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Improving antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste through prolonged fermentation

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ABSTRACT

The antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste were improved through prolonged fermentation (90, 180, and 360 days). The antioxidant ability against 1,1-diphenyl-2-picryhydrazyl (DPPH) radical, hydrogen peroxide, and lipid peroxidation increased significantly with prolonged fermentation and were suggested to be related with the Maillard reaction products formed, as measured by the characteristic browning and fluorescent developments. Polyunsaturated fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the shrimp paste were not substantially damaged for 360 days, while free amino acid content dramatically increased at 90 days. However, excessive fermentation showed slight but significant decrease in free amino acids and increase in ammonia. These results suggest that properly prolonged fermentation would improve antioxidant ability and some nutritional value in the salt-fermented shrimp paste.

1. Introduction

Fish fermentation is one of the most common methods of fish preservation in the Philippines due to the simplicity of technology and low equipment cost. Fish paste and sauce are the most popular products due to their salty, slightly cheese-like flavour and possessing a characteristic appetite-stimulating aroma. Fish paste is obtained through the natural fermentation process of whole fish or shrimp in the presence of 20–25% salt under ambient conditions. On the other hand, fish sauce is a straw yellow to amber clear liquid extracted through the complete hydrolysis of fish/salt mixture for 9–12 months (Lopetcharat, Choi, Park, & Daeschel, 2001). These fermented fish products will remain a part of the diet of most Filipinos due to its desirable flavour and cheap source of protein. Fish fermentation is the transformation of organic substances into simpler compounds such as peptides, amino acids, and other nitrogenous compounds either by the action of microorganisms or endogenous enzymes. Peptides and amino acids are important contributors to the flavour and aroma of fermented products (Raksakulthai & Haard, 1992) but they have also been found as naturally occurring antioxidants. Antioxidant activity has been found in a number of fermented fishery products such as fermented blue mussels (Jung, Rajapakse, & Kim, 2005), fish sauces (Harada et al., 2003; Michihata, 2003), and fermented shrimp paste (Peralta et al., 2005).

In the previous study (Peralta et al., 2005), we found that salt-fermented shrimp paste exhibited antioxidant activity. However, the observed initial antioxidant activity of the product did not show significant increase after 10 days of fermentation. This could be due to the short fermentation period employed. Prolonging the fermentation period of shrimp paste could produce other substances that may contribute to an increase in total antioxidant activity. Studies have shown that while amino compounds such as amino acids and peptides function as a primary antioxidant (Amarowicz & Shahidi, 1997; Hatate, Numata, & Kochi, 1990; Houlihan & Ho, 1985; Klompong, Benjakul, Kantachote, & Shahidi, 2007; Park, Jung, Nam, Shahidi, & Kim, 2001), they also interact with other substances to form compounds exhibiting antioxidant activity such as Maillard reaction products (MRPs) (Kitts & Weiller, 2003; Morales & Jimenez-Perez, 2001; Wang & Gonzalez de Mejia, 2005). MRPs are formed by the reaction between reducing sugars and amino compounds, commonly observed in food processing including fermented fishery products. Their antioxidant activity has been evaluated in sugar–lysine (Jing & Kitts, 2004), glucose–glycine (Yoshimura, Iijima, Watanabe, & Nakazawa, 1997), and sugar–protein (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2004) model systems. Therefore, MRPs having antioxidant activity

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would be formed in shrimp paste as well as other fermented fishery products through prolonged fermentation because of its abundant in free amino acids (Peralta et al., 2005). However, fish fermentation process also involves the use of high salt concentration and incubation at ambient temperature. Fish oils are generally good dietary sources of essential polyunsaturated fatty acids (PUFAs) including eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) (Ackman, 1995) but could be greatly damaged during severe fermentation conditions.

This study aimed to improve antioxidant activity of salt-fermented shrimp paste by prolonging the fermentation for up to 12 months and assessed variation of its nutritional value such as PUFAs and amino acids. We also measured the development of brown colour and fluorescence, associated with the production of MRPs, as a possible contributor to the observed antioxidant activity.

2. Materials and methods

2.1. Chemicals

α-Tocopherol, 1,1-diphenyl-2-picryhydrazyl (DPPH), and 2,2′-azobis (2-amidinopropane) dihydrochrolide (AAPH) were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Methyl linoleate was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Triton X-100 and horseradish peroxidase (HRPO) were from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in this experiment were of analytical grade.

