Title	Effects of the addition of spleen of skipjack tuna (<i>Katsuwonus pelamis</i>) on the liquefaction and characteristics of fish sauce made from sardine (<i>Sardinella gibbosa</i>)
Author(s)	Klomklao, Sappasith; Benjakul, Soottawat; Visessanguan, Wonnop; Kishimura, Hideki; Simpson, Benjamin K.
Citation	Food Chemistry, 98(3), 440-452 https://doi.org/10.1016/j.foodchem.2005.06.013
Issue Date	2006
Doc URL	http://hdl.handle.net/2115/30279
Type	article (author version)
File Information	FC98-3.pdf

[Instructions for use](#)

**Effect of Skipjack Tuna Spleen on the Liquefaction and
Characteristics of Sardine Fish Sauce**

To be submitted to

Food Chemistry

Sappasith Klomklao¹, Soottawat Benjakul^{1*}, Wonnop Visessanguan²,

Hideki Kishimura³ and Benjamin K. Simpson⁴

¹*Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand.*

²*National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholayothin Rd., Klong 1, Klong Luang, Pathumthani, 12120, Thailand.*

³*Graduate School of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, 041-8611, Japan*

⁴*Department of Food Science and Agricultural Chemistry, McGill University, Macdonald campus, 21111 Lakeshore Road, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9.*

*To whom correspondence should be addressed. Tel: 66-7428-6334.

Fax: 66-7421-2289, e-mail: soottawat.b@psu.ac.th

Abstract

The effects of skipjack tuna (*Katsuwonus pelamis*) spleen addition at various levels (0, 10 and 20%) on the liquefaction and characteristics of sardine fish sauce produced with different salt concentrations (15, 20 and 25%) were monitored during fermentation of 180 days. Fish sauce prepared from sardine with spleen supplementation contained greater total nitrogen, amino nitrogen, formaldehyde nitrogen, ammonia nitrogen content, compared to those without spleen addition throughout the fermentation. The rate of liquefaction was dependent upon the amount of spleen added. Reduction of salt content accelerated the hydrolysis of fish protein during fermentation. The liquefaction rate of the lower salt treated samples was generally faster than those treated with higher salt content. Among all treatments, sardine added with 25% spleen and 15% salt exhibited the greatest protein hydrolysis, particularly at early stages, suggesting the combination effects of autolysis and spleen proteinase. The greater liquefaction was coincidental with the development of browning as well as the increase in redness of liquid formed. Acceptability test revealed that the samples were different in color, aroma, taste as well as overall acceptance ($p < 0.05$). Fish sauce samples containing 20% salt without and with 10% spleen addition had the similar acceptability with commercial fish sauce. Therefore, addition of spleen as well as salt reduction can accelerate the liquefaction of sardine for fish sauce production.

Keywords: Fish sauce; Spleen; Fermentation; Acceleration; Proteinase; Sardine; Liquefaction

1. Introduction

Fish sauce fermentation is a common practice in Southeast Asia as a means of preservation and producing value-added products from underutilized fish species. Fish sauce has become more interesting for consumers in Europe, North America and other countries (Brillantes, 1999). As a consequence, fish sauce rapidly expands in both domestic and foreign markets. There are approximately 390 fish sauce factories in Thailand with 64,000 metric ton of fish used annually for fish sauce production (Saisithi, 1994).

Fish sauce is a clear brown liquid hydrolysate from salted fish and is commonly used as a flavor enhancer or salt replacement in various food preparations (Lopetcharat, Choi, Park & Daeschel, 2001). Generally, fish sauce is produced by adding the salt to the fish with the ratios of fish to salt of 2:1 or 3:1. The salt/fish mixture is kept in the concrete tank at the temperature range of 35-40°C. A great variety of raw materials can be used for fish sauce production and the proteolytic enzymes must be sufficient for tissue or protein solubilisation. Fish sauce is generally made from small pelagic species such as anchovies and sardines (Amano, 1962; Saisithi, 1994, Gildberg, 2001). During fermentation, protein are hydrolysed, mainly as a result of autolytic action by the digestive proteases in fish (Orejana & Liston, 1982; Sikorski, Gildberg & Ruiter, 1995). Fermentation process normally takes a long time to ensure the solubilisation as well as the flavor and color development of fish sauce. In Thailand, fish sauce is manufactured through fermentation up to 18 months (Lopetcharat & Park, 2002). To accelerate the solubilisation process, lowering the pH and salt content was conducted to enhance the rapid autolysis, particularly caused by trypsin and chymotrypsin (Gildberg, Espejo-Hermes & Magno-Oregana, 1984; Beddows & Ardesir, 1979; Chaveesuk, Smith & Simpson, 1993). The fish sauce was

obtained after 6 months of fermentation with the addition of 5 to 10% enzyme-rich (trypsin and chymotrypsin) cod intestines into minced capelin (Gildberg, 2001).

