

Activity of naturally occurring antioxidants during heat processing of low-salt fermented shrimp paste

¹Ernestina M. Peralta, ²Augusto E. Serrano Jr.

¹ Institute of Fish Processing Technology, CFOS, UP Visayas, Miagao Iloilo, Philippines;

² Institute of Aquaculture, CFOS, UP Visayas, Miagao, Iloilo, Philippines. Corresponding author: A. E. Serrano Jr., serrano.gus@gmail.com

Abstract. The present study intends to assess the activity of naturally occurring antioxidants in salt-fermented shrimp paste when subjected to thermal processing. Shrimp (*Acetes sibugae*) was mixed with a ratio of 1:7 (salt:shrimp) and allowed to ferment for 15 days at room temperature (28 – 32 °C). Changes in antioxidant activity were measured during fermentation and heat-processed shrimp paste with added ingredients. Diphenyl-1-picrylhydrazyl (DPPH) free radical and hydrogen peroxide scavenging activities from day 1 significantly increased at day 5, with minimal increase as fermentation progressed to day 15 and with no significant differences. DPPH scavenging activity generally increased in the varying heat processing employed in cooking shrimp paste. On the other hand, although hydrogen peroxide scavenging activity significantly increased after 10 min heating, activity decreased in the 30 min boiling and pasteurization except for sample product with no ingredients added. The study showed that the consequence of processing and preservation procedures on the overall antioxidant activity of shrimp paste is generally the result of different reactions which probably took place consecutively or simultaneously. Thus, processing methods may either improve the properties of naturally occurring antioxidants or induce the formation of new compounds having antioxidant properties.

Key Words: Shrimp paste, low-salt, antioxidant activity, heat processing.

Introduction. *Acetes sibugae* and *Acetes intermedius* are two of the species found in the Visayas sea area (located in the central part of the Philippines) and commonly used in the production of salt-fermented and dried shrimp products. Traditionally, shrimp is mixed with a ratio of 1:3 (salt:shrimp) resulting in a salt content of the product of 22 – 25 %. Fermented fish products contain peptides and amino acids that are known to function as naturally occurring antioxidants (Guerard et al 2002; Harada et al 2002; Jung et al 2005). Salt-fermented shrimp paste contain strong antioxidant activity when is fermented for 10 days (Peralta et al 2005) which significantly increased after 60, 180 and 360 days of fermentation (Peralta et al 2007; Peralta 2008; Peralta et al 2008). It suggests that health benefits can be derived from the consumption of the product. However, with the changing lifestyle of consumers, preference has shifted to healthy food such as low-salt, low-fat foods. The change from high to low salt reduces shelf stability of salt-fermented shrimp, and an additional process of heat treatment is commonly employed.

Application of heat as processing technique is widely believed to greatly affect the naturally occurring antioxidants in food. Antioxidants decrease their resistance against oxidation through interaction with other food components during processing, by evaporation, formation of pro-oxidants or their liberation from inactive complexes (Nicoli et al 1997; Pokorny & Schmidt 2001). Astaxanthin extracted from fermented shrimp byproducts undergoes oxidation when exposed to air and full light (Armenta & Legarreta 2009). On the other hand, antioxidant properties can be enhanced during thermal heating through the transformation of antioxidants into a more active compound, *i.e.* the production of Maillard reaction products (MRPs). Thermal treatment induces the formation of MRPs with new antioxidant properties (Nicoli et al 1997). Studies have

shown that during heating of fermented cabbage (Kusznierewicz et al 2008), sugar-tuna stomach hydrolysate (Martinez-Sumaya et al 2005) and sugar-amino acid model systems (Benjakul et al 2005; Jing & Kitts 2004; Yoshimura et al 1997), there is concomitant formation of MRPs with antioxidant capacity. As far as we know, there is no study yet on the effects of heating on the natural antioxidants in Philippine fermented products. The present study intends to assess the activity of naturally occurring antioxidants in salt-fermented shrimp paste subjected to thermal processing.

Material and Method

Preparation of raw material and treatments. Shrimp (*A. sibugae*), a common species found in the Central Philippine sea *i.e.* the Visayan Sea, was purchased from Tigbauan, Iloilo, cleaned and salted with a ratio of 1:7 (salt:shrimp). The mixture was allowed to ferment for 15 days at room temperature (30° – 35 °C) with occasional stirring. Samples from three replicates were withdrawn at days 1, 5, 10 and 15 and were subjected to antioxidant assays.

Shrimp paste was heat processed (sautéed and boiled) with added ingredients/spices (Table 1).

Table 1

Ingredients used for each type of shrimp paste product

<i>Sample code</i>	<i>Ingredients added</i>	<i>Product type</i>
CA	oil, garlic	Regular
CB	oil, garlic, sugar	Sweet
CC	oil, garlic, sugar, vinegar	Traditional
CD	none	Plain

Shrimp pastes were cooked/boiled for either 10 min or 30 min for water reduction under heat medium. Product variants were then packed in glass jar and pasteurized under boiling water for 1 h, cooled and incubated for 15 days. Samples from three replicates of heating time variable were withdrawn for analysis.

Preparation of 80 % ethanol extract from salt-fermented shrimp paste (raw and cooked). Shrimp paste (5 g) was homogenized with 5 mL of water and 20 mL of 95 % ethanol, centrifuged at 1500 rpm for 20 min. The upper layer was recovered and the process was repeated on the precipitate formed and the combined recovered upper layers were made up to 50 mL with 95 % ethanol (Peralta et al 2005). When insoluble materials were observed, the extracted solution was centrifuged again to remove the insoluble materials. The resulted solution, which contained approximately 80 % ethanol, was designated as 80 % ethanol sample extract.