2.2. Preparation of salt-fermented shrimp paste

Shrimp (*Acetes* spp.), purchased from a local market in Atabayan-Iloilo, Philippines, was mixed with salt in a ratio of 3:1 by weight and allowed to ferment for 360 days at ambient temperature (28–35 °C). The salt-fermented shrimp samples were collected at the initial (1 day), 90, 180, and 360 days of fermentation and stored at −30 °C before use. The moisture content of salt-fermented shrimp samples, determined by the oven method at 105 °C, were 62.1%, 63.2%, 62.9%, and 64.4% at 1, 90, 180, and 360 days, respectively. Added amount of sample for the antioxidant assays is expressed as dry weight (D.W.) of the starting salt-fermented shrimp pastes calculated from their moisture contents unless otherwise indicated.

2.3. Preparation of 80% ethanol extract from salt-fermented shrimp paste

The salt-fermented shrimp sample (5 g) was homogenized and mixed with 5 mL of water and 20 mL of 95% ethanol. After centrifugation at 400*g* for 20 min, the upper layer was recovered. The precipitate was again treated with 20 mL of 95% ethanol as described above. The recovered upper layers were combined and adjusted to 50 mL by adding 95% ethanol (Peralta et al., 2005). When insoluble materials were observed, the extracted solution was again centrifuged to remove them. The resulting solution, which contained approximately 80% ethanol, was designated as 80% ethanol extract.

2.4. DPPH radical scavenging activity

Appropriate amount of the 80% ethanol extract was diluted to 9 mg D.W. of shrimp paste/mL with the 80% ethanol solution. Each sample (1.0 mL) was incubated with 0.25 mL of 0.5 mM DPPH in ethanol for 20 min at room temperature and absorbance was read at 517 nm (Peralta et al., 2005). When the insoluble substances were observed in the sample mixture, they were removed by centrifugation before reading the absorbance. The DPPH radical scavenging activity (%) was calculated from the decrease of absorbance at 517 nm by the addition of 80% ethanol extract toward that of control (without antioxidant).

2.5. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured using the method of Bahorun et al. (1996) with slight modification. Appropriate amount of the 80% ethanol extract was added to a test tube. The solvent was removed by evaporation under reduced pressure or with nitrogen gas stream. The dried sample (5.0 mg D.W. of shrimp paste) was well mixed with 50 μL of 80% ethanol, and then was incubated with 0.45 mL of 0.1 M phosphate buffer (pH 7.0) containing 89 mM NaCl and 50 μL of 23 mM hydrogen peroxide for 10 min at 37 °C. To the mixture, 0.5 mL of 0.1 M phosphate buffer (pH 7.0) containing 0.05 mg HRPO and 0.1 mg phenol red was added and kept at room temperature for 15 min. Then, 50 μL of 1.33 M NaOH was added to the mixture. After 10 min, the absorbance was read at 610 nm. When the insoluble substances were observed in the sample mixture, they were removed by centrifugation before reading the absorbance. The hydrogen peroxide scavenging activity (%) was calculated from the decrease of absorbance at 610 nm by the addition of 80% ethanol sample extract toward that of control (without antioxidant).

2.6. Inhibition of methyl linoleate peroxidation

Appropriate amount of the 80% ethanol extract was added to a screw cap test tube, and the solvent was removed as described above. The dried sample (10.0 mg D.W. of shrimp paste) was dispersed with 1.0 mL of 0.025 M phosphate buffer (pH 7.0) containing 0.025 M methyl linoleate and 1.25% Triton X-100 using a sonicator and a Vortex mixer. The methyl linoleate peroxidation was initiated by adding 0.25 mL of 12.5 mM AAPH at 37 °C (Peralta et al., 2005). The peroxidation degree of methyl linoleate was periodically measured by the ferric thiocyanate method (Inatani, Nakatani, & Fuwa, 1983) and expressed as absorbance at 500 nm.

2.7. Measurement of fluorescence and brown colour developments

The 80% ethanol extract was diluted to 3.0 mg D.W. of shrimp sample/mL with 80% ethanol and its fluorescent and brown colour developments were measured using the method of Benjakul, Lertittikul, and Bauer (2005). The fluorescence intensity was measured at an excitation wavelength of 347 nm and emission wavelength of 415 nm using a fluorescence spectrophotometer RF-540 (Shimadzu Co., Kyoto, Japan). The brown colour was measured at 420 nm using a spectrophotometer UV-190 (Shimadzu Co., Kyoto, Japan).

2.8. Free amino acid analysis

The free amino acid contents in the salt-fermented shrimp pastes were estimated using the 80% ethanol extracts (Peralta et al., 2005). The solvent was removed as described above. The dried extract was again dissolved with an appropriate amount of lithium citrate buffer (pH 2.98) (JEOL Ltd., Tokyo, Japan) and filtered through a Millipore filter (0.45 μm). Free amino acids and some related compounds of the filtrate were determined by using an automatic amino acid analyzer system JLC-500/V (JEOL Ltd., Tokyo, Japan).