Fish viscera is a potential source of various proteinases (Simpson, 2000). Apart from other digestive enzymes, Klomklao, Benjakul and Visessanguan (2004) found that skipjack tuna spleen contained high proteolytic activity which was identified as trypsin-like serine proteinase. Accordingly, spleen from skipjack tuna, generally considered as the waste from tuna processing plant, can be used as the novel source of proteinase for acceleration of fish sauce production. However, no information regarding the use of skipjack tuna spleen to accelerate protein hydrolysis and its effect on the characteristics of fish sauce has been reported. Our objective was to determine the effects of skipjack tuna spleen and salt concentration on the hydrolysis of fish protein as well as the chemical and physical changes of fish sauce obtained during fermentation up to 180 days.

2. Materials and Methods

2.1 Chemicals

Sodium caseinate, β -mercaptoethanol (β ME), *L*-tyrosine and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). Trichloroacetic acid, sodium chloride, tris (hydroxymethyl) aminomethane and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), Coomassie Blue R-250 and *N,N,N',N'*-tetramethyl ethylene diamine (TEMED) were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2 Fish sample preparation

Internal organs from skipjack tuna (*Katsuwonus pelamis*), obtained from Chotiwat Industrial Co. (Thailand) Ltd., Songkhla, were packed in polyethylene bag (5 kg each) kept in ice, and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 30 min. Pooled internal organs were then excised and separated into individual organs. Only spleen was collected, immediately frozen and stored at -20°C until used.

Sardine (*Sardinella gibbosa*), with average body weight of 55-60 g, was caught from Songkhla-Pattani Coast along the Gulf of Thailand. The fish, off-loaded approximately 12 h after capture, were placed in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat-Yai within 2 h. The fish samples were kept on ice until needed.

2.3 Fish sauce preparation

Sardine and frozen spleen were ground separately using a meat grinder with 5 mm plate. Ground sardine (200 g) was mixed with the different levels of solar salt (15,

20 and 25% w/w). For each salt level, one batch was used as the control and the others were mixed with different levels of ground spleen (0, 10 and 20% w/w). The mixtures were transferred to glass bottles and covered with polyethylene film. The containers were placed in an incubator at 37°C. The liquid formed was taken for analysis at 5, 10, 20, 30, 60, 90, 120, 150 and 180 days.

2.4 Collection of liquid

After incubating for the designated time, the samples were centrifuged for 15 min at 7,700×g using a Sorval Model RC-Plus centrifuge (Newtown, CT). The fat layer was separated from the aqueous layer, which was again filtered using a Whatman filter paper No. 4. The filtered liquid obtained was used for analysis.

2.5 Measurements of proteinase activity

At the time designated, fish sauce sample was dialysed with 10 volumes of distilled water at 4°C for 24 h to remove salt prior to the proteolytic activity assay. Proteolytic activity was measured using casein-TCA-Lowry assay (Klomklao et al., 2004). To initiate the reaction, 200 µl of dialysed fish sauce sample was added into assay mixtures containing 2 mg of casein, 200 µl of distilled water and 625 µl of reaction buffer (0.1 M glycine-NaOH, pH 9.0). Enzymatic reaction was conducted at 55°C for 15 min and terminated by adding 200 µl of 50% (w/v) trichloroacetic acid (TCA). Unhydrolysed protein substrate was allowed to precipitate for 15 min at 4°C, followed by centrifuging at 7,000×g for 10 min (Hettich zentrifugen, Berlin, Germany). The oligopeptide content in the supernatant was determined by the Lowry assay (Lowry, Rosebrough, Fan & Randall, 1951) using tyrosine as a standard. Activity was expressed as tyrosine equivalents in TCA-supernatant. One unit of

activity was defined as that releasing 1 mmole of tyrosine per min (mmol/Tyr/min). A blank was run in the same manner, except the enzyme was added after addition of 50 %TCA (w/v).

2.6 Chemical analysis

2.6.1 Total nitrogen

Total nitrogen content of fish sauce samples was measured using Kjeldahl method (AOAC, 1999). Total nitrogen content was expressed as g nitrogen/l.

2.6.2 Ammonia nitrogen, formaldehyde nitrogen and amino nitrogen

Amino nitrogen, ammonia nitrogen and formaldehyde nitrogen contents were determined as described by Thai Industrial Standard (1983).

Formaldehyde nitrogen was determined by the titration method. One ml of sample was mixed with 9 ml of distilled water and titrated to pH 7.0 with 0.1 N NaOH. Ten ml of formaldehyde solution (38% v/v, pH 9.0) was then added to the neutralised samples. Titration was continued to pH 9.0 with 0.1 N NaOH. Formaldehyde nitrogen content was calculated as follows:

$$\text{Formaldehyde nitrogen content (g/l)} = \text{ml (NaOH}_{\text{pH } 7 - \text{pH } 9}) \times 0.1 \times 14$$

