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RESEARCH PAPER

Influence of Different Salt Concentrations and Addition of Commercial Enzyme on the Chemical Characteristics of Fish Sauce from Round Scad (*Decapterus maruadsi*)

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Abstract

The effects of salt concentrations and addition of Flavourzyme on the chemical characteristics of fish sauce were studied. The fermentation mixtures were prepared by mixing fish with salt at one of two levels (25% or 30%) and Flavourzyme supplementation at 0%, 0.1%, 0.3%, and 0.5% based on fish weight, and fermented at the ambient temperature ranging from 25 to 32 °C for three months. The results showed that the fish sauces with 25% salt contained total nitrogen of 17.4 - 25.3 g/L, amino acid nitrogen of 8.1 - 13.5 g/L, and ammonia nitrogen of 1.2 - 1.9 g/L. For fish sauces with 30% salt, these contents were 16.3 - 24.2 g/L, 7.2 - 12.2 g/L and 1.1 - 1.5 g/L, respectively. The fish sauces with 25% salt had amino acid nitrogen contents of 8.1 g/L for sample without Flavourzyme and 10.9 - 13.5 g/L for samples with Flavourzyme. The higher enzyme levels gave higher yield, total nitrogen, and amino acid nitrogen contents but lower ammonia nitrogen and pH values. This study suggested that the fish sauce produced using 25% salt and 0.3% Flavourzyme was appropriate. Flavourzyme could be a potential enzyme applied to improve the chemical properties of fish sauce.

Introduction

Fish sauce is a fermented traditional product in many Southeast Asian countries. It is a clear, reddish-brown liquid with a salty taste and characteristic flavor produced by the hydrolysis of fish protein. The hydrolysis of fish protein during fermentation is conducted by endogenous proteases in fish and also by proteases produced by bacteria (Gildberg & Thongthai, 2001). There are many different names for fish sauce, such as *Nam-pla* (Thailand), *Nuoc-mam* (Vietnam), *Patis* (Philippines), *Shottsuru* (Japan), *Aek-jeon* (Korean), *Budu* (Malaysia), and *Yu-lu* (China) (Lopetcharat & Park, 2002; Hariono *et al*., 2005; Jiang *et al*., 2007). Fish sauce is generally made from small species of fish, such as anchovy, sardine, scad, and other low-value fish species. In addition, raw materials such as tuna, mackerel, shrimp, squid, and clams were also used for fish sauce production (Ilmi *et al*., 2024). Traditionally, fish sauce is often produced by mixing fish with salt and fermenting at ambient tropical temperatures. The commercial production of fish sauce varies in fish species and salt ratios depending on the production area. This influences the chemical properties and quality of fish sauce. During the fermentation, the fish proteins are hydrolyzed by endogenous proteases in the fish muscle and digestive tract, as well as proteases produced by halophilic bacteria (Gildberg & Thongthai, 2001). The disadvantage of traditional fish sauce production is the very long production time, about 6 - 12 months or longer. Thus, the addition of commercial proteolytic enzymes and a decrease in salt concentration were applied in the production process to accelerate the fermentation process and shorten the production time. Some

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researchers used proteolytic enzymes such as Trypsin, Papain, and Protamex to accelerate the fermentation process (Rianingsih *et al*., 2016; Wenno & Loppies, 2019; Lee *et al*., 2020). Flavourzyme is a commercially available enzyme that has been popularly used in the hydrolysis of fish proteins. It enhances the hydrolysis of peptides to free amino acids during fermentation, minimizes bitterness, and improves the flavour of protein hydrolysate (Nilsang *et al*., 2005). Thus, Flavourzyme was added to round scad in fish sauce production in this study to evaluate its effect on the chemical properties of fish sauce. The chemical characteristics of fish sauce are evaluated by criteria such as total nitrogen, amino acid nitrogen, ammonia nitrogen content, pH value, and sodium chloride content. There is very little literature regarding the production and properties of fish sauce made from round scad, while that of fish sauce made from anchovy or sardine is very available. The purpose of this study was to determine the influence of different salt concentrations and the addition of Flavourzyme on the chemical characteristics of fermented round scad sauce.

