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Food Chemistry 98 (2006) 440–452
Food Chemistry
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Effects of the addition of spleen of skipjack tuna (*Katsuwonus pelamis*) on the liquefaction and characteristics of fish sauce made from sardine (*Sardinella gibbosa*)

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Received 28 March 2005; received in revised form 8 June 2005; accepted 8 June 2005

Abstract

The effects of the addition of spleen of skipjack tuna (*Katsuwonus pelamis*), at levels of 0%, 10% and 20%, on the liquefaction and characteristics of fish sauce produced from the sardine (*Sardinella gibbosa*) with different salt concentrations (15%, 20% and 25%) were monitored during fermentation for 180 days. Fish sauces prepared from sardine with spleen supplementation contained greater total nitrogen, amino nitrogen, formaldehyde nitrogen and ammonia nitrogen than did those without spleen addition throughout the fermentation. The rate of liquefaction was dependent on 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 were those treated with higher salt content. Among all treatments, sardine with 25% spleen and 15% salt added exhibited the greatest protein hydrolysis, particularly at the early stages, suggesting the combined 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. An acceptability test revealed that the samples were different in colour, aroma, taste and overall acceptance ($p < 0.05$). Fish sauce samples containing 20% salt, without and with 10% spleen addition had similar acceptabilities to commercial fish sauce. Therefore, the 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 preserving and producing value-added products from underutilised fish species. Fish sauce has become more interesting for consumers in Europe, North America and other countries (Brillantes, 1999). As a consequence, fish sauce is rapidly expanding in both domestic and foreign markets. There are approximately 390 fish sauce factories in Thailand with 64,000 metric ton of fish being 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 flavour enhancer or salt replacement in various food preparations

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doi:10.1016/j.foodchem.2005.06.013

(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 a concrete tank in 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; Gildberg, 2001; Saisithi, 1994). During fermentation, proteins are hydrolysed, mainly as a result of autolytic action by the digestive proteases in fish (Orejana & Liston, 1982; Sikorski, Gildberg, & Ruiter, 1995). The fermentation process is normally continued for a long time, to ensure the solubilisation as well as the flavour and colour development of fish sauce. In Thailand, fish sauce is manufactured by fermentation for up to 18 months (Lopetcharat & Park, 2002). To accelerate the solubilisation process, lowering the pH and salt content was conducted to enhance rapid autolysis, particularly that caused by trypsin and chymotrypsin (Beddows & Ardeshir, 1979; Chaveesuk, Smith, & Simpson, 1993; Gildberg, Espejo-Hermes, & Magno-Orejana, 1984). The fish sauce was obtained after 6 months of fermentation with the addition of 5–10% enzyme-rich (trypsin and chymotrypsin) cod intestines to 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 a trypsin-like serine proteinase. Accordingly, the spleen from skipjack tuna, generally considered as the waste from tuna processing plants, can be used as a 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 the spleen of skipjack tuna and salt concentration on the hydrolysis of fish protein as well as on the chemical and physical changes of fish sauce obtained during fermentation for up to 180 days.

2. Materials and methods

2.1. Chemicals

Sodium caseinate, β-mercaptoethanol (βME), 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

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

Sardine (*Sardinella gibbosa*), with an 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 a 5 mm plate. Ground sardine (200 g) was mixed with 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 (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 7700*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 against 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 the casein–TCA–Lowry assay (Klomklao et al., 2004). To initiate the reaction, 200 μl of dialysed fish sauce sample were 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). The 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 7000*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 that 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 the 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 the 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 M NaOH. Ten ml of formaldehyde solution (38% v/v, pH 9.0) were then added to the neutralised samples. Titration was continued to pH 9.0 with 0.1 M NaOH. Formaldehyde nitrogen content was calculated as follows:

$$\text{Formaldehyde nitrogen content(g/l)} = \text{ml}(\text{NaOH}_{\text{pH7–pH9}}) \times 0.1 \times 14$$