To determine ammonia nitrogen, 50 ml of 10-fold diluted samples were transported to Kjeldahl flask containing 100 ml of distilled water and 3 g of MgO. The mixture was distilled to release volatile nitrogen into 50 ml of 4% boric acid containing methyl red bromocresol green. The distillate was finally titrated with 0.05 H₂SO₄ until the end-point was obtained. Ammonia nitrogen content was calculated as follows:

Ammonia nitrogen content (g/l) = $5.6 \times 0.05 \times Y$

where Y is the volume of H₂SO₄ (ml)

Amino nitrogen content was calculated using the following formula:

Amino nitrogen content (g/l) = Formaldehyde nitrogen content – Ammonia nitrogen content

2.7 Physical analysis

2.7.1 Color

Color characteristics of the samples were determined by measuring the L* a* and b* values using Hunter Lab (Color Flex, Hunter Associates Laboratory, Verginia, USA), according to the CIE Lab scale. The system provides the values of three color components: L* (black-white component, luminosity), a* (+red to –green component) and b* (+yellow to –blue component). Samples (15 ml) were pipetted into a glass Petri dish (5 cm diameter). The sample was illuminated with D65-artificial daylight (10° standard angle) according to the procedure provided by the manufacture. The color determination was conducted in four replications for each sample.

2.7.2 Nonenzymatic browning

Nonenzymatic browning of samples was determined by measuring melanoidin pigment formation using the method of Hoyle and Merritt (1994) with a slight modification. Five ml of sample were mixed with 50 ml of 50% (v/v) ethanol and the mixture was stirred for 1 h at 25°C. The sample was then centrifuged for 30 min at 7,700×g using a Sorval Model RC-B Plus centrifuge (Newtown, CT). The supernatant obtained was subjected to absorbance measurement at 420 nm using a UV-1601

spectrophotometer (Shimadzu, Kyoto, Japan). A_{420} was used as an index for browning intensity.

2.8 Acceptability test

The fish sauce samples obtained after 180 days of fermentation as well as commercial fish sauce (first grade Nampla) were evaluated for acceptance by an untrained 50-member panel according to the method of Chamber and Wolf (1996). The panelists were graduate students in Food Technology from the Faculty of Agro-Industry, Prince of Songkla University, with age ranging from 20 to 35 years. All panelists had sensorial acquaintance with fish sauce. Panelists were asked to give acceptance scores for 4 attributes: color, aroma, taste and overall acceptance using the 9-point hedonic scale. A nine-point hedonic scale, in which a score of 1 represented extreme dislike, 5 represented neither like nor dislike and 9 represented like extremely, was used for evaluation. The fish sauce (10 ml) was poured into glass and the samples were covered with aluminum foil. Cooked chicken in uniform strips (1 cm width) was used for dipping fish sauce samples. Samples were coded with three-digit random numbers and presented to the panelists at ambient temperature. Fish sauce samples were served to each panelist in a random order. During evaluation, the panelists were situated in private booths. Room-temperature drinking water and sliced white bread were used to rinse the mouth between samples.

2.9 Statistical Analysis

A completely randomized design was used throughout this study and the experiments were done in duplicate. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's Multiple Range Test

(Steel & Torrie, 1980). Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for Windows; SPSS Inc.).

3. Results and Discussion

3.1 Proteinase activity

Changes in proteinase activities of all fish sauce samples from different treatments during fermentation are shown in Fig. 1. The activity decreased continuously as the fermentation time increased ($p < 0.05$) (Fig. 1). However, the rate of changes varied, depending on the salt concentration and spleen level added. No changes in activity were found in the samples added with 20 % spleen and 25% salt in the first 20 days of fermentation ($p > 0.05$). The decrease in activity might be due to the denaturation of enzymes, particularly in presence of high salt content. The loss in activity was also thought to be due to the inhibition by end products, such as amino acid and short chain peptides (Orejana et al., 1982).

From the result, different spleen levels and salt concentrations resulted in different proteinase activity ($p < 0.05$). At the same level of salt concentration, the fish sauce with 20% spleen showed the highest activity followed by those with 10% and without spleen, respectively. Spleen was reported to consist of a large amount of proteinase (Klomklao et al., 2004). Salt concentrations directly affected proteolytic activity in fish sauce sample. Generally, the activity decreased with increasing NaCl concentration. The 'salting out' effect was postulated to result in the enzyme denaturation. The water molecule was drawn from the proteinase molecule, leading to the aggregation of those enzymes (Klomklao et al., 2004). Increased ionic strength caused by high salt along with the extended incubation time at high temperature (37°C) possibly resulted in the increased denaturation and loss in enzyme activity

