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Physico-chemical and color characteristics of salt-fermented fish sauce from anchovy *Stolephorus commersonii*

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Abstract. Fish sauce is a salt-fermented product and considered as an economically important traditional fishery product in most Southeast Asian countries. The manufacturing processes involve various species of fish and salt to fish ratios. This study was conducted to assess the changes in the physico-chemical and color characteristics of laboratory-prepared salt-fermented fish sauce made from anchovy, *Stolephorus commersonii* (Lacepède, 1803), with 1:3.5 salt to fish ratio and fermented for 270 days at 28-30°C. One-way ANOVA showed a highly significant effect ($p < 0.05$) of fermentation period on pH, total volatile base-nitrogen (TVB-N), protein and color characteristics of the product but no significant effect on salt content. The 270-day anchovy sauce had pH, TVB-N, total protein and sodium chloride contents of 6.05±0.01, 147.35±1.88 mgN $100g^{-1}$, 12.65±0.07% and 19.01±0.04%, respectively. Salt (as sodium chloride) and protein contents are two important quality criteria of the product and the present study reveals that the 1:3.5 salt to fish ratio, which is commonly used in the commercial manufacturing of Philippine fish sauce resulted to a sodium chloride content of the product which is lower than the 20-25% standards for fish sauce while protein content is higher than the 1-8% required minimum amount. While the salt content is below 20%, the TVB-N values were within the set acceptable limit. The color description of the product was achieved at 270 days of fermentation, which was a clear liquid with the hues of yellow and red.
Key Words: fishery product, Philippine patis, fermentation, CIE L* a* b*.

Introduction. Fish sauce is a salt-fermented traditional fishery product in many Southeast Asian countries. It is a translucent, clear amber yellow or brown liquid, with a salty taste and fish flavor obtained from fermentation of a mixture of fish and salt, and the fermentation takes not less than 6 months (Codex Alimentarius 2013). The product is intended for direct consumption as a seasoning, or condiment, or ingredient for many Asian dishes. It is considered as high salt product due to its 20-25% sodium chloride content (Mackie et al 1971; Gopakumar 1997). It also contains free amino acids, essential fatty acids, considerable amount of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) (Oh 1999; Cho et al 2000; Montaño et al 2001; Dincer et al 2010; Ulfah 2013) that are beneficial to human health.

The process of fish sauce production normally involves the use of small species of fish like anchovy (*Stolephorus* sp.), sardines (*Sardinella* sp.), mackerel (*Rastrelliger* sp. and *Scomber scombrus*), gambusia (*Affinis affinis*), Pacific whiting (*Merluccius* sp.) and other low value fish species, and the methods vary according to their practices (Essuman 1992; Tungkawachara et al 2003; Ibrahim 2010; Olubunmi et al 2010). Anchovy is used in salt-fermented fish sauce in Southeast Asian region due to its recognized high quality and it fetches good market price. There are two species of anchovy that are widely used, *Stolephorus indicus* (van Hasselt, 1823) and *S. commersonii* (Lacepède, 1803). The commercial manufacturing of fish sauce varies in fish species and salt ratios and this greatly affects the quality of the final product. Every country in Southeast Asia has their own formulation and the physico-chemical qualities vary from one formulation to the other. In the Philippines, it is manufactured using various species of fish like anchovies,

sardines, mackerel and other small fish species mixed with varying salt to fish ratios ranging from 1:1 to 1:5 (Sanchez 2008). Malaysian fish sauce *budu* uses anchovies and salt (7:3 w/w) and the method of processing includes boiling of the mixture (Ulfah 2013). Other countries like Thailand, Vietnam, Laos, Myanmar and Cambodia use the same species of fish with that of the Philippines but have their own practices in the processing of salt fermented fish sauce product (Mackie et al 1971; Gopakumar 1997). Due to these variations, the quality of fish sauce varies from one processor to the others. The biochemical characteristics of fish sauce largely depend on the quality of raw materials, salt concentration or ratio of salt to fish, method of processing and length of fermentation methods as well as the fish species. Thus, this study measures changes in the physico-chemical and color characteristics of salt-fermented anchovy sauce with 1:3.5 salt:fish ratio over the 270-day fermentation period.