2.9. Extraction of total lipid and fatty acid analysis

Total lipid (TL) was extracted from the homogenate of salt-fermented shrimp paste with chloroform and methanol mixture according to the method of Bligh and Dyer (1959). Fatty acid constituents in the TL were converted to fatty acid methyl esters using 5% hydrochloric methanol (90 °C, 1.5 h) (Hatate, Ohgai, Murase, Miyake, & Suzuki, 1998). They were then analyzed by a gas–liquid chromatography using a GC-14A (Shimadzu Co., Kyoto, Japan) equipped with a capillary column Omegawax 320 (30 m × 0.32 mm, film thickness 0.25 μm, Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector. The carrier gas was helium at a flow rate of 1.6 mL/min and the split ratio was 50 to 1. The injection and detection ports were set to 250 and 260 °C with an oven temperature program of 180–230 °C at 1 °C/min. The fatty acid methyl esters were identified by comparing the retention times with authentic ones.

2.10. Statistical analysis

The data in Tables were expressed as mean ± SD($n-3$). Analysis of variance was performed by ANOVA procedure. Duncan's new multiple-range test was used to determine the differences of means. p values <0.05 were regarded as significant. Regression analysis was used with commercial PC program of Microsoft Excel 98.

3. Results

3.1. Antioxidant activity of salt-fermented shrimp paste

Table 1 shows the changes in DPPH radical and hydrogen peroxide scavenging activity of 80% ethanol extracts from salt-fermented shrimp paste samples at 1, 90, 180, and 360 days. The initial DPPH radical scavenging activity at one day significantly increased up to 360 days. Similarly, the hydrogen peroxide scavenging activity of the 80% ethanol extracts increased at 180 days and declined, but not significantly at 360 days ($p > 0.05$). Thus, prolonged fermentation of shrimp paste contributed to a significant increase in antioxidant activity ($p < 0.05$).

Fig. 1 shows the behavior of the 80% ethanol extracts in inhibiting methyl linoleate peroxidation. The absorbance at 500 nm, which indicates the degree of methyl linoleate peroxidation, of the control (without antioxidant) increased rapidly while the addition of the 80% ethanol extracts as well as a known antioxidant, α-tocopherol, suppressed the increase. α-Tocopherol concentration-dependently inhibited lipid peroxidation and its addition of 15 μg appeared to be comparable activity with shrimp samples at 90, 180, and 360 days. However, their activity was not so different among them but was stronger than the initial (1 day). This suggests that prolong fermentation contributed to an increase in the ability to suppress lipid peroxidation.

Table 1
Changes in antioxidant activity and Maillard reaction products (MRPs) formation of 80% ethanol extracts from salt-fermented shrimp pastes during fermentation for 1, 90, 180, and 360 days

Fermentation period (day)	Antioxidant activity		Index of MRPs formation	
	DPPH radical (9 mg D.W./ assay system)*	Hydrogen peroxide (5 mg D.W./ assay system)	Browning (3 mg D.W./mL)	Fluorescence (3 mg D.W./mL)
1	24.3 ± 1.9a	29.2 ± 1.5a	0.098 ± 0.009a	20.0 ± 0.6a
90	41.4 ± 6.6b	44.4 ± 3.6b	0.136 ± 0.004b	31.1 ± 0.9b
180	50.2 ± 0.5c	56.0 ± 3.7c	0.211 ± 0.007c	86.6 ± 0.3c
360	61.5 ± 0.9d	51.1 ± 3.6c	0.259 ± 0.005d	76.9 ± 3.0d

* Added amount of sample was expressed as dry weight (D.W.) of the starting salt-fermented shrimp pastes calculated from their moisture contents. The moisture contents of salt-fermented shrimp pastes determined by the oven method at 105 °C were 62.1%, 63.2%, 62.9%, and 64.4% at 1, 90, 180, and 360 days, respectively. Values in the same column with the different letters are significantly different ($p < 0.05$). Results are mean ± SD for $n = 3$.