(Tungkawachara, Park & Choi, 2003). The activity of trypsin-like enzyme in protein hydrolysate made from 75% fish viscera and 25% salt at 27°C was only 10% after 50 days (Gildberg, 1992). From the result, it was noted that no proteolytic activity of sample without spleen addition was observed after 10 days of fermentation in presence of high salt (25% salt). Nevertheless, some proteinase activity of the samples without spleen was still found in presence of lower salt at every fermentation time. This indicated the role of endogenous proteinases in sardine, which play as essential role in autolysis of this species. Thermostable proteinases were still active and able to degrade myofibrillar protein in the commercial salted anchovy containing 16-17% salt (Ishida, Niizelei & Nagayama, 1994). The activity of acid proteinases from sardine was reduced with the addition of 3.42 M salt (Noda & Murakami, 1981). Nevertheless, Fang and Chiou (1989) reported that salt up to 3.42 M had no effect on pepsin, trypsin and chymotrypsin activities from tilapia. Thus, salt stability of proteinases depends upon fish species as well as salt concentrations. High salt concentration (25%) prolonged fish sauce shelf life but it inhibited peptidase activity and hence retarded protein hydrolysis (Gildberg, 1989). However, salt reduction from 25% to 5-15% accelerated autolysis during fish sauce fermentation (Sikorski et al., 1995). From the results, spleen supplementation as well as salt reduction from 25% to 15-20% resulted in the increased proteolytic activity and the retarded loss in proteolytic activity of fish sauce sample, respectively.

3.2 Total nitrogen content

Total nitrogen content of all fish sauce samples from different treatments throughout the fermentation period is depicted in Fig. 2. The total nitrogen content of samples without spleen addition increased with increasing fermentation time ($p < 0.05$),

except that the sample with 15% salt had the constant TN after 20 days. For the samples with 10% or 20% spleen addition, total nitrogen increased rapidly within the first 20 days in presence of 20 or 25% salt ($p < 0.05$). Thereafter, no marked changes were observed ($p > 0.05$). With 15% salt addition, TN of all samples with spleen addition reached the plateau since 5 days of fermentation, indicating the effective hydrolysis of protein into liquid form. High salt concentrations decreased the percentage protein conversion, presumably due to the lowering of proteolytic activity. The activity of splenic proteinases from skipjack tuna decreased with increasing NaCl concentration (Klomklao et al., 2004). Addition of the higher amount of salt slowed down the breakdown of the fish meat by autolysis or microbial activities (Gildberg, 1989). After 180 day of fermentation, the highest TN of fish sauce sample was found in the sample with 20% spleen addition, particularly at 15% NaCl (Fig. 2), possibly due to the greater degree of hydrolysis. High content of tryptic enzymes in spleen increased the protein hydrolysis (Klomklao et al., 2004) together with the autolytic caused by sardine proteinases in presence of low salt (15%). It is well established that tryptic enzymes are essential for the tissue solubilisation during fish sauce fermentation (Gildberg, 2001). Total nitrogen in fish sauce is mainly from protein nitrogen and non protein nitrogen compounds such as free amino acids, neucleotide, peptides, ammonia, urea and TMAO. These components contribute to the specific aroma and flavor (Finne, 1992; Shahidi, 1994). Total nitrogen content is an objective index used to classify the quality of nampla, Thai fish sauce (Lopetcharat et al., 2002). High quality nampla must have a total nitrogen content of 20 gN/l based on the Kjeldahl method (Thai Industrial Standard, 1983). From the results, a total nitrogen content, equivalent to or higher than 20 g N/l, was obtained from fish sauce containing higher spleen and lower salt concentration (15-20%) at the early stage of

fermentation (5-10days). The results showed that spleen from skipjack tuna was suitable as a supplement of proteolytic enzymes to accelerate the liquefaction, especially at low salt concentration. Thus, the addition of spleen could shorten the fermentation time in the manufacture of fish sauce

3.3 Formaldehyde nitrogen

Formaldehyde nitrogen content increased gradually during the first two months of fermentation ($p < 0.05$) (Fig. 3). No changes in formaldehyde nitrogen contents were observed after 90 days of fermentation ($p > 0.05$). Formaldehyde nitrogen content is used as a convenient index of the degree of protein hydrolysis (Chaveesuk et al., 1993). Comparison of all treatments showed that samples with higher spleen level and lower salt concentration, especially samples with 20% spleen and 15% salt added, contained greater formal nitrogen content, compared with the samples with the lower spleen or higher salt content. Among all treatments, the sample without spleen addition had the lowest formaldehyde nitrogen content. This suggested the lower hydrolysis of muscle proteins. However, with 15% salt, formaldehyde nitrogen of sample without spleen addition reached the maximum within 60 days. This result indicated that endogenous proteinase in sardine activity involved in liquefaction at low salt concentration. The addition of spleen as well as low salt concentration could increase the conversion of insoluble to soluble. This result was in accordance with that of total nitrogen (Fig. 2).