Materials and Methods

Materials

The raw material used for fish sauce production was round scads (*Decapterus maruadsi*) caught in the Nha Trang Sea, Vietnam. Fresh round scads were washed and minced using a grinder.

Flavourzyme is a fungal protease/peptidase complex produced by the submerged fermentation of a selected strain of *Aspergillus oryzae.* Flavourzyme contains both endoprotease and exopeptidase activities. The optimal pH for the enzyme complex is in the range of 5.0 - 7.0. Flavourzyme is standardized in Leucine Amino Peptidase Units per gram (LAPU/g). The declared activity of Flavourzyme is 500 LAPU/g.

Fish Sauce Production

An experimental diagram of fish sauce production from round scad is indicated in Figure 1. Eight batches of fish sauce were experienced. The minced round scad was mixed with different levels of salt (25% and 30%, w/w). 1000 g of round scad was mixed with 250 g of salt (25% w/w), and 1000 g of round scad was mixed with 300 g of salt (30% w/w). For each salt level, the four batches were added with Flavourzyme 0%, 0.1%, 0.3%, and 0.5% based on fish weight (w/w). The mixtures were transferred to glass jars, covered with polyethylene plastic, closed hermetically, and fermented at the ambient temperature ranging from 25 to 32°C and natural pH (initial pH 6.5) for 3 months with weekly stirring of the mixture. At the end of the fermentation period, the mixtures in glass jars were filtered with cloth to obtain the fish sauces. These fish sauces were then filtered through filter papers, and the filtrates were used for chemical analysis.

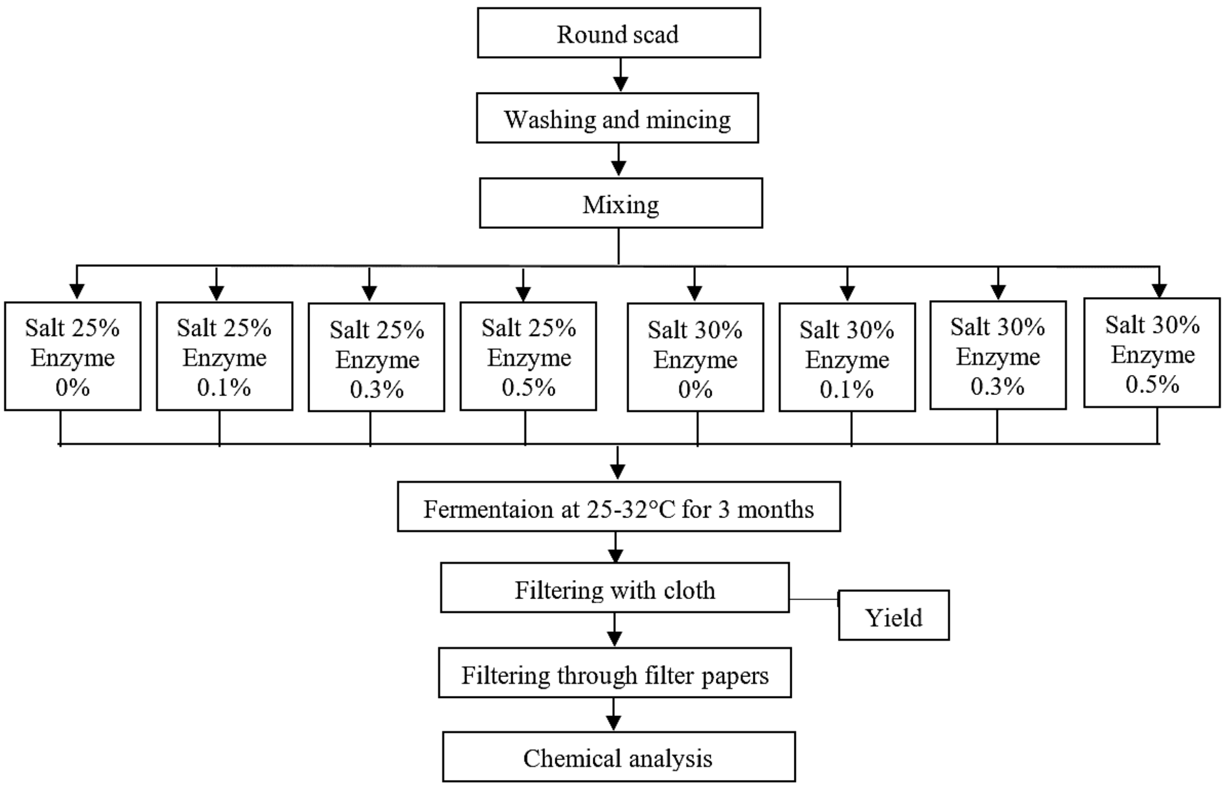

Figure.1. Experimental diagram of fish sauce production from round scad.