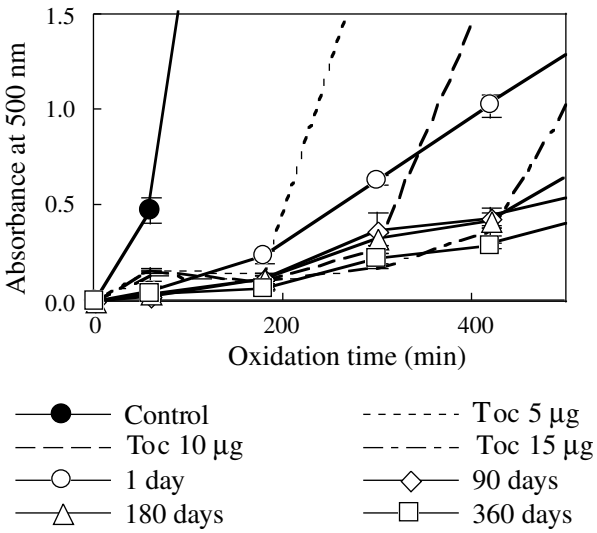

Fig. 1. Typical inhibitory pattern of methyl linoleate peroxidation by 80% ethanol extracts from salt-fermented shrimp pastes during fermentation for 1, 90, 180, and 360 days. Sample (10 mg D.W. of shrimp paste) was incubated in 1.25 mL of 0.02 M phosphate buffer (pH 7.0) including 0.02 M methyl linoleate, 1% Triton X-100, and 2.5 mM AAPH as radical initiator at 37 °C. Lipid peroxidation degree was periodically measured by thiocyanate method and expressed as absorbance at 500 nm. α-Tocopherol (Toc, 5, 10, and 15 μg) was used as positive control. Plots show mean ± SD for $n = 3$.

3.2. Brown colour and fluorescence development

Table 1 also presents the absorbance readings of the brown colour and fluorescence intensity of the 80% ethanol extracts at various fermentation periods. Both values as an index of the formation of MRPs showed significant increase during fermentation compared to the initial ones ($p < 0.05$), although fluorescence intensity decreased slightly from 180 to 360 days. The increasing tendency of MRPs during fermentation was similar to that of the observed antioxidant activity (Table 1). Fig. 2 shows the regression analyses between MRPs formation and antioxidant activity. Browning showed good correlation both with DPPH radical scavenging activity ($r = 0.969$) and hydrogen peroxide scavenging activity ($r = 0.846$) (Fig. 2a). Fluorescent intensity also related significantly with DPPH radical scavenging activity ($r = 0.858$) and hydrogen peroxide scavenging activity ($r = 0.909$) (Fig. 2b). This suggested that the formation of MRPs was responsible for the increase in antioxidant activity of the salt-fermented shrimp paste during prolonged fermentation.

3.3. Free amino acid analysis of salt-fermented shrimp paste

Table 2 presents the free amino acid content of 80% ethanol extracts at various fermentation periods. The initial total free amino acid content of 3600 mg/100 g of sample wet weight dramatically increased to 8239 mg at 90 days, but it did not significantly increase from 90 to 180 days ($p > 0.05$). At 360 days, it significantly decreased as compared to 90 days ($p < 0.05$). The major free amino acids present in the sample extracts were taurine, glycine, alanine, lysine, and arginine, and most of them significantly increased up to 90 or 180 days ($p < 0.05$). However, at 360 days, the amino acids such as taurine, arginine and glutamic acid showed significant

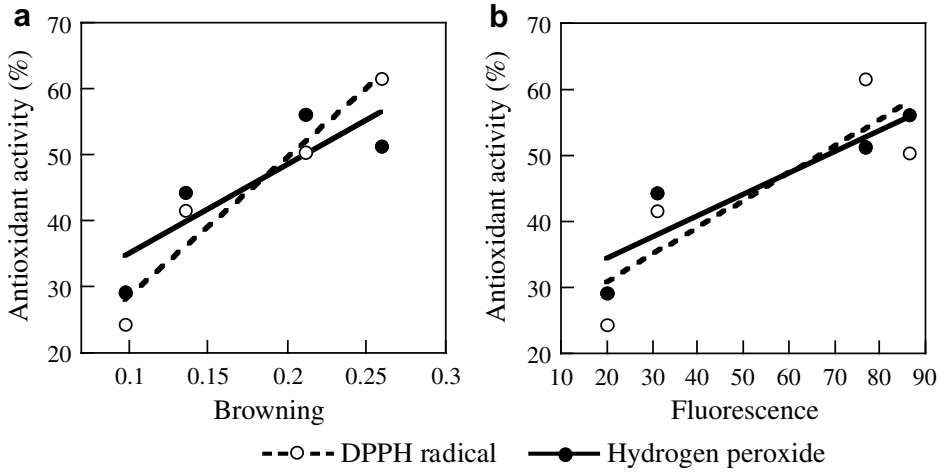

Fig. 2. Correlation between index of MRPs formation and antioxidant activity. (a) Correlation of browning with DPPH radical (r = 0 969) and with hydrogen peroxide (r = 0.846), (b) Correlation of fluorescence with DPPH radical (r = 0.858) and with hydrogen peroxide (r = 0.909). Mean shown in Table 1 was used for regression analysis.