3.4 Ammonia nitrogen

The ammonia nitrogen content of fish sauce samples during fermentation of 180 days is depicted in Fig. 4. The ammonia nitrogen content of all samples increased as fermentation time increased ($p < 0.05$). Generally, the ammonia nitrogen contents were different among all samples. The ammonia nitrogen content indicates the breakdown of soluble protein and peptides into free amino acid and volatile nitrogen (Lopetcharat et al., 2002; Chaveesuk et al., 1993). The increased ammonia nitrogen content could be due to fish enzymes that were active during fermentation (Beddows, Ardeshir & Daud, 1980). The higher ammonia nitrogen content was observed in samples with decreasing NaCl concentration ($p < 0.05$) (Fig. 4). With 20% salt, no marked differences in ammonia nitrogen content were found and the lower contents were observed, when compared with sample with lower salt content. This suggested that the ammonia or volatile compounds generated by spoilage microorganism might be reduced in presence of high salt. The increase in ammonia nitrogen indicated the deamination or decomposition of nitrogenous compounds as well as proteins in the fish and spleen. At low salt concentrations, the spoilage might take place, particularly with increasing fermentation time. This was associated with the faint odor of sample with low salt content (15%). Nevertheless, no apparent spoilage occurred with the samples having a salt concentration of 10% or more (Beddows et al., 1979). The constant ammonia nitrogen content in all samples after 60 days might be owing to the balance between the formation and its reaction with other components, especially via Maillard reaction. The formation of Schiff base is the reaction of amine with aldehyde or ketone group (Lopetcharat et al., 2002). The further reaction from Schiff base between amine and aldehyde or ketone, which is well known as the Maillard reaction, is believed to play an important role in color and flavor development of fish sauce.

Ammonia was suggested as one of the key components of volatile bases giving ammonical notes (Dogan & Howard, 1975). However, there is no evidence that ammonia is the aroma-active component for ammonical notes in fish sauce (Lopetcharat et al., 2002). Thus, salt content affected the formation of volatile degradation components as monitored by ammonia nitrogen content.

3.5 Amino nitrogen

Changes in amino nitrogen content in fish sauce samples from different treatments during 180 days of fermentation are shown in Fig. 5. Generally, similar pattern of changes in TN and amino nitrogen contents were observed throughout the fermentation time of 180 days. The results suggested that the nitrogenous compounds were hydrolysed to small fragments, particularly those with amino acids. The amino nitrogen content represents the amount of primary amino groups in fish sauce. According to the Thai Industrial Standard, amino nitrogen content must be 40 to 60% of total nitrogen (Thai Industrial Standard, 1983). The results suggested that addition of 10% and 20% spleen to the fish mixture increased the rate of liquefaction via protein hydrolysis during fermentation as evidenced by the increased amino nitrogen content. Furthermore, effect of salt concentrations on the formation of amino nitrogen was similar to that found in total nitrogen content (Fig. 2). Addition of the higher amount of salt could retard the hydrolysis of fish proteins caused by autolysis or spleen proteinase. Therefore, the addition of spleen was shown to accelerate the production of fish sauce with high content of amino acid.

3.6 Color

Fish sauce samples obtained from different treatments with various levels of spleen and salt concentrations had different color characteristics (Table 1). The color of fish sauce samples was developed gradually as the fermentation time increased. Generally, samples with spleen addition had the increase in a^* and b^* values but decrease in L^* -values when the fermentation time increased ($p < 0.05$). The higher spleen content resulted in the higher color intensity. At the same levels of spleen addition, samples with higher salt content showed the greater color intensity. Color formation was likely to be due to both the formation of low molecular weight compounds and the presence of melanoidines with high molecular weight (Ames, 1992). From the result, the sample with 20% spleen addition and low salt content exhibited more redness as shown by the greater a^* -value. The development of red color was coincidental with decrease in lightness (L^* -value), particularly when the fermentation time increased. Maillard reaction might contribute to the increase a^* value (redness). Most of the nitrogenous compounds in fish sauce are free amino acids and small peptides, which contribute to brown color development (Lopetcharat et al., 2002). Even though reducing sugar content in fish is low, carbohydrate derivatives, such as glucose-6-phosphate and other substances present in the metabolic pathways, can also act as reactants to initiate the maillard reaction (Kawashima & Yamanaka, 1996). From the result, the fish sauce sample with 20% spleen and 15% salt showed the greatest a^* value with the low L^* value.

3.7 Nonenzymatic browning

Increase in browning was observed in all samples during fermentation (Fig. 6). Browning of all samples was highest at day 180, suggesting the brown pigment

formation at the extended fermentation period. The browning contributes to the color of fish sauce. After 3 months of fermentation, greater browning was found in the samples produced by mixing fish with 20% spleen. Lee, Homma and Aida (1997) reported that fish and soy sauces became darker with melanoidine produced by Maillard reaction during storage. From the result, fish sauce produced with low salt concentration showed the highest browning. The increase in browning was found to depend on salt concentration. The higher concentration of salt used, the lower the increase in browning was found. The increase in browning was generally in agreement with the increase in a^* and b^* values and the decrease in L^* -value (Table 1). The increase in absorbance at 420 nm was used as an indicator for browning development in the final stage of browning reaction (Ajandouz, Tchiakpe, Ore, Benajiba & Puigserver, 2001). The brown color in fish sauce was caused by nonenzymatic browning reaction such as Maillard reaction (Lopetcharat et al., 2002). The reducing sugar and oxidation products, such as aldehyde could react with free amino acids, which could be released to a higher extent with increasing fermentation time. Therefore, the addition of spleen and salt content not only affected the hydrolysis, but also influenced the color development in fish sauce.