Chemical Analysis

The contents of moisture and ash were determined by the method of AOAC (2000). The total nitrogen content was determined by the Kjeldahl method (AOAC, 2000), and the crude protein content was estimated by multiplying the total nitrogen content by 6.25. The lipid content was determined according to the method of Folch *et al*. (1957). The ammonia nitrogen and formaldehyde nitrogen contents were determined as described by Klomklao *et al*. (2006). Amino acid nitrogen was calculated as the difference between formaldehyde and ammonia nitrogen contents (Klomklao *et al*., 2006). The sodium chloride was determined as described by Le *et al*. (2021). The pH values of the fish sauce samples were measured with a pH meter. The yield of fish sauce was calculated as the percentage of the weight of fish sauce divided by the weight of round scad.

Statistical Analysis

A statistical program (SPSS, SPSS Inc., Chicago, IL, USA) and Microsoft Excel were used for data processing and statistical analysis. The data were subjected to an analysis of variance (ANOVA). Duncan's multiple range test is a multiple comparison procedure that was used to determine if significant differences existed between treatment means. Differences in treatment means were considered significant at $P<0.05$. All experiments were conducted with three replicates. The values were presented as mean ± standard deviation.

Results

Proximate Chemical Compositions of Round Scads

The proximate chemical compositions of round scads are given in Table 1. The round scads had a large amount of moisture (74.5%), a high content of protein (17.2%), a low quantity of lipid (4.1%), and a low content of ash (3.3%).

Yield of Fish Sauce

The yield of fermented fish sauces with and without Flavourzyme in the different salt concentrations is presented in Table 2. The salt concentration and enzyme addition during the fish sauce fermentation affected the yield of fish sauce. The yield of fish sauce with 30% salt levels was higher than that with 25% salt levels at the same enzyme concentration. The yield of fish sauces with Flavourzyme addition was higher than that without Flavourzyme addition. The higher salt and enzyme levels gave a higher yield of fish sauce. Indeed, after 3 months of fermentation, for the fish sauce samples without Flavourzyme supplementation, the yields of fish sauces with 25% and 30% salt levels were 40.2% and 41.1%, respectively, while the yield of fish sauce with 25% salt and Flavourzyme addition reached 41.7-42.8%. In the case of 30% salt, these values were 42.7-43.5%.

Total Nitrogen

One of the most important quality parameters for fish sauce is the total nitrogen content (Lopetcharat & Park, 2002). The total nitrogen content of fermented fish sauces with and without Flavourzyme in the different salt concentrations is presented in Table 2. The total nitrogen content in fish sauce was greatly influenced by the level of salt. Indeed, the total nitrogen content of fish sauce samples with 25% salt was significantly higher than that of fish sauce samples with 30% salt at the same level of Flavourzyme. After 3 months of fermentation, the total nitrogen content reached 17.4 g/L for the fish sauce sample with 25% salt and 16.3 g/L with 30% salt and without Flavourzyme addition. The total nitrogen contents in fish sauce samples with Flavourzyme addition were significantly higher than those in fish sauces without Flavourzyme addition. The fish sauces with Flavourzyme addition had total nitrogen contents of 21.6-25.3 g/L for the samples with 25% salt and 20.2-24.2 g/L for the samples with 30% salt. In comparing the total nitrogen of fish sauce among three Flavourzyme concentrations, the fish sauce with 0.5% Flavourzyme had the highest value, followed by that with 0.3%, and then 0.1%. However, there was no significant difference in total nitrogen content between the fish sauce samples added Flavourzyme at 0.3% and 0.5%.