Table 2
Changes in free amino acid content of 80% ethanol extracts from salt-fermented shrimp pastes during fermentation for 1, 90, 180, and 360 days

Amino acid	Fermentation period (day)			
	1	90	180	360
	(mg/100 g wet weight of shrimp paste)			
o-Phosphoserine	24 ± 2a	23 ± 0a	35 ± 4b	40 ± 0c
Taurine	827 ± 70a	896 ± 7ab	919 ± 45b	730 ± 12c
Urea	134 ± 33	22 ± 19	83 ± 106	33 ± 30
Aspartic acid	33 ± 5a	440 ± 19b	493 ± 13c	611 ± 13d
Threonine	91 ± 5a	268 ± 3b	248 ± 2c	256 ± 4d
Serine	47 ± 8a	172 ± 2b	139 ± 3c	181 ± 4d
Asparagine	52 ± 17a	0 ± 0b	0 ± 0b	0 ± b
Glutamic acid	140 ± 17a	102 ± 2b	67 ± 2c	40 ± 3d
Glutamine	94 ± 22a	823 ± 24b	778 ± 15c	725 ± 9d
Glycine	222 ± 7a	371 ± 8b	383 ± 7b	330 ± 2c
Alanine	257 ± 11a	433 ± 23b	375 ± 16c	254 ± 1a
Citrulline	153 ± 41a	450 ± 22b	528 ± 35c	760 ± 4d
α-Aminobutyric acid	0 ± 0a	56 ± 2b	45 ± 1c	62 ± 1d
Valine	127 ± 7a	499 ± 16b	422 ± 9c	428 ± 5c
Methionine	72 ± 1a	165 ± 27b	183 ± 3bc	205 ± 3c
Cystathionine	9 ± 2a	14 ± 8a	35 ± 1b	36 ± 1b
Isoleucine	105 ± 17a	448 ± 18b	320 ± 3c	301 ± 2c
Leucine	241 ± 30a	889 ± 43b	667 ± 10c	603 ± 3d
Tyrosine	93 ± 5a	138 ± 28b	173 ± 15c	156 ± 6b
Phenylalanine	92 ± 15a	374 ± 12b	348 ± 15c	308 ± 4d
Ornithine	1 ± 1a	140 ± 5b	154 ± 4c	208 ± 5d
Histidine	25 ± 1a	75 ± 1b	44 ± 1c	24 ± 3a
Lysine	241 ± 4a	620 ± 11b	614 ± 10b	625 ± 11b
Tryptophan	18 ± 16a	81 ± 8b	40 ± 35a	52 ± 16a
Anserine	0 ± 0a	37 ± 32b	9 ± 15ab	0 ± 0a
Arginine	363 ± 9a	412 ± 8b	370 ± 11a	276 ± 28c
Proline	133 ± 13a	280 ± 33bc	236 ± 35b	314 ± 9c
Ammonia	6 ± 5a	65 ± 4b	119 ± 5c	163 ± 4d
Total	3600 ± 230a	8239 ± 306b	7783 ± 351bc	7658 ± 131c

Values in the same line with the different letters are significantly different (p < 0.05). Results are mean ± SD for n = 3.

Table 3
Changes in fatty acid composition (%) of total lipids from salt-fermented shrimp pastes during fermentation for 1, 90, 180, and 360 days

Fatty acid	Fermentation period (day)			
	1	90	180	360
14:0	3.7 ± 0.1	3.7 ± 0.2	3.5 ± 0.1	3.5 ± 0.1
15:0	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
16:0	20.2 ± 1.8	20.5 ± 1.5	17.9 ± 3.3	18.4 ± 1.9
16:1*n*−7	9.2 ± 0.3	9.4 ± 0.5	10.1 ± 0.9	10.0 ± 0.6
16:2*n*−4	1.3 ± 0.2	1.3 ± 0.1	1.1 ± 0.2	1.2 ± 0.2
16:3*n*−4	0.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
18:0	6.1 ± 0.9	6.2 ± 0.8	5.1 ± 1.2	5.4 ± 0.8
18:1*n*−9	6.6 ± 0.1	6.6 ± 0.1	7.1 ± 0.9	7.2 ± 0.3
18:1*n*−7	2.4 ± 0.0	2.4 ± 0.0	2.6 ± 0.3	2.6 ± 0.1
18:2*n*−6	1.9 ± 0.0	1.9 ± 0.0	2.0 ± 0.3	2.0 ± 0.1
18:3*n*−3	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.2	1.6 ± 0.0
18:4*n*−3	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.1	0.8 ± 0.1
20:0	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
20:4*n*−6	5.1 ± 0.1	5.1 ± 0.1	5.3 ± 0.8	5.4 ± 0.2
20:5*n*−3	13.2 ± 0.4	13.4 ± 0.3	13.7 ± 1.7	13.7 ± 0.6
22:0	1.7 ± 0.1	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.2
22:4*n*−3	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.0
22:5*n*−3	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0
24:0	0.5 ± 0.0ab	0.4 ± 0.0a	0.7 ± 0.2b	0.4 ± 0.1a
22:6*n*−3	16.1 ± 0.7	16.5 ± 0.5	17.9 ± 0.4a	16.7 ± 0.7
24:1	0.6 ± 0.0	0.6 ± 0.1	0.8 ± 0.4	0.5 ± 0.1
Others	5.6 ± 1.3	4.3 ± 1.2	4.7 ± 0.6	5.2 ± 0.8

