. (2004), anchovies fermented at room temperature obtained a total nitrogen level of 2.1–2.3 g/100 mL after 25 weeks, but anchovies fermented at 40 °C reached a total nitrogen content of 2.0 g/100 mL after 7 weeks. This study has also shown that increasing the fermentation temperature to 45 °C enhanced the formation of total nitrogen content of fish sauce. Total nitrogen in the fish sauce was reported to be 1.0% and 1.7% after 240 days of fermentation at 25 °C and 45 °C, respectively. At temperatures higher than 45 °C, the total nitrogen concentration was just 1.3%. Increasing fermentation temperature, favors the hydrolysis process. However, the protease activity sharply decreased at temperatures over 45 °C (Kim et al., 2012). As shown in Fig. 1(b), the total nitrogen content of fish sauce increases as fermentation time increases. This might be explained by the combined effect of fish muscle autolysis and microbial degradation, which is commonly associated with the formation of fish sauce (Kilinc et al., 2006). Total nitrogen is an objective parameter for determining the quality of the fish sauce. The total nitrogen in fish sauce mainly consists of protein nitrogen and nonprotein nitrogen materials (Lopetcharat et al., 2001). The total nitrogen concentration of high-quality fish sauce must be at least 15 g/L (Thai Industrial Standard, 1983). The Codex Standard for Fish Sauce, clearly stated that the total nitrogen concentration should not be < 10 g/L (Codex Alimentarius Commission, 2013). The results showed that total nitrogen concentration in all treatments of fish sauce ranged from 10.54 to 24.65 g/L, which is higher than the minimum standard level.

3.3. Amino nitrogen

Table 2 displays the ANOVA findings for each variable's influence on the amino nitrogen content of fish sauce. Salt concentration and fermentation time had significant linear effects, while fermentation temperature had no significant effect ($p > 0.05$).

The estimated response surface for amino nitrogen in fish sauce samples is shown in Fig. 2. The fermentation time exhibited a positive linear effect on the amino nitrogen content in all treatments of fish sauce (Fig. 2 (a)). The amino nitrogen content increased with increasing fermentation time. Degradation of polypeptides by microbial and endogenous enzymes leads to the generation of low molecular weight peptides and free amino acids, and consequently amino nitrogen content increases (Park et al., 2001). According to Udomsil et al. (2015), the fermentation time and the presence of microbial proteinases can affect the amino nitrogen content. Other researchers also reported that as fermentation time progresses, the amino nitrogen content increases (Shim et al., 2022). In the fish sauce samples, the salt concentration had a negative linear influence on the amino nitrogen content (Fig. 2 (b)). The degradation of fish proteins induced by autolysis or proteinases might be slowed down by high salt concentrations. This conclusion is consistent with Kim et al. (2012), who discovered that salt concentration affected amino nitrogen level. After 240 days of fermentation, the amino nitrogen content of fish sauce with 25 and 35% salt was 810 and 618 mg/100g, respectively. Other researchers observed that when the amount of salt was increased, the level of amino nitrogen reduced considerably (Auttanak et al., 2022; Klomklao et al., 2006). The amino nitrogen content of fish sauce indicates the number of primary amino groups in fish sauce (Klomklao et al., 2006). As per the Thai Industrial Standard, the amount of amino nitrogen in fish sauce must be between 40 and 60% of the total nitrogen (Thai Industrial Standard, 1983). According to the Codex Standard, the amino nitrogen content of fish sauce shall not exceed 40% of total nitrogen (Codex Alimentarius Commission, 2013).

3.4. pH

Table 2 shows the ANOVA results for the pH values response. Due to a p-value of 0.0006 and a non-significant lack of fit, this model is suitable for forecasting. The pH values are a function of the significant linear

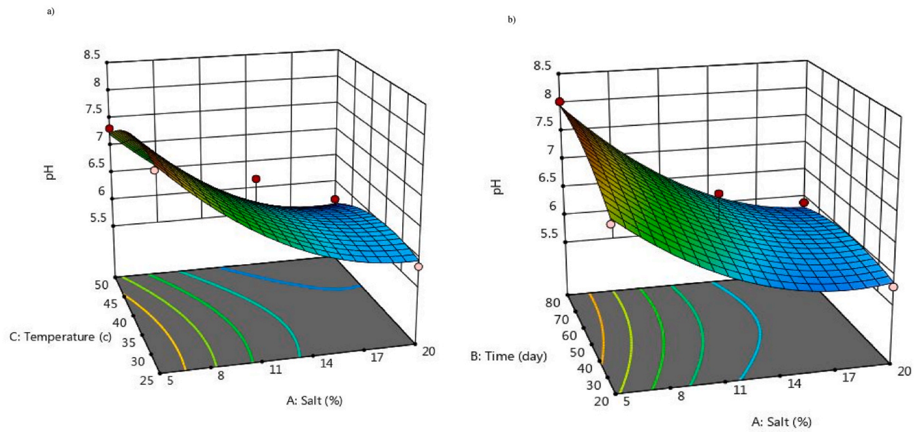

Fig. 3. pH response surfaces for (a) salt content and temperature at 50 days, (b) salt content and time at 37.5 °C.