Amino Acid Nitrogen

The study results indicated that the amino acid nitrogen contents of fermented fish sauces ranged from 7.2 to 13.5 g/L (Table 2). The fish sauce with 25% salt had a higher content of amino acid nitrogen than that with 30% salt at the same level of Flavourzyme. The fish sauces with Flavourzyme supplementation had a higher content of amino acid nitrogen than those without Flavourzyme addition. The amino acid nitrogen content of fish sauce with 25% salt without Flavourzyme addition was 8.1 g/L, but fish sauces with Flavourzyme

Table 1. Proximate chemical compositions of round scads (*Decapterus maruadsi*)

Chemical compositions	Content (%)
Moisture	74.5±0.4
Crude protein	17.2±0.3
Lipid	4.1±0.3
Ash	3.3±0.2

* Data are given as mean values ± SD (n=3).

Table 2. Chemical characteristics of round scad (*Decapterus maruadsi*) fish sauce

Parameters	Salt 25%				Salt 30%			
	Flavourzyme				Flavourzyme			
	0 %	0.1 %	0.3 %	0.5 %	0 %	0.1 %	0.3 %	0.5 %
Yield (%)	$40.2^{a}\pm0.1$	$41.7^{c}\pm0.2$	$42.5^{d}\pm0.2$	$42.8^{e}\pm0.1$	$41.1^{b}\pm0.1$	$42.7^{de}\pm0.2$	$43.3^{f}\pm0.2$	$43.5^{f}\pm0.2$
Total nitrogen (TN) (g/L)	$17.4^{b}\pm0.3$	$21.6^{d}\pm0.2$	$24.8^{fg}\pm0.5$	$25.3^{g}\pm0.5$	$16.3^{a}\pm0.5$	$20.2^{c}\pm0.3$	$23.7^{e}\pm0.6$	$24.2^{ef}\pm0.6$
Amino acid nitrogen (AAN) (g/L)	$8.1^{b}\pm0.2$	$10.9^{d}\pm0.4$	$12.9^{f}\pm0.3$	$13.5^{f}\pm0.4$	$7.2^{a}\pm0.4$	$9.8^{c}\pm0.3$	$11.6^{e}\pm0.6$	$12.2^{e}\pm0.5$
AAN to TN ratio (%)	$46.6^{b}\pm0.4$	$50.5^{de}\pm1.4$	$52.0^{ef}\pm0.7$	$53.4^{f}\pm0.8$	$44.2^{a}\pm0.9$	$48.5^{c}\pm0.9$	$48.9^{cd}\pm1.3$	$50.4^{cde}\pm1.3$
Ammonia nitrogen (g/L)	$1.9^{e}\pm0.2$	$1.6^{d}\pm0.1$	$1.4^{bcd}\pm0.1$	$1.2^{ab}\pm0.1$	$1.5^{cd}\pm0.1$	$1.3^{abc}\pm0.1$	$1.1^{a}\pm0,2$	$1.1^{a}\pm0.1$
pH	$5.9^{d}\pm0.1$	$5.6^{bc}\pm0.1$	$5.5^{ab}\pm0.1$	$5.5^{ab}\pm0.2$	$5.8^{cd}\pm0.1$	$5.4^{ab}\pm0.1$	$5.4^{ab}\pm0.1$	$5.3^{a}\pm0.1$
Sodium chloride (g/L)	$253.7^{c}\pm2.0$	$251.5^{bc}\pm1.2$	$248.8^{ab}\pm1.6$	$246.4^{a}\pm2.6$	$272.2^{f}\pm1.6$	$269.9^{ef}\pm1.7$	$267.6^{de}\pm1.3$	$265.1^{d}\pm1.4$

* Data are given as mean values ± SD (n = 3). The mean values in the same row with different superscript letters are significantly different (P<0.05).

addition had amino acid nitrogen contents of 10.9-13.5 g/L. For the fish sauces with 30% salt, the amino acid nitrogen content was 7.2 g/L for sample without Flavourzyme and 9.8-12.2 g/L for samples with Flavourzyme addition. For fish sauces without enzyme supplementation and with 25% and 30% salt, the amino acid nitrogen to total nitrogen ratios were 46.6% and 44.2%, respectively. These ratios reached 50.5-53.4% for fish sauces with 25% salt and 48.5-50.4% for fish sauces with 30% salt and enzyme addition. At the same level of salt, the fish sauce with 0.5% Flavourzyme had the highest amino acid nitrogen content, followed by that with 0.3% Flavourzyme and then 0.1% Flavourzyme. There was no significant difference in amino acid nitrogen content between the fish sauces with 0.3% and 0.5% Flavourzyme. This study revealed that the fish sauce produced with Flavourzyme supplementation at 0.3% was enough and appropriate.