3.8 Acceptability

The scores for color, aroma, taste and overall acceptance of all fish sauce samples from different treatments in comparison with the commercial fish sauce (Nampla) are shown in Table 2. Varying levels of spleen and salt added affected the acceptability of fish sauce samples obtained. At the 20% salt level, all samples obtained either with or without spleen addition showed no differences in all attributes. Nevertheless, fish sauce samples obtained with 20% spleen showed the lower score

for aroma, compared with the commercial fish sauce. Among fish sauce samples containing 20% salt, there were no differences in the scores of color, aroma, taste and overall acceptance. Also, all attributes of fish sauce samples obtained were mostly comparable to those of commercial fish sauce. For the color score, fish sauce sample with the same salt level had the higher score when the greater amount of spleen was added ($p < 0.05$). However, the fish sauce with 25% salt showed the lowest score for color liking ($p < 0.05$). Panelists generally preferred the dark brown color of commercial fish sauce and fish sauce prepared from sardine with higher spleen content and lower salt concentration (15-20%) to the light brown color of fish sauce sample containing no spleen with 25% salt. For aroma acceptance, fish sauce sample with 15% salt showed the lowest aroma score ($p < 0.05$), especially that without spleen. At low salt concentration, protein hydrolysis might be accelerated by microbial proteinase and the decomposition of nitrogen compounds might occur, leading to the formation of offensive volatile compounds such as ammonia or volatile compounds. This was associated with the faint odor of sample with low salt content. Commercial fish sauce possessed the greater aroma scores than most sardine fish sauce samples ($p < 0.05$). However, fish sauce sample containing 20% salt without and with 10% spleen and that with 25% salt and 10% spleen showed the comparable aroma and overall acceptance scores to commercial fish sauce. Commercial fish sauce had a slightly stronger aroma score than the lower salt and higher spleen supplemented sauces, probably due to the greater degree of hydrolysis which was associated with the formation of tasty products such as free amino acid. The darker color and slightly stronger aroma and taste of the commercial fish sauce, Nampla, could be attributed to the longer fermentation/ageing period. Additionally, it may also result from the addition of molasses to Nampla prior to bottling (Chaveesuk et al., 1993). Generally,

sensory evaluation is frequently applied in estimating the quality of fish sauce. Therefore, fish sauce from different treatments had the differences in acceptability which were related with physical and chemical properties of the products.

4. Conclusion

Addition of spleen accelerated the hydrolysis of fish protein during fermentation, especially at low salt concentration. Based on the biochemical and chemical aspects as well as the acceptability, fish sauce could be made from sardine with spleen addition at the level of 10% in the presence of 20 % salt. This process led to the greater rate of liquefaction and rendered the acceptable fish sauce.

Acknowledgments

Financial support from Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0216/2546) to Sappasith Klomklao.

References

- Ajandouz, E.H., Tchiakpe, L.S., Ore, F.D., Benajiba, A., & Puigserver, A. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. *Journal of Food Science*, 66, 926-931.
- Amano, K. (1962). Influence of fermentation of the nutritive value on fish with special reference to fermented fish products of Southeast Asia. In E. Heen, & R. Kreuzer (Eds.), *Fish in nutrition*, (pp. 180-200). London: Fishing News.
- Ames, J.M. (1992). The Maillard reaction. In B.J.F. Hudson (Ed.), *Biochemistry of food proteins*, (pp. 99-153). London, Elsevier Applied Science.

- AOAC. (1999). In K. Helrich (Ed.), *Official Method of Analysis*. (15th ed.). Washington, DC: Association of Official Analytical Chemists.
- Beddows, C.G., & Ardeshir, A.G. (1979). The production of soluble fish protein solution for use in fish sauce manufacture I. The use of added enzymes. *Journal of Food Technology*, 14, 603-612.
- Beddows, C.G., Ardeshir, A.G., & Daud, W.J. (1980). Development and origin of the volatile fatty acids in Budu. *Journal of the Science of Food and Agriculture*, 31, 86-92.
- Brillantes, S. (1999). Histamine in fish sauce-health and safety considerations. *INFOFISH Intern.* 4, 51-56.
- Chamber, I.V.E., & Wolf, M.B. (1996). Sensory testing methods (2nd ed.). Philadelphia: American Society for Testing and Materials.
- Chaveesuk, R., Smith, J.P., & Simpson, B.K. (1993). Production of fish sauce and acceleration of sauce fermentation using proteolytic enzymes. *Journal of Aquatic Food Product Technology*, 2(3), 59-77.
- Dougan, J., & Howard, G.E. (1975). Some flavoring constituents of fermented fish sauce. *Journal of the Science of Food and Agriculture*, 26, 887-894.
- Finne, G. (1992). Non-protein nitrogen compounds in fish and shellfish. In J.G. Flick & Martin, R.E. (Eds.), *Advances in seafood biochemistry, composition and quality*, (pp. 393-401). Lancaster: Technomic Publishing.
- Fang, L.S., & Chiou, S.F. (1989). Effect of salinity on the activities of digestive proteases from the tilapia fish, *Oreochromis niloticus*, in different culture environments. *Comparative Biochemistry and Physiology Part A*, 93, 439-443.