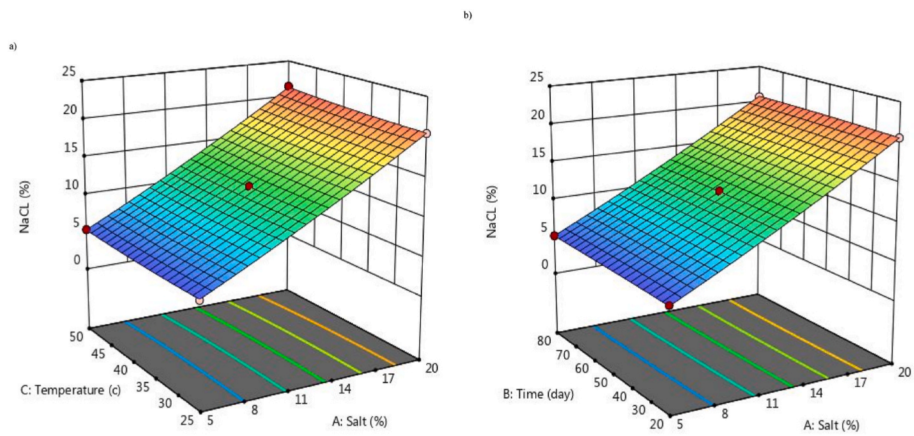

Fig. 4. NaCl response surfaces for (a) salt content and temperature at 50 days, (b) salt content and time at 37.5 °C.

influence of the fermentation temperature. The salt content had a quadratic significant effect on the pH ($p < 0.05$). However, there was no significant influence of time on pH levels ($p > 0.05$). The pH of fish sauce is regarded as one of the important quality factors. The pH values of fish sauce ranged from 5.56 to 8.02. Zarei et al. (2012) also reported the similar trend for pH of Iranian fish sauce between 4.89 and 7.55. According to the Codex Standard, the pH of fish sauce should be between 5.0 and 6.5. The response surfaces for pH values of fish sauces at different salt content, time and temperature are presented in Fig. 3 (a - b). The salt concentration and temperature showed a negative effect on the pH values of fish sauce samples. As the salt content and temperature increased, the pH values decreased ($p < 0.05$). The pH change in fish sauce could be attributed to the release of amino acids from polypeptides and proteins, the production of ammonium, and interconversions between amino acids and other molecules (Lopetcharat et al., 2001).

3.5. NaCl

One of the most important components of fish sauce is salt. Salt concentration ranged from 20 to 25% in fish sauce prepared in South

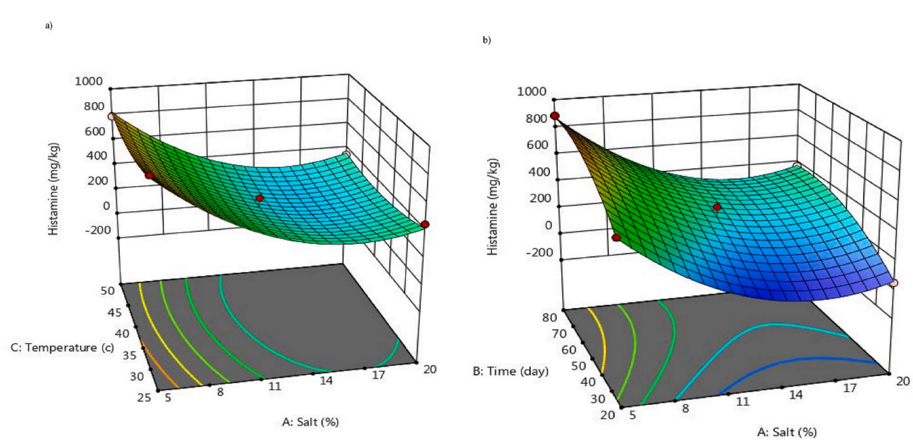

Fig. 5. Histamine content response surfaces for (a) salt content and temperature at 50 days, (b) salt content and time at 37.5 °C.

East Asian countries (He et al., 2020; Zhu et al., 2021). The Higher salt concentration caused a significant decrease in the fermentation rate. In addition, too high salt concentrations are not conducive to good health (Xu et al., 2008). According to Zarei et al. (2012), Iranian fish sauce has a salt concentration of 4.87–17.1%. The findings in Table 2 demonstrated that the linear effect of temperature, salt content and quadratic term of salt content were significant on the NaCl ($p < 0.05$).