Ammonia Nitrogen

Ammonia is one of the main components of the volatile bases that give off the ammonia smell. Ammonia is always present in fish sauces; it is one of the products of the degradation of nitrogenous substances, particularly amino acids (Lopetcharat & Park, 2002). Thus, not only peptides and free amino acids but also ammonia were formed during the fermentation process. The ammonia nitrogen content of fermented fish sauces is shown in Table 2. The ammonia nitrogen content of fish sauce was affected by salt concentration. The ammonia nitrogen contents of fish sauces with 30% salt were lower than those of fish sauces with 25% salt. The fish sauces with Flavourzyme supplementation had a lower content of ammonia nitrogen than those without Flavourzyme. The ammonia nitrogen content of fish sauce had a decreasing trend when the enzyme concentration increased. In this study, the ammonia nitrogen content of fermented fish sauces ranged from 1.1 to 1.9 g/L.

pH

The pH of fish sauce is also an important characteristic in identifying the quality of fish sauce. As can be seen from Table 2, the pH values of fermented fish sauces were within the range of 5.3 to 5.9. At the same enzyme concentration, there was no significant difference in pH value between fish sauces with 25% salt and those with 30% salt. The pH value of fish sauces with Flavourzyme addition was significantly lower compared to fish sauces without Flavourzyme.

Sodium Chloride

The use of salt in fish sauce manufacturing inhibits microbial activity and prevents the spoilage of fish during the fermentation process. The sodium chloride contents of fermented fish sauces were within the range of 246.4 to 272.2 g/L (Table 2). The 25% addition of salt to the round scad gave the fish sauces sodium chloride contents of 246.4-253.7 g/L. The 30% addition of salt to the round scad gave the fish sauces sodium chloride contents of 265.1-272.2 g/L.

Discussion

The species of fish and their chemical compositions affect the quality of fish sauce. Generally, species of fish with a high moisture content give the fish sauce a low total nitrogen content. Moreover, the species of fish having a high protein content give fish sauce a high amino acid content (Tungkawachara *et al*., 2003). The round scad with a high protein content and a low lipid content was suitable for fish sauce production due to its low oxidation. The formation of volatile components, such as aldehydes, ketones, and alcohols, largely depends on the lipid contents of fish and fatty acid oxidation during fish sauce fermentation. Xiao *et al*. (2020) indicated that fatty fish species with a high content of lipids are highly susceptible to oxidation. The excessive oxidation of fatty acids induces off-flavors in fermented fish. Ding *et al.* (2020) showed that the flavour of fish sauces made from three different fish species was significantly different.

In this study, the Flavourzyme addition and high salt level led to an increase in the yield of fish sauce. These results were in line with the study of Rianingsih *et al*. (2016), who indicated that the yield of fish sauce from sea catfish viscera with Trypsin enzyme addition was higher than that without Trypsin addition. The higher level of salt gave a higher yield of fish sauce.

These authors showed that the yield of fish sauce with 15% salt was 40.55% for the sample without Trypsin addition and 45.58% for the sample with 0.3% Trypsin addition. In the case of 20% salt, the yield of fish sauce was 43.74% for the sample without Trypsin and 48.54% for the sample with a 0.3% Trypsin addition.

It has been shown that proteases play an important role in the proteolysis and solubilization of proteins in fish during fish sauce fermentation. Pepsin, Trypsin, and Cathepsin are responsible for the solubilization of proteins in fish (Tungkawachara *et al*., 2003; Klomklao *et al*., 2006). The addition of enzymes during the fish sauce fermentation accelerated the hydrolysis of fish protein, thus, increasing the yield of the final product. Study results demonstrated that the addition of Flavourzyme speeded up the process of round scad liquefaction by increasing proteolysis. The high salt concentration contributed to an increase in the liquefaction rate of the fish during the fermentation. It was probably due to the diffusion-osmosis phenomenon. The salt enters the tissue of the fish, and water from the fish is released as a result of osmotic pressure, leading to an increase in the yield of fish sauce (Rianingsih *et al*., 2016).