To determine ammonia nitrogen, 50 ml of 10-fold diluted samples were placed in a 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 M H_2SO_4 until the end-point was obtained. Ammonia nitrogen content was calculated as follows:

$$\text{Ammonia nitrogen content(g/l)} = 5.6 \times 0.05 \times Y,$$

where Y is the volume of H_2SO_4 (ml)

Amino nitrogen content was calculated using the following formula:

$$\text{Amino nitrogen content(g/l)} = \text{formaldehyde nitrogen content} - \text{ammonia nitrogen content}$$

2.7. Physical analysis

2.7.1. Colour

Colour characteristics of the samples were determined by measuring the L^* a^* and b^*-values using a Hunter Lab instrument (Colour Flex, Hunter Associates Laboratory, Verginia, USA), according to the CIE Lab scale. The system provides the values of three colour 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 manufacturer. The colour 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 milligrams 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 7700*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 of 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 panellists were graduate students in Food Technology from the Faculty of Agro-Industry, Prince of Songkla University, of age ranging from 20 to 35 years. All panellists had sensorial acquaintance with fish sauce. Panellists were asked to give acceptance scores for four attributes: colour, aroma, taste and overall acceptance using the nine-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 aluminium 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 were presented to the panellists at ambient temperature. Fish sauce samples were served to each panellist in a random order. During evaluation, the panellists were seated 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 randomised 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

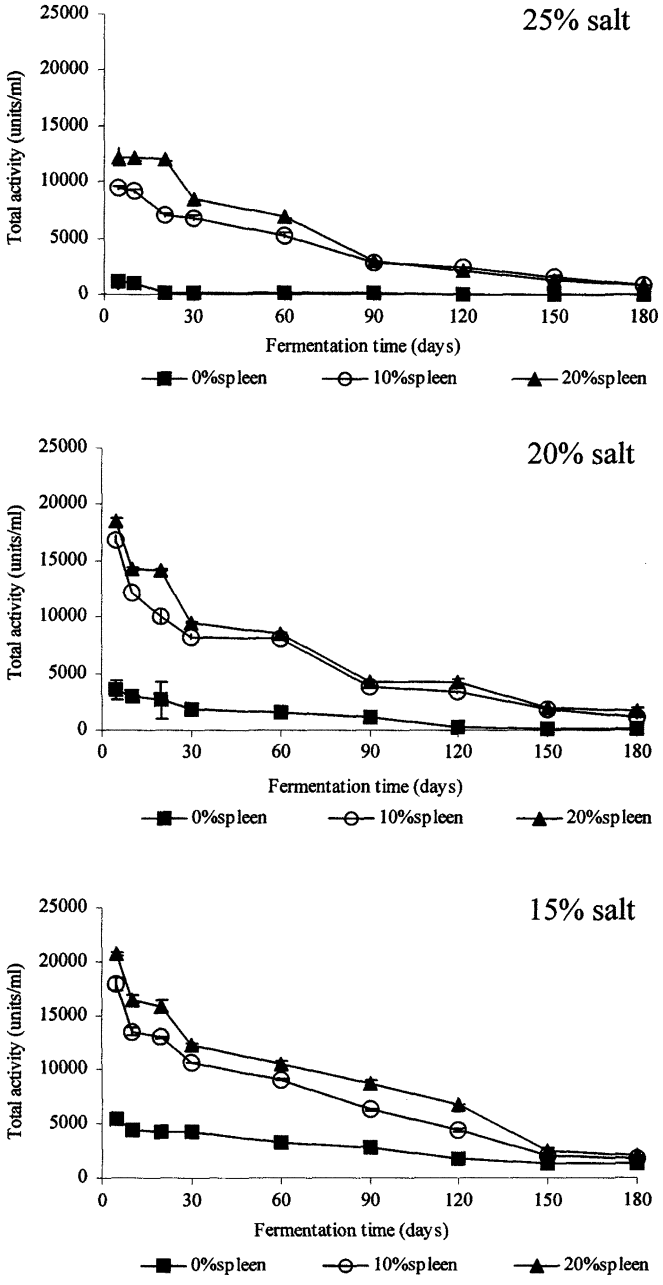

Fig. 1. Proteinase activity of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

in activity were found in the samples containing 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 the presence of a 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 & Liston, 1982).