Material and Method

Preparation of fish sauce sample. Newly-caught anchovy, *S. commersonii*, with a mean total length of 9.26±0.467 cm, mean weight of 15.05±1.05 g, caught within the waters of Panay Gulf, Philippines were used in the study. Approximately twenty (20) kilograms of anchovy were immediately iced and kept inside the styrophore boxes and transported, in less than 15 minutes, to the University of the Philippines Visayas Laboratories. The fish samples were cleaned (ungutted), washed with potable water and drained. The salt and fish, with 1:3.5 ratio (w/w), were mixed thoroughly and packed in previously sterilized glass jars, covered with cheese cloth and tied with rubber bands. All packed samples were kept at room temperature of about 28 to 30^{o}C and allowed to ferment for 270 days, with weekly stirring of the mixture.

Sampling procedure. Salt-fermented anchovy was mixed thoroughly using stainless steel ladle and a representative sample of 200 g was collected at day 1, 7, 14, 21, 28, 35, 45, 55, 90, 120, 150, 180, 210, 240 and 270 days of fermentation. The sample was placed in 4-fold cheese cloth, hydraulic pressed and filtered using filter papers (Whatman No. 41) and these were subjected for physico-chemical analyses. All analyses were carried out in triplicates. For color evaluation, fish sauce samples were analyzed at day 28, 120, 150, 210 and 270 days. Analyses were done in replicates.

Physico-chemical analyses. A 5 mL fish sauce sample mixed to 50 mL neutralized distilled water was used for pH determination. The pH was measured directly using a digital waterproof pH meter (pHTestr BNC Oakton Singapore). Salt as sodium chloride was determined by titration according to modified Volhard method and AOAC 935.47 (AOAC 2005). Crude protein was determined by the Kjeldhal method using the Kjeltec system. Measurements of total volatile base nitrogen compounds were conducted using Conway microdiffusion procedure wherein total volatile base-nitrogen (TVB-N) was estimated in a trichloroacetic acid extract (Siang & Kim 1992).

Color evaluation. Samples of salt-fermented anchovy sauce which were collected at various fermentation stages of 28, 120, 150, 210 and 270 days, were brought to the Industrial Technology Development Institute of the Department of Science and Technology for color evaluation. The color characteristics of the samples were determined using the CIE L* a* b* scale with the CR-200 Chroma Meter (Minolta Japan). Fish sauce samples were placed in an optical glass cell and reflectance measurements were obtained from the average of 2 readings for each sample, and done in replicates. The color difference values and the total color difference values were computed. The use of the unit of color difference Delta E (ΔE) from the National Bureau of Standards (CIE 1978; Clydesdale 1978) was recommended. The equation below estimates the overall changes in color over fermentation time based on L* a* b* values determined at day 28, as initial value.

Color difference values: ΔL* = L*- L*initial; Δa*= a* - a* initial; Δb* = b* - b* initial

Total color difference values: $\Delta E_{ab} = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$

where

L* = lightness;
a* = chromaticity of red and green;
b* = chromaticity of blue and yellow.

Statistical analysis. The results obtained were analyzed statistically using the One-way ANOVA and a post-hoc analysis, the Tukey's test, when there were significant differences among the group. All statistical analyses were performed using the statistical software, SPSS version 14.0 (Chicago, Illinois, USA). The level of significance was set at α level of 0.05, $p < 0.05$ (Sokal & Rohlf 2012). All values were presented as mean±standard deviation (SD) and statistical significance is indicated with appropriate letters on the data tables.

Results and Discussion

Physico-chemical characteristics. One-way ANOVA showed a significant effect of fermentation period on the pH values, TVB-N and total protein ($p < 0.05$) of salt-fermented anchovy sauce with 1:3.5 salt to fish ratio, however the salt content was not significantly influenced ($p > 0.05$) (Table 1). The 270-day fermentation period affected the physico-chemical characteristics of salt-fermented anchovy sauce stored at ambient condition of 28-30°C.