Values in the same line with the different letters are significantly different (p < 0.05). Results are mean ± SD for n = 3.

decrease in content as compared to 90 or 180 days (p < 0.05). The decline in the free amino acid content could be due to its degradation to amines, volatile acids, and other nitrogenous substances as by-products of bacterial metabolism or enzymatic decomposition. An example is ammonia. Ammonia, an index of the degradation, has distinctly increased during prolonged fermentation (p < 0.05) (Table 2). However, the observed decline in amino acids would be also responsible for the formation of MRPs, as manifested by the increase in brown colour and fluorescence intensity (Table 1). The automatic amino acid analyzer used in this study can also determine content of two antioxidant dipeptides, anserine and carnosine (Kohen, Yamamoto, Cundy, & Ames, 1988), but their content was very small or undetectable through fermentation. Therefore, the antioxidant dipeptides would not be correlated with antioxidant activity in the salt-fermented shrimp pastes.

3.4. Fatty acid analyses of salt-fermented shrimp paste

The TL was extracted from the salt-fermented shrimp pastes at various fermentation periods to estimate their fatty acid composition (Table 3). The TLs of salt-fermented shrimp pastes contained large amount of PUFAs and the major fatty acids were 16:0, 16:1*n*−7, 18:0, 18:1*n*−9, 20:4*n*−6, 20:5*n*−3 (EPA) and 22:6*n*−3 (DHA). Their composition in the salt-fermented shrimp pastes did not significantly change during fermentation for 360 days (p < 0.05) except DHA (%) at 180 days that was slightly higher than other samples. The observed initial and increased antioxidant

activity (Table 1 and Fig. 1) would have inhibited the oxidation of PUFAs through prolonged fermentation. It can be concluded that the PUFAs including EPA and DHA have remained intact despite the severe fermentation conditions.

4. Discussion

Free radicals are generally reactive and attack other molecules such as lipids, proteins and sugars. This may result to oxidative damage such as deterioration of foods, protein modification, and enzyme inactivation. Antioxidant substances can prevent or delay these oxidative damages by inhibiting the production of primary catalyst of lipid peroxidation or by interfering with the propagation step of lipid oxidation by reacting with free radicals (Noguchi & Niki, 1999). Salt-fermented shrimp was found to exhibit antioxidant activity, which did not increase after 10 days fermentation. This antioxidant activity would be mainly due to the original antioxidants in the shrimp paste but not to the fermentative products (Peralta et al., 2005). In order to improve its antioxidant activity, we prolonged the fermentation time for up to 12 months like fish source (Lopetcharat et al., 2001) and investigated changes in antioxidant activity and some nutritional value at 1, 90, 180, and 360 days of fermentation.

The 80% ethanol extracts of salt-fermented shrimp paste exhibited significant DPPH radical and hydrogen peroxide scavenging activity and ability to inhibit methyl linoleate peroxidation as the fermentation progressed (Table 1, Fig. 1). Fermentation, a process of protein hydrolysis, produces peptides and amino acids. Several amino acids such as tyrosine, methionine, histidine, lysine and tryptophan are generally known to exhibit antioxidant activity (Kitts & Weiller, 2003; Houlihan & Ho, 1985). This study did not show any significant increase in free amino acid after 90 days of fermentation of shrimp paste, while DPPH radical and hydrogen peroxide scavenge activity increased after then (Tables 1 and 2). Therefore, the amino acids would not directly influence the observed increase in antioxidant activity but likely participate in the production of antioxidant substances such as MRPs.

MRPs formed in food have been reported to exhibit high antioxidant activity (Jing & Kitts, 2004). In this study, the browning and fluorescent intensity, an index of MRPs formation, increased significantly as fermentation prolonged (Table 1) and showed the good correlation with antioxidant activity such as hydrogen peroxide and DPPH radical scavenging activity ($r > 0.8$) (Fig. 2). Some reports have also shown the participation of MRPs formation in antioxidant activity. Porcine plasma protein and reducing sugar (glucose, fructose and galactose) heated for 5 h showed increasing activity with increasing browning (Benjakul et al., 2005). In soy sauce, the brown colour, as products of Maillard reaction, was recognized as the compound contributing to the antioxidant activity (Moon, Lee, Lee, & Trakoontivakorn, 2002). It was also found that light coloured soy sauce has low antioxidant activity while the dark coloured ones showed higher activity. These reports support our speculation that MRPs formation in shrimp paste would be responsible for the increase in antioxidant activity during prolonged fermentation. The antioxidant activity observed in this study, especially substances showing antioxidant ability in inhibiting lipid peroxidation (Fig. 1), would effectively prevent the PUFAs of shrimp paste from undesirable oxidation during prolonged fermentation of 360 days (Table 3). On the other hand, MRPs can also decrease digestibility and possibly form toxic and mutagenic compounds. These undesirable effects of MRPs have been widely studied in food model systems and mostly associated with the use of high temperature such as autoclave temperature in forming the MRPs (Kitts & Hu, 2005). Because the MRPs formation in this study was performed at ambient temperature using a traditional processing in the Philippines, they would be less toxic. However, further research on possible formation of toxic MRPs in salt-fermented shrimp paste is required to determine its safety aspect.