Evidently, different spleen levels and salt concentrations resulted in different proteinase activities ($p < 0.05$). At the same level of salt concentration, the fish sauce with 20% spleen showed the hightest 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 the fish sauce sample. Generally, the activity decreased with increasing NaCl concentration. The 'salting out' effect was postulated to cause the enzyme denaturation. A water molecule is drawn from the proteinase molecule, leading to the aggregation of those enzymes (Klomklao et al., 2004). Increased ionic strength caused by high salt concentration, along with the extended incubation time at high temperature (37 °C), possibly resulted in the increased denaturation and loss of 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 results, it was noted that no proteolytic activity of sample without spleen addition could be observed after 10 days of fermentation in the presence of high salt (25% salt). Nevertheless, some proteinase activity of the samples without spleen was still found in the presence of less salt at every fermentation time. This indicated the role of endogenous proteinases in sardine, which play an essential role in autolysis of this species. Thermostable proteinases were still active and able to degrade myofibrillar protein in commercial salted anchovy containing 16–17% salt (Ishida, Niizelei, & Nagayama, 1994). The activity of acid proteinases from sardine was reduced by 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 or 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 an increased proteolytic activity and a retarded loss of proteolytic activity of the fish sauce sample.

3.2. Total nitrogen content

Total nitrogen contents of all fish sauce samples from different treatments throughout the fermentation period are depicted in Fig. 2. The total nitrogen content of samples without spleen addition increased with increasing fermentation time ($p < 0.05$), except for the sample with 15% salt, which had a constant total nitrogen content after 20 days. For the samples with 10% or 20% spleen addition, total nitrogen increased rapidly within the first 20 days in the presence of 20% or 25% salt ($p < 0.05$). Thereafter, no marked changes were observed ($p > 0.05$). With 15% salt addition, total nitrogen content of all samples with spleen addition reached a plateau after 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 a higher amount of salt slowed down the breakdown of the fish meat by autolysis or microbial activities (Gildberg, 1989). After 180 days 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. A high content of tryptic enzymes in spleen increased the protein hydrolysis (Klomklao et al., 2004), together with the autolysis caused by sardine proteinases in the 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 nonprotein nitrogen compounds, such as free amino acids, nucleotide, peptides, ammonia, urea and TMAO. These components contribute to the specific aroma and flavour (Finne, 1992; Shahidi, 1994). The total nitrogen content is an objective index used to classify the quality of nampla, Thai fish sauce (Lopetcharat et al., 2001). High quality nampla must have a total nitrogen content of 20 gN/l based on the Kjedahl method (Thai Industrial Standard, 1983). From the results, a total nitrogen content, equivalent to or above 20 gN/l, was obtained from fish sauce with higher spleen and lower salt concentrations (15–20%) at the early stage of fermentation (5–10 days). Those results showed that spleen from skipjack tuna was suitable as a supplement to 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 ($p < 0.05$)