Table 1

Chemical changes in salt-fermented fish sauce made from anchovy (*Stolephorus commersonii*), during fermentation

Fermentation days	*pH*	*Salt as NaCl (%)*	*TVB-N (mg 100g^{-1})*	*Total protein (%)*
1	6.99±0.08^{ab}	18.51±0.60	21.97±1.00^{g}	13.58±0.44^{abc}
7	6.96±0.02^{ab}	19.68±0.35	24.17±2.49^{g}	12.85±0.96^{bc}
14	6.90±0.01^{ab}	19.59±0.42	25.59±1.51^{g}	15.12±0.42^{ab}
21	7.00±0.04^{a}	19.47±0.13	23.46±2.30^{g}	14.16v0.77^{abc}
28	7.01±0.00^{a}	19.45±0.20	34.82±2.58^{f}	14.78±1.42^{abc}
35	6.95±0.04^{ab}	19.22±0.27	46.05±2.04^{e}	15.55v0.67^{a}
45	6.92±0.03^{ab}	19.74±0.17	45.81±3.38^{e}	15.36±1.75^{a}
55	6.91±0.02^{ab}	19.46±0.05	54.38±0.65^{d}	14.16v0.77^{abc}
90	6.87±0.03^{abc}	19.59±0.40	86.21±0.95^{c}	14.16±0.77^{abc}
120	6.80±0.01^{bc}	19.43±0.06	136.54±1.48^{b}	13.74v0.94^{abc}
150	6.84±0.02^{abc}	19.61±0.47	147.50±2.09^{a}	13.97±1.91^{abc}
180	6.67±0.01^{c}	19.41v0.11	151.65±4.55^{a}	14.56±1.52^{abc}
210	5.96±0.01^{d}	19.39±0.09	147.95±0.74^{a}	14.40v1.31^{abc}
270	6.05±0.03^{d}	19.01v0.04	147.35±1.88^{a}	12.65±0.07^{c}

*Values are shown as mean±standard deviation of triplicates; superscripts in each column with different letters are significantly different ($p < 0.05$).

The pH of the fermentation mixture influences the extraction of fish proteins and the maximum extraction of protein takes place between pH 7.0 and 9.0 (Sanchez 2008). The same author stated that the decrease in pH in salt-fermented fish sauce samples was probably due to dissociation of amino acids and small peptides in the presence of salt. In the present study, the decrease in pH was observed on the first two weeks of fermentation process, increased on the following days and thereby decreased on 180 days of fermentation and onwards. The drop in pH value on the first few weeks of fermentation was also observed in the study of El Hag et al (2012) and Kilinc et al (2006) wherein similar pattern of decreasing pH values in their fermented fish samples during the early stages of fermentation using debs, *Labeo sp*. with 25% salt and sardines with

10% salt added, respectively. The pH in salt-fermented fish sauce product is a very important characteristic in identifying between good and low quality fish sauce. Good quality fish sauce can only be produced from good quality raw material where pH of fresh fish is almost neutral. After fish dies, decomposition occurs through enzymatic digestions of fish muscle and gradually increased the pH of the flesh. The use of proper concentration of salt in the fermentation process inhibits decomposition of fish.

The pH of salt-fermented anchovy sauce significantly decreased over the 270 day fermentation period, from its initial pH of 6.99±0.08 to its final value of 6.05±0.03. The pH value in day 1, however, was not significantly different from day 7 until 150 days of fermentation. The significant drop in pH was observed during the 210 until 270 days of fermentation, with pH 5.96±0.01 and 6.05±0.03, respectively. It was observed that the initial pH of the fish sauce samples until 150 days of fermentation was at the near neutral values of 6.99-6.84. It also demonstrates the rapid decrease from pH 6.67 on day 180 to pH 6.05 on day 270. The pH difference may be due to protein hydrolysis which resulted from free hydrogen ions, free amino acids, and amino acid of oligopeptides (Tungkawachara et al 2003). The same authors reported the pH of fermented Pacific whiting fish sauce, fermented for 9 months (270 days) ranged between 7.05 (0 month) and 5.42 (9 months). Lopetcharat & Park (2002) reported the pH of fish sauce using the same fish species fermented for 40 days ranged between 6.1 and 6.3. The pH 6.08, a value which was almost similar to the final pH in this study, was observed in 150 day salt-fermented fish sauce from gambusia (Ibrahim 2010).