- Gildberg, A. (1989). Accelerate fish sauce fermentation by initial alkaline at low salt concentration. In S. Miyachi, J. Karube & Y. Ishida (Eds.). *Current topic in marine biotechnology*, (pp. 101-104). Tokyo: Fuji Technol Press.
- Gildberg, A. (1992). Recovery of proteinase and protein hydrolysate from fish viscera. *Bioresource Technology*, 39, 271-276.
- Gildberg, A. (2001). Utilization of male Arctic capelin and Atlantic cod intestines for fish sauce production-evaluation of fermentation conditions. *Bioresource Technology*, 76, 119- 123.
- Gildberg, A., Espejo-Hermes, J., & Magno-Orejana, F. (1984). Acceleration of autolysis during fish sauce fermentation by adding acid and reducing the salt content. *Journal of the Science of Food and Agriculture*. 35, 1363-1369.
- Hoyle, N.T., & Merritt, J.H. (1994). Quality of fish protein hydrolysates from herring (*Clupea harengus*). *Journal of Food Science*, 59, 76-79.
- Ishida, M., Niizelei, S., & Nagayama, F. (1994). Thermostable proteinase in salted anchovy muscle. *Journal of Food Science*, 59, 781-785.
- Kawashima, K., & Yamanaka, H. (1996). Free amino acids responsible for the browning of cooked scallop adductor muscle. *Fisheries Science*, 62, 293- 296.
- Klomklao, S., Benjakul, S., & Visessanguan, W. (2004). Comparative studies on proteolytic activity of spleen extracts from three tuna species commonly used in Thailand. *Journal of Food Biochemistry*, 28, 355-372.
- Lee, Y.S., Homma, S., Aida, K. (1997). Characterization of melanoidin in soy sauce and fish sauce by electrofocusing and high performance gel permeation chromatography. *Nippon Shokuhin Kogyo Gakkaishi*, 34, 313-319.

- Lopetcharat, K., & Park, J.W. (2002). Characteristics of fish sauce made from Pacific whiting and surimi by-products during fermentation storage. *Journal of Food Science*, 67, 511-516.
- Lopetcharat, K., Choi, Y.J., Park, J.W., & Daeschel, M.A. (2001). Fish sauce products and manufacturing: a review. *Food Reviews International*, 17, 65-68.
- Lowry, O.H., Rosebrough, N.J., Fan, A.L., & Randall, R.J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry*, 193, 256-275.
- Noda, M., & Murakami, K. (1981). Studies on proteinases from the digestive organs of sardine. Purification and characterization of two acid proteinases from the stomach. *Biochimica et Biophysica Acta (BBA) - Enzymology*, 658, 27-32.
- Orejana, F.M., & Liston, J. (1982). Agents of proteolysis and its inhibition in patis (fish sauce) fermentation. *Journal of Food Science*, 47, 198-203, 209.
- Saisithi, P. (1994). Traditional fermented fish: fish sauce production. In A.M. Martin (Ed.), *Fisheries processing biotechnology application*, (pp. 111-131). London: Chapman and Hall.
- Sikorski, Z.E., Gildberg, A., & Ruiter, A. (1995). Fish products. In P. Ruiter (Ed.), *Fish and fishery products*, (pp. 315-346). Wallingford: Cap International.
- Shahidi, F. (1994). Proteins from seafood processing discards. In Z.E. Sikorski, B.S. Pan & F. Shahidi (Eds.), *Seafood protein* (pp. 171-193). New York: Chapman and Hall.
- Simpson, B.K. (2000). Digestive proteinases from marine animals. In N.F. Haard & B.K. Simpson (Eds.), *Seafood enzymes: Utilization and influence on postharvest seafood quality*, (pp. 531-540). New York: Mercel Dekker.

Steel, R.G.J., & Torrie, J.H. (1980). *Principles and procedures of statistics: A biometrical approach* (2nd ed., p. 633). New York: McGraw-Hill.

Thai Industrial Standard. (1983). *Local fish sauce standard*. Bangkok: Dept. of Industry.

Tungkawachara, S., Park, J.W. & Choi, Y.J. (2003). Biochemical properties and consumer acceptance of Pacific whiting fish sauce. *Journal of Food Science*, 68, 855-860.