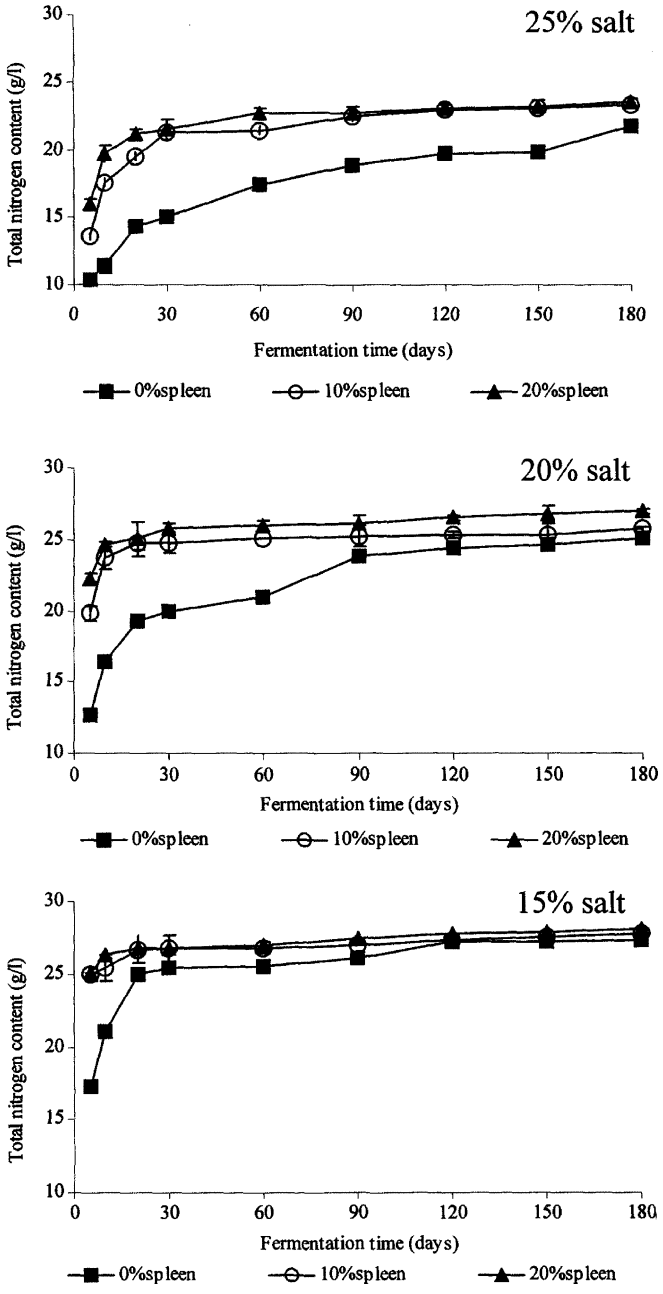

Fig. 2. Total nitrogen contents of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

(Fig. 3). No changes in formaldehyde nitrogen contents were observed after 90 days of fermentation ($p > 0.05$). Formaldehyde reacts with amino acids, liberating one H^+ ion from the amino group, which is potentiometrically titrated with a sodium hydroxide solution. Therefore, formaldehyde nitrogen content is useful for measuring total free amino acid (Angeles Navarrete del Toro & García-Carreño, 2002). 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 levels and lower salt concentrations, especially samples with 20% spleen and 15% salt added, had greater formaldehyde nitrogen contents, than did samples with lower spleen or higher salts content. This result suggested that protein hydrolysis took place to a greater extent, resulting in greater free amino group content, in the presence of spleen. Among all treatments, the samples without spleen addition had the lowest formaldehyde nitrogen contents. This suggested lower hydrolysis of muscle proteins. However, with 15% salt, formaldehyde nitrogen content of the sample without spleen addition, reached a maximum within 60 days. This result indicated that endogenous proteinases in sar-