The total nitrogen of fish sauce includes amino acid nitrogen, peptide nitrogen, and ammonia nitrogen, as well as the nitrogen of other substances such as urea, trimethylamine oxide, and nucleotides (Jiang *et al*., 2007; Tungkawachara *et al*., 2003). During the fermentation process, the fish proteins are hydrolyzed into peptides and amino acids. The proteases of fish play an important role in proteolysis during fish sauce fermentation (Orejana & Liston, 1982). The microbial enzymes also contribute to proteolysis during the fish sauce fermentation. The addition of Flavourzyme led to a higher content of total nitrogen in the fermented fish sauce compared to the sample without Flavourzyme addition. This result was similar to that reported by Le *et al*. (2021), who indicated that the addition of Protex 51FP also led to a higher content of total nitrogen in anchovy fish sauce compared to fish sauce without Protex 51FP addition. According to Le *et al*. (2021), the total nitrogen content of anchovy fish sauces after 6 months of fermentation was within the range of 18.67 - 21.23 g/L. Chaveesuk *et al*. (1994) also stated that the herring fish sauce with Trypsin and Chymotrypsin additions had significantly higher total nitrogen and amino acid contents than fish sauce without the added enzyme. The addition of enzymes reduced the fermentation time to two months without affecting the quality of the fish sauce (Chaveesuk *et al*., 1994). Saisithi *et al*. (1966) showed 18 g/L of total nitrogen in Nam-pla after 9 months of fermentation. Beddows *et al*. (1979) reported 17.7 g/L of total nitrogen in Budu after 154 days of fermentation. Wang *et al*. (2017) indicated that the anchovy sauce contained 0.89 g/100 mL of amino acid nitrogen. According to Gildberg and Thongthai (2001), the amino acid nitrogen to total nitrogen ratio is used as an indicator of hydrolysis degree. Tungkawachara *et al*. (2003) reported that the amino acid nitrogen to total nitrogen ratio of fish sauce from Pacific whiting was about 50-52% after 3 months of fermentation. The total nitrogen and amino acid nitrogen contents in the different types of fish sauce have been variable and depend on the raw material as well as the processing conditions such as temperature, salt concentration, and fermentation time.

The higher amount of salt (30%) inhibited the activity of proteolytic enzymes and retarded the hydrolysis of fish proteins; hence, the fish sauce had lower contents of total nitrogen and amino acid nitrogen. It would be recommended that using salt at 25% was appropriate for fish sauce fermentation. Gildberg *et al*. (1984) also demonstrated the inhibitory action of the salt on proteolysis, in particular when the salt contents exceeded 25%.

The addition of Flavourzyme accelerated the hydrolysis of fish proteins into peptides and free amino acids. A higher amount of total nitrogen in fish sauces with Flavourzyme supplementation showed that proteolytic activity was well conducted during the fermentation process. The release of a higher amount of amino acids in fish sauce with enzyme addition was mainly due to the breakdown of peptides by Flavourzyme. According to Chae *et al*. (1998), Flavourzyme was a peptidase enzyme that speeded up the hydrolysis of peptides into amino acids. The results of this study demonstrated that Flavourzyme was useful in accelerating the hydrolysis of fish proteins during fermentation and increasing the amino acid nitrogen content.