Our findings showed that salt concentration and temperature had positive impacts on the NaCl level of the fish sauce samples (Fig. 4(a)). It has been demonstrated that the activity of fish proteases is fully dependent on ambient salt. As the salt concentration rises, enzyme activity is declined and fish tissue hardens (Beddows, 1998). Therefore, lowering the salt content improves the rate of fermentation and protein breakdown, as well as the nutritional value and sensory quality of the fish sauce (Hjalmarsson et al., 2007). However, our findings showed that the salt reduction should not be less than 5%, because samples with 5% salt exhibited poor sensory properties. At low salt concentrations, spoilage microorganisms can produce ammonia or volatile compounds (Klomklao et al., 2006). On the other hand, the fermentation time had no significant influence on the salt content of the fish sauce ($p > 0.05$) (Table 2) (Fig. 4(b)). These results correspond to those of other studies (Dissaraphong et al., 2011).

3.6. Histamine

In this research, the influences of salt, time, and temperature on the level of histamine in the fish sauce were investigated. The histamine model was significant with a p-value <0.0001 (Table 2). The results showed that salt content, time and temperature had significant quadratic and linear effects on histamine level (Table 2) ($p < 0.05$). The concentration of histamine in fish sauce increased as fermentation time progressed. While the concentration of histamine in fish sauce decreased with increasing salt content and temperature (Fig. 5). Zarei et al. (2012) found that histamine is the most prevalent biogenic amine in Iranian fish sauce. The presence of biogenic amines depends on the type of used fish (Park et al., 2001). Sardines and anchovies are rich in the amino acid histidine (Abe, 1995). As a result, histamine production is dependent on the presence of microorganisms which have decarboxylase enzyme activity and their growth conditions (Visciano et al., 2014; Zaman et al., 2011). The growth of these bacteria is inhibited at 20–25% of salt content and at high temperature (45–55 °C) (Dissaraphong et al., 2006; Kimura et al., 2001). Previous research has also demonstrated that salt aids in the breakdown of histamine by *Staphylococcus nepalensis 5-5*. This bacterium was able to decompose 16.77% and 2.51% of histamine in the concentration of 10 and 20% salt, respectively (Ma et al., 2022).

According to our findings, the fermentation temperature exhibited a considerable influence on histamine level (Fig. 5(a)). While Yongsawatdigul et al. (2004) observed that fermentation temperatures of 45 °C and ambient temperature did not influence on biogenic amines production. On the other hand, Kim et al. (2012) compared fish sauce samples fermented at 35, 40, 45 and 55 °C. They discovered that fish sauce sample fermented at 35 °C contained the least amount of histamine.

Histamine concentration rises with time, as demonstrated in Fig. 5 (b). Shim et al. (2022) also reported that the concentration of histamine in fish sauce increased with increasing the fermentation time. Other studies also confirmed that the amount of produced histamine rises as fermentation time progresses (Moon et al., 2013; Rabie et al., 2009; Zaman et al., 2011). Increasing the histamine level with extended fermentation time could be related to increased fish tissue breakdown or hydrolysis. Consequently, microorganisms get access to more free amino acids, which can be used as precursors for biogenic amines synthesis (Shukla et al., 2014).

In general, the histamine level in some of the treatments in our investigation was lower than the limits set by the European Union (EU) and Codex Alimentarius.

Table 3
Predicted and experimental values of the response variables at optimum formulation.

Response variables	Predicted values	Experimental values[a]
Total nitrogen	21.43	21.40 ± 0.1
Amino nitrogen	10.442	10.41 ± 0.1
pH	6.257	6.24 ± 0.02
NaCl	12.679	12.75 ± 0.1
Histamine	236.249	235.27 ± 1.2

[a] mean ± standard deviation.

3.7. Optimization procedure and verification of results

Numerical optimization was done using Design Expert software following the response surface evaluation. The target was to increase amino nitrogen and total nitrogen content while reducing histamine and sodium chloride levels. The optimal parameters were chosen from among the generated solutions with the highest desirability. According to the final results of this optimization, fish sauce prepared with 12.65% salt and 39.15 °C fermentation temperature for 39.79 days has a high total nitrogen content and a low histamine percentage. The model appeared to match the experimental data well since there was no significant difference between the estimated and observed values ($p < 0.05$) (Table 3).

4. Conclusion

The response surface methodology was applied in this research to optimize the parameters impacting fish sauce production with lower salt and histamine levels. The estimated regression model indicated that all of the input parameters had a significant influence ($p < 0.05$) on the outputs. Through experiments, the model was verified under optimal conditions and proved to be a good fit. The findings revealed that some samples have low salt and histamine levels with high total nitrogen and amino nitrogen levels. As a result, the optimum conditions obtained from this study could be adopted to produce a fish sauce that matches Thai and Codex standards in terms of chemical properties and histamine level.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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