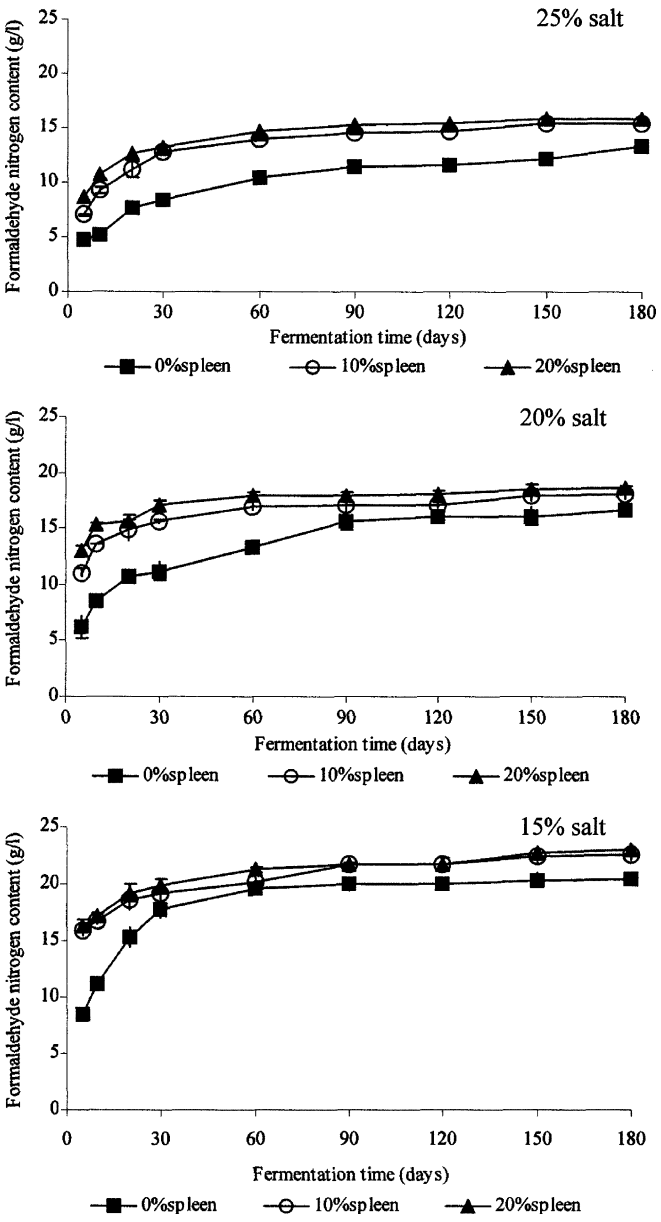

Fig. 3. Formaldehyde nitrogen contents of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

dine are 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 nitrogen. 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 acids and volatile nitrogen (Chaveesuk et al., 1993; Lopetcharat et al., 2001). The increased ammonia nitrogen content could be due to fish enzymes that were active during fermentation (Beddows, Ardeshir, & Daud, 1980). Higher ammonia nitrogen contents were observed in samples with decreasing NaCl concentrations ($p < 0.05$) (Fig. 4). With 20% salt, no marked differences in the ammonia nitrogen content were found and lower contents were observed, when compared with samples with a lower salt content. This suggested that the ammonia or volatile compounds generated by spoilage microorganism might be reduced at high salt concentrations. The increase in ammonia nitrogen indicated the