The pH values of most of the commercially available Southeast Asian salt-fermented fish sauces were within the range of 4.66-5.91 (Cho et al 2000). The Tropical Product Institute of the United Kingdom recommended a standard pH of 5.8 in high grade fish sauce while the Myanmar food standards and specifications for fish sauce recommended a pH ranged between 6.0 and 7.5 (Yap & Lin 1986). The same authors revealed that market samples of Thai and Chinese fish sauce are between 5.4 and 5.6, and 5.3 and 5.8, respectively. Codex Alimentarius (2013) stated a standard pH ranged of 5.0-6.5 for fish sauce products. In the present study, the pH value of the 270-day fermented anchovy sauce was 6.05 and it is within the recommended value.

Figure 1 shows the increasing values in the TVB-N during the 270 days fermentation period. The measurement of TVB-N indicates the degree of proteolysis of samples caused by spoilage bacteria, autolytic enzymes, deamination of amino acids and nucleotide catabolites (FDA 2004). The TVB-N values of salt-fermented fish sauce from anchovy had significantly increased ($p < 0.05$) during the 270-day fermentation period but the values fluctuate over time of fermentation. It had an initial value of 21.97±0.99 and reached a level of 147.35±1.88 mg 100 g^{-1} on the 270 days of fermentation. The values were within the acceptable limits set, which are at 100-200 mg 100 g^{-1} for salted and dried fish products (FDA 2004) and within the range of 14.1-338.6 mg 100 mL^{-1} TVB-N which were found in most Southeast Asian fish sauce products (Cho et al 2000).

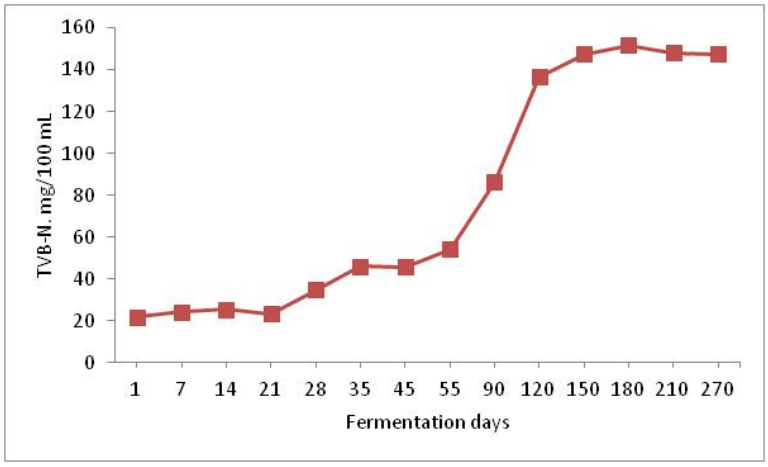

Figure 1. Changes in total volatile base-nitrogen (TVB-N) of salt-fermented anchovy sauce over the 270-day fermentation period.

The increase in TVB-N was due to the presence of microorganisms in the salt-fermented fish sauce samples. Similar increasing pattern was also observed in the study of Besas & Dizon (2012) wherein the TVB-N of the fish sauce samples increased during the 7 month fermentation and was affected by salt concentration and fish species. The fish sauce samples from tuna (*Thunnus albacares*) viscera with 10%, 17.5% and 25% NaCl had TVB-N values of 226.80, 81.60 and 76.41 mg 100 g^{-1}, respectively. In the present study, the fish sauce sample had salt concentration in the range of 18.51±0.60 to 19.01±0.04% and the low TVB-N values could be attributed to the preservative action of salt, though values increased over time of fermentation. The same trend was also reported in the study of Tsai et al (2006) wherein TVB-N increased during fermentation.