The major free amino acids in shrimp paste are taurine, glycine, alanine, leucine, lysine and arginine (Table 2), and most of them increased dramatically during fermentation. Lysine, proline, alanine, glycine, serine, glutamic acid, and leucine have been shown to be the important taste compounds of shrimp (Raksakulthai & Haard, 1992) and the increase in these amino acids would further enhanced its desirable flavour. However, a decrease in glutamic acid, a substance responsible for the 'umami' taste in most fermented fish products (Kim, Shahidi, & Heu, 2003; Lopetcharat et al., 2001), was observed as fermentation progress. This decline can cause changes in the sensory characteristics of shrimp paste. Increase in ammonia, one of the major odor components, was also observed during the fermentation (Table 2). These results suggest that, although accumulation of free amino acids in shrimp paste is predominant, undesirable changes might occur during prolonged fermentation. Biogenic amines, influencing safety of fermented food, could be also produced in shrimp paste during prolonged fermentation. Although this study did not deal with biogenic amines, histamine, posing health hazard due to its toxic effect (Arnold & Brown, 1978), must be low in content because of the low level in its precursor, histidine (Table 2). It was also reported that histamine decreased continuously in content during fermentation time and this attributed to the presence of histamine-decomposing bacteria (Sanceda, Suzuki, Ohashi, & Kurata, 1999).

In this study, we did not evaluate variation in salt-fermented shrimp paste between the initial (1 day) and 90 days of fermentation. However, considering the obtained results, the antioxidant activity and MRPs would increase during this period while the PUFA must be intact (Fig. 1, Tables 1 and 3). The free amino acid content also increased and might reach the maximum earlier than 90 days of fermentation. The maximum value would be almost the same as those of 90 and 180 days since no significant change in total amino acid content was observed between 90 and 180 days (Table 2). Regarding antioxidant dipeptides, carnosine and anserine were not significantly detected in the salt-fermented shrimp pastes (Table 2). However, we should study some other bioactive peptides because shrimp paste could produce them as well as observed in various fish protein hydrolysates (Amarowicz and Shahidi, 1997; Hatate et al., 1990; Jung et al., 2005; Klompong et al., 2007).

5. Conclusions

Salt-fermented shrimp paste, which is normally processed for 10 days in the Philippine, could increase in antioxidant activity and free amino acid content by prolonging fermentation time. The increase in antioxidant activity was suggested to depend on formation of MRPs, having antioxidant activity. PUFAs such as EPA and DHA in the shrimp paste remained almost intact during prolonged fermentation while excessive prolongation such as for 360 days brought about a slight decrease in free amino acids. Thus, properly prolonged fermentation would improve antioxidant ability and some nutritional value such as amino acids in the salt-fermented shrimp paste without loss in the PUFAs.

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References

Ackman, R. G. (1995). Composition and nutritive value of fish and shellfish lipids. In A. Ruiter (Ed.), *Fish and fishery products: Composition, nutritive properties and stability* (pp. 77–115). UK Biddles Ltd. Guildford: CAB International.

Amarowicz, R., & Shahidi, F. (1997). Antioxidant activity of peptide fractions of capelin protein hydrolysates. *Food Chemistry, 4*, 355–359.

Arnold, S. H., & Brown, W. D. (1978). Histamine toxicity from fish products. *Advances in Food Research, 24*, 113–154.

Bahorun, T., Gressier, B., Trotin, F., Brunet, C., Dine, T., Luyckx, M., et al. (1996). Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneimittel-Forschung, 46*, 1086–1089.

Benjakul, S., Visessanguan, W., Phongkanpai, V., & Tanaka, M. (2004). Antioxidative activity of caramelization products and their preventive effect on lipid oxidation in fish mince. *Food Chemistry, 90*, 231–239.

Benjakul, S., Lertittikul, W., & Bauer, F. (2005). Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system. *Food Chemistry, 93*, 189–196.