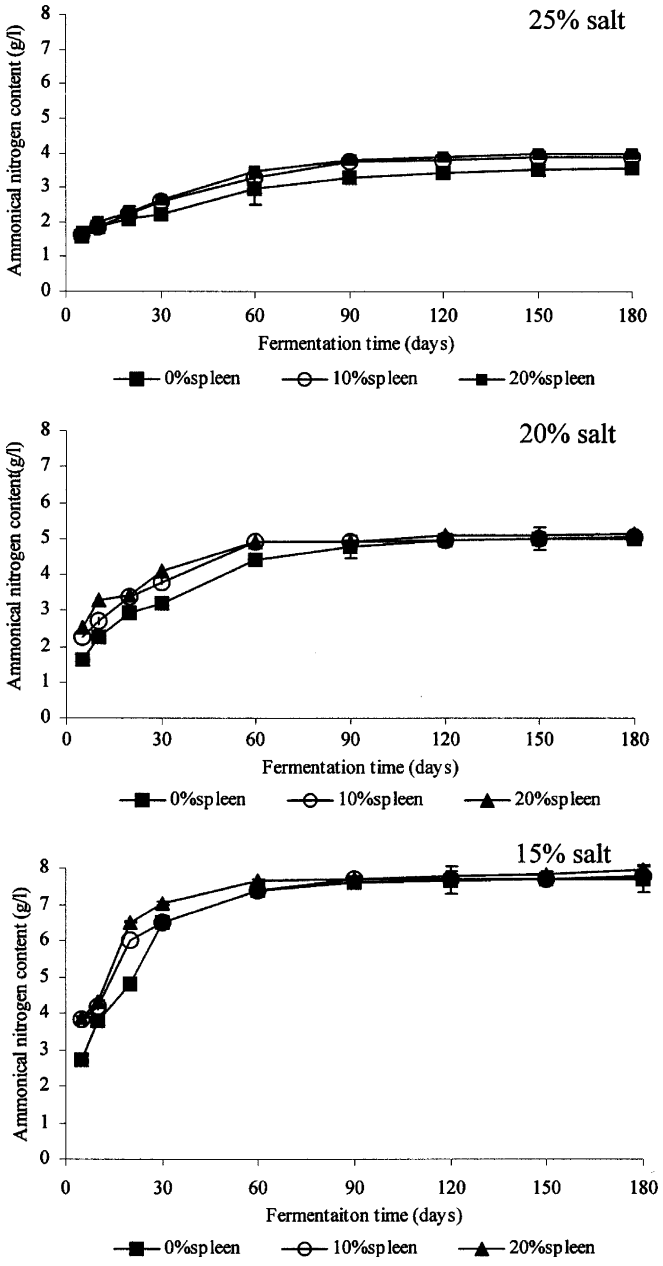

Fig. 4. Ammonia nitrogen contents of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

deamination or decomposition of nitrogenous compounds, as well as proteins, in the fish and spleen. At low salt concentrations, spoilage might take place, particularly with increasing fermentation time. This was associated with the faint odour of samples with low salt content (15%). Nevertheless, no apparent spoilage occurred in the samples with a salt concentration of 10% or more (Beddows & Ardeshir, 1979). The constant ammonia nitrogen content in all samples, after 60 days, might be due to the balance between formation and reaction with other components, especially via the Maillard reaction. The formation of Schiff base is the reaction of amine with aldehyde or ketone groups (Lopetcharat et al., 2001). The further reaction of Schiff base, between amine and aldehyde or ketone, which is well known as the Maillard reaction, is believed to play an important role in colour and flavour development of fish sauce. Ammonia was suggested as one of the key components of volatile bases giving ammoniacal notes (Dougan & Howard, 1975). However, there is no evidence that ammonia is the aroma-active component for ammoniacal notes in fish sauce (Lopetcharat et al., 2001). Thus, the salt content affected the formation of volatile degradation components as monitored by the 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 patterns of changes in total nitrogen contents 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 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 40–60% of total nitrogen (Thai Industrial Standard, 1983). The results suggested that the addition of 10% and 20% of spleen to the fish mixture increased the rate of liquefaction via protein hydrolysis during fermentation, as evidenced by the increased amino nitrogen content. Furthermore, the effects of salt concentrations on the formation of amino nitrogen were similar to those found for total nitrogen content (Fig. 2). An addition of higher amounts of salt could retard the hydrolysis of fish proteins caused by autolysis or