Huss (1995) stated that TVB-N is used to measure seafood quality but it reflects later stage of advanced spoilage. The high level in TVB-N was formed due to protein degradation through microbial and enzymatic activities and this resulted from the formation of nitrogenous compounds such as ammonia. The total volatile base nitrogen is formed in fermented fish products due to autolysis and microbial spoilage of fish during processing. TVB-N is the combined amount of ammonia, dimethylamine and trimethylamine and this value is often used as a rejection limit in regulations and commercial specifications. The increase in nitrogenous basic compounds is caused by microbial proteolytic enzymes and consequently the utilization of amino acids by fermenting microorganisms (Dissaraphong et al 2006).

The salt content of the anchovy sauce samples did not significantly change during fermentation and the values were within the range of 18.51±0.60-19.74±0.17% NaCl. Most of the salt-fermented fish sauce products found in different Southeast Asian countries had salt content within the range of 24.1-30.6% (Cho et al 2000). The present study revealed that the salt content in salt-fermented anchovy sauce with 1:3.5 salt to fish ratio fluctuates during the 270-day fermentation period. There was an abrupt increase in salt content on the 2nd day of fermentation, after which the values fluctuates over time, and had the final salt content as NaCl of 18±51.60%. This value is lower compared to some salt-fermented fish sauce products with higher than 20% NaCl (Cho et al 2000; Tungkawachara et al 2003). This study, however, used the 1:3.5 salt to fish ratio, similar to the traditional salt-fermented fish sauce in Malaysia called *budu*, (Ulfah 2013). It is interesting to note, however, that although the salt content is below 20%, the TVB-N value of the sample is within the acceptable limit.

The total protein decreased during fermentation, but the values were fluctuating over time. The initial value was 13.58±0.44 and had a final protein content of 12.65±0.073 on the 270 days of fermentation. The content was significantly affected by the days of fermentation ($p < 0.05$). The protein content obtained in this study is in agreement with the result on fish sauce from gambusia, with 1:3 salt:fish ratio, and fermented for 5 months which had 12.37±0.77% (Ibrahim 2010). This finding is also similar to the observation of Cho et al (2000) wherein total protein of some Southeast Asian fish sauce products were within the range of 0.9-13.7%. Philippine standard for crude protein in fish sauce products is 8.0% for special and 4.0% for regular fish sauce products. This study shows that the protein content of fish sauce product with 1:3.5 salt to fish ratio met the standards set for high quality fish sauce.

During fermentation, there is a possibility that crude protein content decreased (Sanchez 2008) and this may be due to some changes such as Maillard reactions or by the reactions of myoblobin as affected by bacterial action and this can also be accounted due to changes in pH (Lawrie 1998). While it is true that fish muscle protein could swell by the presence of salt, however, protein denaturation occurs if salt concentration increased during fish fermentation process (Hamm 1994).

Color description. Most studies on the evaluation and description of color of salt-fermented fish sauce are based on visual assessment. In most cases, color of fish sauce is used as indicator of quality, where straw yellow or amber color is seldom differentiated from dark brown liquid color.

Similarly, the color characteristics were significantly affected by the fermentation period ($p < 0.05$). Table 2 shows the color characteristics of salt-fermented fish sauce as measured by CR-200 Chroma Meter (Minolta Japan). Color properties of fish sauce samples from anchovy vary according to the fermentation period. There is a clear difference in the color of the samples as shown by the mean CIE L*a*b* values wherein the L* values decreased, and the a* and b* values increased during the 270 days fermentation.

