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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).