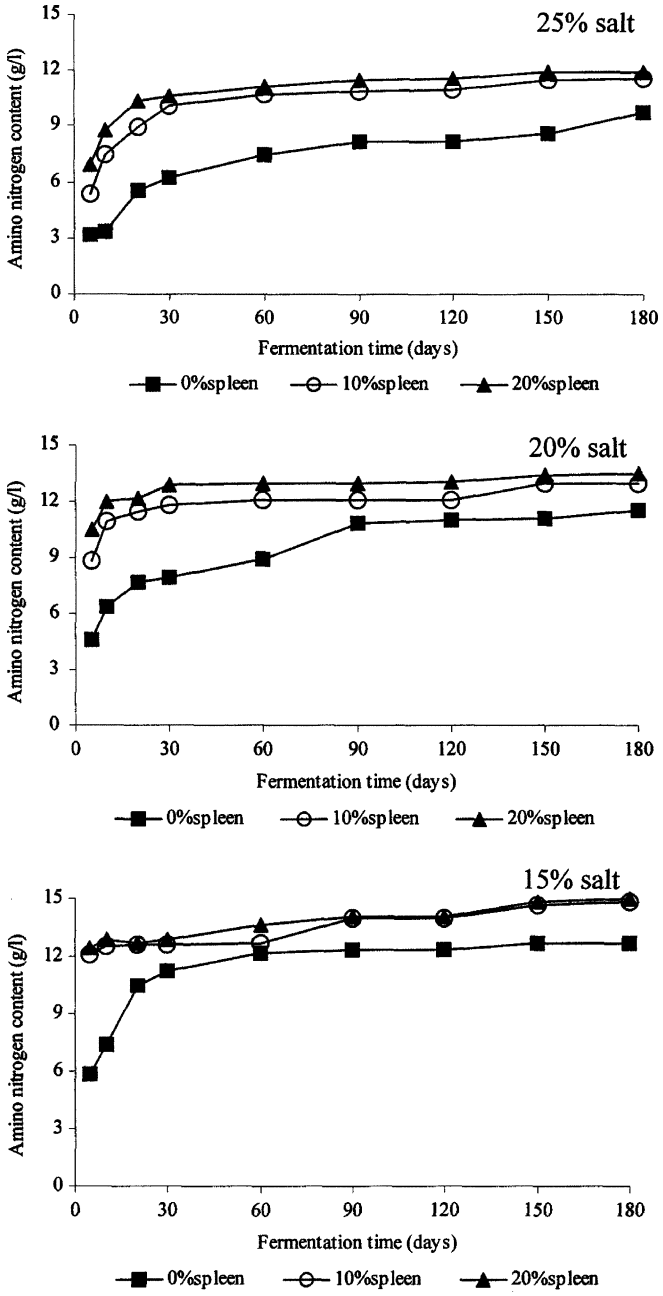

Fig. 5. Amino nitrogen contents of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

spleen proteinase. Therefore, the addition of spleen was shown to accelerate the production of fish sauce with a high content of amino acid.

3.6. Colour

Fish sauce samples obtained from different treatments with various levels of spleen and salt concentrations had different colour characteristics (Table 1). The colour of fish sauce samples developed gradually as the fermentation time increased. Generally, samples with spleen addition had increases in the a^* and b^*-values but a decrease in the L^*-values when the fermentation time increased ($p < 0.05$). Higher spleen content resulted in a higher colour intensity. At the same levels of spleen addition, samples with higher salt contents showed greater colour intensity. Colour formation was likely due to both the formation of low molecular weight compounds and the presence of melanoidins of high molecular weight (Ames, 1992). From the result, the sample with 20% spleen addition and a low salt content exhibited more redness, as shown by the greater a^*-value. The development of the red colour was coincidental with a decrease in the lightness (L^*-value), particularly when the fermentation time increased. Maillard reaction might contribute to the increase in the a^*-value (redness). Most of the nitrogenous compounds in fish sauce are free amino acids and small peptides, which contribute to the brown colour development (Lopetcharat et al., 2001). 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 results, the fish sauce sample with 20% spleen and 15% salt showed the greatest a^*-value with a low L^*-value.

3.7. Nonenzymatic browning

An increase in browning was observed in all samples during fermentation (Fig. 6). Browning of all samples was highest at day 180, suggesting that brown pigment formed during the extended fermentation period. The browning contributes to the colour 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

Table 1
L^*, a^* and b^*-values of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days

Colour[a] 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

[a] Values are the means of four determinations.