Bligh, E. G., & Dyer, W. J. (1959). Rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Harada, K., Okano, C., Kadoguchi, H., Okubo, Y., Ando, M., Kitao, S., et al. (2003). Peroxyl radical scavenging capability of fish sauces measured by the chemiluminescence method. *International Journal of Molecular Medicine, 12*, 621–625.

Hatate, H., Numata, Y., & Kochi, M. (1990). Synergistic effect of sardine myofibril protein hydrolyzates with antioxidants. *Nippon Suisan Gakkaishi, 56*, 1011.

Hatate, H., Ohgai, M., Murase, N., Miyake, N., & Suzuki, N. (1998). Accumulation of fatty acids in *Chaetoceros gracilis* (Bacillariophyceae) during stationary growth phase. *Fisheries Science, 64*, 578–581.

Houlihan, C. M., & Ho, C. (1985). Natural antioxidants. In D. B. Martin & T. H. Smouse (Eds.), *Flavor chemistry of fats and oils* (pp. 117–143). USA: American Oil Chemists' Society.

Inatani, R., Nakatani, N., & Fuwa, M. (1983). Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis* L.) and their derivatives. *Agricultural and Biological Chemistry, 47*, 521–528.

Jing, H., & Kitts, D. D. (2004). Antioxidant activity of sugar–lysine Maillard reaction products in cell free and cell culture systems. *Archives of Biochemistry and Biophysics, 429*, 154–163.

Jung, W., Rajapakse, N., & Kim, S. (2005). Antioxidative activity of a low molecular weight peptide derived from the sauce of fermented blue mussel, *Mytilus edulis*. *European Food Research and Technology, 220*, 535–539.

Kim, J., Shahidi, F., & Heu, M. (2003). Characteristics of salt-fermented sauces from shrimp processing byproducts. *Journal of Agriculture and Food Chemistry, 51*, 784–792.

Kitts, D. D., & Weiller, K. (2003). Bioactive proteins and peptides from food sources: Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design, 9*, 1309–1323.

Kitts, D. D., & Hu, C. (2005). Biological and chemical assessment of antioxidant activity of sugar–lysine model Maillard reaction products. *Annals New York Academy of Sciences, 1043*, 501–512.

Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry, 102*, 1317–1327.

Kohen, R., Yamamoto, Y., Cundy, K. C., & Ames, B. N. (1988). Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain. *Proceedings of the National Academy of Sciences of the United States of America, 85*, 3175–3179.

Lopetcharat, K., Choi, Y. J., Park, J. W., & Daeschel, M. A. (2001). Fish sauce products and manufacturing: A review. *Food Reviews International, 17*, 65–88.

Michihata, T. (2003). Components of fish sauce Ishiru in Noto peninsula and its possibilities as a functional food. *Foods and Food Ingredients Journal of Japan, 208*, 683–692.

Moon, G., Lee, M., Lee, Y., & Trakoontivakorn, G. (2002). Main component of soy sauce representing antioxidative activity. *International Congress Series, 1245*, 509–510.

Morales, F. J., & Jimenez-Perez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to color and fluorescence. *Food Chemistry, 72*, 119–125.

Noguchi, N., & Niki, E. (1999). Chemistry of active oxygen species and antioxidants. In A. M. Papas (Ed.), *Antioxidant status, diet, nutrition and health* (pp. 3–20). Florida: CRC Press.

Park, P., Jung, W., Nam, K., Shahidi, F., & Kim, S. (2001). Purification and characterization of antioxidative peptides from protein hydrolyzate of lecithin-free egg yolk. *Journal of the American Oil Chemists' Society, 78*, 651–656.

Peralta, E., Hatate, H., Watanabe, D., Kawabe, D., Murata, H., Hama, Y., et al. (2005). Antioxidative activity of Philippine salt-fermented shrimp paste and variation of its contents during fermentation. *Journal of Oleo Science, 54*, 553–558.

Raksakulthai, N., & Haard, N. (1992). Correlation between the concentration of peptides and amino acids and the flavor of fish sauce. *ASEAN Food Journal, 7*, 286–290.

Sanceda, N. G., Suzuki, E., Ohashi, M., & Kurata, T. (1999). Histamine behavior during the fermentation process in the manufacture of fish sauce. *Journal of Agricultural and Food Chemistry, 47*, 3596–3600.

Wang, W., & Gonzalez de Mejia, E. (2005). A new frontier in soy bioactive peptides that may prevent age-related chronic diseases. *Comprehensive Reviews in Food Science and Food Safety, 4*, 63–78.

Yoshimura, Y., Iijima, T., Watanabe, T., & Nakazawa, H. (1997). Antioxidative effect of Maillard reaction products using glucose–glycine model system. *Journal of Agriculture and Food Chemistry, 45*, 4106–4109.