Table 1

L*, a* and b*-values of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Color ¹ Characteristics	Day	25% salt			20% salt			15% salt		
		0% spleen	10% spleen	20% spleen	0% spleen	10% spleen	20% spleen	0% spleen	10% spleen	20% spleen
L*	5	74.72	74.08	71.46	71.14	69.35	69.13	67.96	53.09	56.75
	10	74.79	74.87	73.36	71.50	71.56	70.80	68.49	61.19	58.80
	20	74.62	72.61	71.54	69.58	68.65	66.91	64.69	62.98	61.73
	30	72.77	69.71	67.99	65.93	65.06	64.54	60.19	55.38	56.03
	60	66.92	62.67	61.32	58.13	56.84	54.82	57.41	52.77	49.17
	90	61.60	59.96	54.73	49.01	47.83	46.06	48.56	46.74	42.23
	120	59.72	57.66	51.26	46.63	44.26	39.73	41.72	41.53	35.67
	150	58.79	55.77	45.86	41.53	40.92	38.41	37.74	36.05	31.58
	180	50.49	48.12	45.68	31.85	31.80	31.67	31.65	31.51	30.62
a*	5	-0.56	-1.82	-2.71	0.80	-0.78	-1.94	0.74	8.22	6.26
	10	-1.11	-3.80	-3.91	0.93	-2.35	-2.53	0.72	3.21	6.08
	20	-2.34	-3.27	-3.31	0.64	-0.98	0.15	4.24	5.69	6.10
	30	-2.32	-1.41	-0.59	2.91	2.54	3.51	8.98	10.27	13.39
	60	1.36	4.49	6.92	11.69	13.98	16.35	12.11	14.32	20.46
	90	4.89	9.06	13.26	18.25	19.74	20.55	18.84	19.95	23.50
	120	7.02	9.95	15.95	19.11	21.99	27.39	19.75	22.50	28.01
	150	7.61	10.07	19.31	19.91	23.22	28.56	20.15	25.18	28.75
	180	16.18	18.14	22.63	23.89	26.18	28.75	24.22	28.83	30.44
b*	5	19.08	23.25	29.62	26.94	33.64	37.44	29.25	33.73	41.69
	10	21.53	26.98	33.08	33.22	37.54	40.87	32.54	45.96	52.58
	20	25.48	34.48	39.61	36.87	42.68	49.17	40.87	48.38	55.62
	30	30.35	41.51	47.29	45.13	51.62	54.29	49.67	52.77	60.78
	60	44.12	51.81	56.88	54.79	60.49	60.21	53.04	58.42	63.46
	90	51.69	57.29	62.50	57.98	63.64	64.53	61.36	61.74	63.95
	120	56.08	57.80	64.02	56.19	61.45	62.56	58.64	58.76	61.47
	150	58.30	63.02	67.57	55.57	60.18	60.32	51.75	58.00	60.58
	180	58.09	54.65	64.76	52.03	52.85	60.15	51.19	52.42	60.54

¹Values are the mean from four determinations.

Table 2

Acceptability score of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Treatments	Attributes			
	Color	Aroma	Taste	Overall acceptance
25% salt + 0% spleen	5.06±1.67 ^d	5.04±1.91 ^{de}	6.04±1.62 ^{bcd}	5.49±1.42 ^d
25% salt + 10% spleen	6.51±1.41 ^{cb}	5.78±1.69 ^{abc}	6.35±1.49 ^{abcd}	6.45±1.29 ^{abc}
25% salt + 20% spleen	6.47±1.67 ^c	5.63±1.81 ^{bcd}	6.12±1.84 ^{abcd}	6.12±1.62 ^{bc}
20% salt + 0% spleen	7.33±1.14 ^a	5.86±1.79 ^{ab}	6.59±1.66 ^{ab}	6.51±1.46 ^{ab}
20% salt + 10% spleen	7.35±1.45 ^a	5.84±1.82 ^{ab}	6.73±1.58 ^a	6.76±1.39 ^a
20% salt + 20% spleen	7.37±1.23 ^a	5.59±1.36 ^{bcd}	6.55±1.78 ^{ab}	6.69±1.42 ^a
15% salt + 0% spleen	6.90±1.28 ^{abc}	4.75±2.03 ^e	5.71±1.92 ^d	5.53±1.72 ^d
15% salt + 10% spleen	6.92±1.25 ^{abc}	5.16±1.84 ^{cde}	6.00±1.97 ^{bcd}	6.08±1.60 ^{bc}
15% salt + 20% spleen	7.27±0.98 ^a	5.35±1.88 ^{bcd}	5.86±1.95 ^{cd}	5.92±1.79 ^{cd}
Commercial fish sauce	6.96±1.30 ^{ab}	6.39±1.97 ^a	6.37±1.64 ^{abc}	6.73±1.67 ^a

Means scores range from 0 (dislike extremely) to 10 (like extremely).

Different superscripts in the same column indicate significant differences ($p < 0.05$).

Figure legends

Fig. 1 Proteinase activity of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 2 Total nitrogen content of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 3 Formaldehyde nitrogen content of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 4 Ammonia nitrogen content of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 5 Amino nitrogen content of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 6 Browning intensity of fish sauce samples produced from sardine mixed with different skipjack tuna spleen and various salt concentrations during fermentation of 180 days.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6