Table 2

Color characteristics of salt-fermented fish sauce from anchovy

Fermentation days	*Color characteristics[a] (L* a* b* values)*		
	L*	a*	b*
28	96.25 ± 0.07^{a}	-1.29 ± 0.06^{b}	4.29 ± 0.12^{e}
120	87.95 ± 0.92^{ab}	-2.83 ± 0.21^{bc}	12.45 ± 0.21^{d}
150	82.06 ± 2.09^{bc}	-4.29 ± 0.42^{c}	48.01 ± 0.01^{c}
210	75.85 ± 0.08^{c}	-2.41 ± 0.01^{b}	50.68 ± 0.0^{b}
270	68.41 ± 1.19^{d}	$+4.90 \pm 0.92^{a}$	66.14 ± 1.07^{a}

[a] CIE L*a*b* values wherein L* represents lightness or grayscale axis whose values run from 0 (black) to 100 (white); a* and b* values are based on coloraxes which run from positive to negative: on the a-a' axis, positive values indicate amounts of red while negative values indicate amounts of green; on the b-b' axis, yellow is positive and blue is negative. For both a* and b* axes, zero is neutral gray.
*Values are shown as mean±standard deviation of triplicates; Superscripts in each column with different letters are significantly different ($p < 0.05$).

The L* values which were decreasing signify that the lightness of the samples were from lighter to darker as fermentation progressed until 270 days. The light yellow color was achieved when fish sauce was produced at 270 days, at the time the salt-fermented fish sauce ripened. The initial result of a* and b* values shows that color properties of samples at 28 days to 210 days, and fish sauce samples at 270 days were different. The a* values or the chromaticity of red and green, of the fish sauce samples at 28 days to 210 days had exhibited a slight to moderate green hue (-1.285 to -4.29) while fish sauce samples at 270 days had a dominant red hue (+4.9). The b* values or the chromaticity of blue and yellow, the yellow hue (+b*) was the dominant component in all fish sauce samples but the intensity decreases as the ripening of the fish sauce progresses.

The study of Lopetcharat & Park (2002) on the color properties of fish sauce from Pacific whiting revealed that unripened fish sauce produced from Pacific whiting had different color properties compared to the commercial anchovy fish sauce based on a* and b* values. Yellow hue (b*) was the dominant component in both commercial fish sauce and fish sauce made with Pacific whiting. Regarding a* values, the commercial fish sauce from anchovy exhibited more red hue while unripened Pacific whiting fish sauce possessed a slight green hue. It was further stated that transformation of hue from green to red is expected to occur during the ripening process of fish fermentation. Similarly, the present study exhibited the same findings in terms of the dominant red and yellow hues of the 270 days fish sauce from anchovy, as reflected in the mean values of a* and b*, respectively.

This study also revealed that fermented fish sauce for 210 days at 28-30^{o}C storage condition was not yet ripened. It is based on the description that fish sauce is a clear liquid sauce, with straw yellow to amber in color (PNS 413:1993). It is also evident in this study that color intensity of salt-fermented fish sauce changes according to fermentation period and as affected by the maturity of the product. It may be due to lipids in the fish sauce samples wherein lipids in the fish are broken down in the process to yield fatty acids which may act as precursors not only for flavors and aroma compounds but may also participate in the browning reaction or Maillard reaction that takes place progressively during fermentation (Dougan & Howard 1975; Saisithi et al 1966; Saisithi 1994).

Conclusions. The results of this study showed that fermentation period of 270 days significantly affects the physico-chemical and color characteristics of salt-fermented anchovy sauce with 1:3.5 salt to fish ratio, except for the salt content which was not significantly affected. Salt (as sodium chloride) and protein contents are two important quality criteria of the product and the present study reveals that the 1:3.5 salt to fish ratio, which is commonly used in the commercial manufacturing of salt-fermented fish sauce resulted to a sodium chloride content of the product which is lower than the Codex Alimentarius standard of not less than 20% salt content for fish sauce. However, the results also revealed that the TVB-N value which is an index of spoilage in dried and salted products, is within the set acceptable limits. The protein content is higher than the 1-8% required minimum amount. The color description of the product was achieved at 270 days of fermentation, which was a clear liquid with the hues of yellow and red. The product has achieved the desirable color of clear amber yellow. It is recommended that the purity of commercially available salt that will be used in fish fermentation has to be established.

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