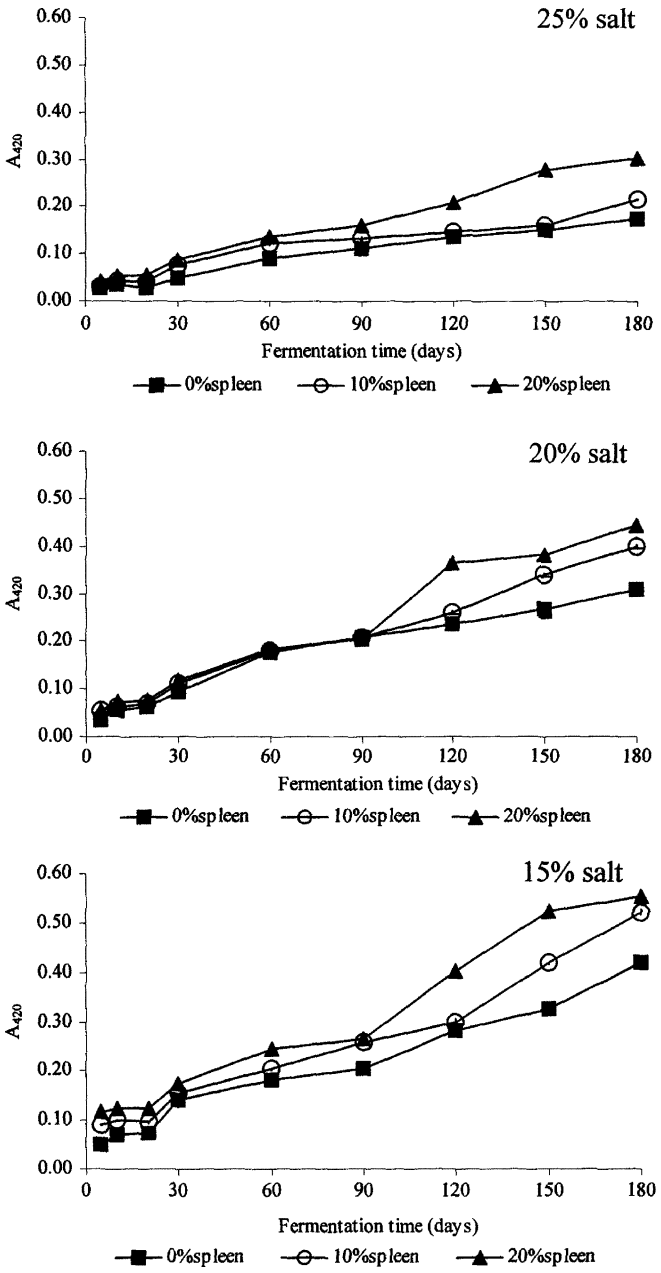

Fig. 6. Browning intensity of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days.

sauces became darker with melanoidin produced by the Maillard reaction during storage. From the results, fish sauce produced with a low salt concentration showed the highest browning. The increase in browning was found to depend on salt concentration. The higher the concentration of salt used, the lower was the increase in browning. 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 of browning development in the final stage of the browning reaction (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001). The brown colour in fish sauce was caused by a nonenzymatic browning reaction, such as the Maillard reaction (Lopetcharat et al., 2001). 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, not only affected the hydrolysis, but also influenced the colour development in fish sauce.

3.8. Acceptability

The scores for colour, aroma, taste and overall acceptance of all fish sauce samples from different

Table 2
Acceptability scores of fish sauce samples produced from sardine with different skipjack tuna spleen and salt concentrations during fermentation for 180 days

Treatments	Attributes			
	Colour	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^{bcde}	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 ($p < 0.05$).

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 the fish sauce samples obtained. At the 20% salt level, all samples obtained, either with or without spleen addition, showed no differences in any attributes. Nevertheless, fish sauce samples obtained with 20% spleen showed a lower score for aroma, than did commercial fish sauce. Among fish sauce samples containing 20% salt, there were no differences in the scores of colour, aroma, taste and overall acceptance. Also, all attributes of fish sauce samples obtained were mostly comparable to those of commercial fish sauce. Fish sauce samples with the same salt level had higher colour scores when a greater amount of spleen was added ($p < 0.05$). However, the fish sauce with 25% salt showed the lowest score for colour liking ($p < 0.05$). Panellists generally preferred the dark brown colour of commercial fish sauce and fish sauce prepared from sardine with higher spleen content and lower salt concentrations (15–20%) to the light brown colour 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 without spleen. At low salt concentration, protein hydrolysis might be accelerated by microbial proteinase and decomposition of nitrogen compounds might occur, leading to the formation of offensive volatile compounds such as ammonia or other volatile compounds. This was associated with the faint odour of samples with low salt content. Commercial fish sauce had greater aroma scores than most sardine fish sauce samples ($p < 0.05$). However, the fish sauce sample containing 20% salt, without and with 10% spleen, and that with 25% salt, and 10% spleen, showed 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 acids. The darker colour 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 sauces from different treatments showed differences in acceptability which were related to physical and chemical properties of the products.

4. Conclusion

The 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 a greater rate of liquefaction and rendered acceptable fish sauce.

Acknowledgement

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

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