The higher salt level (30%) and Flavourzyme addition gave the fermented fish sauce a lower ammonia nitrogen content. These study results were similar to those of Rianingsih *et al*. (2016), who reported that the higher salt and trypsin enzyme addition gave lower ammonia nitrogen content in fish sauce from sea catfish viscera. According to Klomklao *et al*. (2006), the ammonia content of fish sauce depends on the initial quality of the raw material and salt concentration. These authors showed that high ammonia nitrogen contents were reported in fish sauces with a low salt concentration. Le *et al*. (2021) indicated that the ammonia nitrogen contents of anchovy fish sauces were 2.89 - 3.55 g/L after 6 months of fermentation. The formation of ammonia or volatile base compounds in fish sauce might take place through the activity of spoilage microorganisms (Klomklao *et al*., 2006). The growth of specific spoilage bacteria such as *Shewanella putrefaciens, pseudomonas, Photobacterium phosphoreum*, and *Vibrioaceae* is associated with the formation of ammonia and trimethylamine (Gram & Huss, 1996). For the fish sauces with 30% salt, the low content of ammonia nitrogen could be due to the preservative action of salt. The high salt concentration inhibited the growth of microorganisms that produce ammonia; thus, the ammonia nitrogen content was low in fish sauces with 30% salt. The same trend was also reported in the study of Besas and Dizon (2012), wherein total volatile base nitrogen decreased as the salt concentration increased. The fish sauce samples from tuna viscera with 10%, 17.5%, and 25% salt had total volatile base nitrogen contents of 226.80, 81.60, and 76.41 mg/100 g, respectively (Besas & Dizon, 2012).

The fish sauces with Flavourzyme addition had lower pH values compared to those without Flavourzyme. The addition of enzymes accelerated the hydrolysis of fish proteins, enhanced free hydrogen ions, and decreased the pH value. The low pH value of fish sauces with Flavourzyme addition is probably due to the formation of organic acids such as lactic acid and acetic acid produced by microorganisms during fermentation. With the lower pH value, it might inhibit the putrefaction bacteria that produce ammonia, leading to a lower ammonia nitrogen content in fish sauce. Mueda (2015) showed that the pH of anchovy fish sauce decreased from the initial pH of 6.99 to the final value of 6.05 after 270 days of fermentation. Fatimah *et al*. (2017) stated that the pH of Bakasang, an Indonesian fish sauce, ranged from 5.66 to 6.73. The pH of fish sauce from Pacific whiting was 5.42 after 9 months of fermentation (Tungkawachara *et al*., 2003). A pH of 6.08 was observed in the fish sauce from Gambusia after 150 days of fermentation (Ibrahim, 2010). The difference in pH of fish sauces was due to different protein hydrolysis and free hydrogen ions.

The sodium chloride contents of the fermented fish sauces in this study were higher than those of the fish sauce from anchovies (Le *et al*., 2021) and sea catfish viscera (Rianingsih *et al*., 2016). Le *et al*. (2021) reported that the sodium chloride content of the anchovy fish sauce was within the range of 218.53 - 231.24 g/L. Rianingsih *et al*. (2016) showed the sodium chloride content of fish sauces made from sea catfish viscera ranged from 17.94 to 24.78%. The salt content of the fish sauce made from tuna loin by-products was 26.57% (Wenno & Loppies, 2019). The high content of sodium chloride in the fish sauce can protect the product against microorganisms and prolong the shelf life of the fish sauce. The low content of sodium chloride (below 20%) can potentially lead to low-quality fish sauce.

Conclusions

The salt and Flavourzyme levels greatly affected the hydrolysis of fish proteins and the chemical characteristics of fish sauce made from round scad. The total nitrogen and amino acid nitrogen contents of fish sauce with 25% salt were higher than those with 30% salt. The fish sauces with 25% salt and the addition of Flavourzyme had contents of total nitrogen of 21.6-25.3 g/L and amino acid nitrogen of 10.9-13.5 g/L. But for fish sauces with 30% salt and the addition of Flavourzyme, these contents were 20.2-24.2 g/L and 9.8-12.2 g/L, respectively. The fish sauces with Flavourzyme addition had a higher amino acid nitrogen content than those without Flavourzyme. The addition of commercial Flavourzyme accelerated the hydrolysis of fish protein, leading to an increase in the yield and chemical quality of fish sauce. This study indicated that the fermented fish sauces with a salt level of 25% and Flavourzyme additions of 0.3% or 0.5% had high quality.

Ethical Statement

Not applicable.

Funding Information

Not applicable

Author Contribution

Huong Thi My Nguyen performed the experiments, analyzed data, and wrote the paper.

Conflict of Interest

The author declare no competing financial interest.

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