Analytical method. Antioxidant activity assay.

A. DPPH radical scavenging activity. This assay involves the use of a free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is purple in color that gives a strong maximum absorption at 517 nm. The color turns from purple to yellow as the molar absorptivity of DPPH radical is reduced when the odd electron of DPPH radical becomes paired with hydrogen from free radical scavenging antioxidant to form the reduced DPPH-H. Appropriate amount of the 80 % ethanol extract was diluted to 3 mg (dry weight) of shrimp paste mL⁻¹ with 80 % ethanol solution. Each sample (1.0 mL) was incubated with 0.25 mL of 0.1mM DPPH for 20 min at room temperature (Peralta et al 2007). 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 the control (without antioxidant).

B. Measurement of hydrogen peroxide scavenging activity. Being a non-radical oxygen-containing reactive agent, hydrogen peroxide can form a hydroxyl radical, a known highly reactive oxygen radical, in the presence of transition metal ions and initiate lipid

peroxidation by abstracting the hydrogen atom from unsaturated fatty acids (Gutteridge & Halliwell 1994). Hydrogen peroxide scavenging activity was measured using the method described by Bahorun et al (1996) with modification. After concentrating the 85 % ethanol extract, an appropriate amount (50 μ L) was incubated in 0.45 mL of 0.1 M phosphate buffer (pH 7.0) containing 89 mM NaCl and 23 μ M H₂O₂ for 10 min at 37 °C. To the mixture was added 0.5 mL of 0.1 M phosphaste buffer (pH 7.0) containing 0.05 mg HRPO and 0.1 mg phenol red and kept at room temperature for 15 min. Then 50 μ L of 1.33 M NaOH was added to the mixture. Optical density was read at 610 nm after 10 min.

Brown color development. Browning reactions are some of the important phenomena occurring in food during processing and storage. The reaction is classified as non-enzymatic browning which involves sugar, amino acids or protein that condense and progress into a complex reaction products collectively known as Maillard reaction products (MRPs) (Jing & Kitts 2004). The 80 % ethanol sample extract was diluted to 3.0 mg (dry weight) sample mL⁻¹ with 80 % ethanol using the method of (Benjakul et al 2005). Optical density was measured at 420 nm.

Statistical analysis. One-way analysis of variance was used to test for significant difference among treatment means of DPPH and H₂O₂ scavenging activities and optical densities (O.D.) indicating brown color development. If significance was detected, a post hoc test was done using Duncan's multiple range test (DMRT) at 0.05 alpha level.

Results

Antioxidant activity assay. Changes in activity during fermentation of low-salt fermented shrimp paste. DPPH radical scavenging activity was evaluated in salt-fermented shrimp paste collected every five days (day 1, 5, 10, and 15) for 15 days. Results showed that the radical scavenging activity of 80 % ethanol extract from shrimp paste significantly increased with increasing sample concentration during fermentation (Table 2). Significant increase in activity was observed from day 1 to day 5 which could be due to the active breakdown of protein to peptides and amino acids by the initial bacterial load in shrimp paste. The prolonged fermentation from 5 - 15 days resulted in a slight increase in activity but was not significantly different between treatments.

Table 2
Changes in DPPH radical and hydrogen peroxide (H₂O₂) scavenging activity of 80 % ethanol extract from salt-ferment shrimp paste during fermentation

Sample	DPPH radical scavenging activity (%)		H ₂ O ₂ scavenging activity (%)
	7.5 mg*	10 mg*	3.0 mg*
Day 1	13.87 \pm 2.48 ^a	20.21 \pm 1.86 ^a	0.83 ^a
Day 5	20.84 \pm 2.84 ^b	31.56 \pm 4.05 ^b	na
Day 10	22.17 \pm 1.75 ^b	33.69 \pm 4.17 ^b	7.3 ^b
Day 15	24.0 \pm 2.75 ^b	34.47 \pm 1.30 ^b	8.1 ^b

*amount of sample used expressed as dry weight calculated from their moisture content. The moisture content was determined by oven method at 105 °C. Results are mean \pm SD for n=3. Values in the same column with different letters are significantly different (p<0.05), na – not analyzed.

Similar to DPPH scavenging activity, hydrogen peroxide scavenging activity significantly increased from day 1 to day 10 but did not significantly change at day 15 (Table 2).

Activity in heat processed salt-fermented shrimp paste. The initial DPPH free radical and hydrogen peroxide scavenging activity of 80 % ethanol extract (3 mg) of uncooked salt-fermented shrimp was 10.3 % and 8.1 %, respectively. Results showed that heat treatment of all samples (10 min) resulted in a significant increase in DPPH radical scavenging activity. In contrast, scavenging activity in samples treated with

extended heating time resulted in a marked decrease although not significantly different from each other. Hydrogen peroxide scavenging activity of the samples significantly increased after heat treatment, but significantly decreased when heating was prolonged (Table 3).

Table 3

DPPH radical and hydrogen peroxide (H₂O₂) scavenging activity of 80 % ethanol extract (3 mg*) of heat processed salt-ferment shrimp paste

Sample code	DPPH free radical scavenging activity (%)			H ₂ O ₂ scavenging activity (%)		
	Time (min)					
	10	30	90**	10	30	90**
CA	15.2	13.0	21.8	10.1	11.0	8.5
CB	17.0	13.0	18.1	20.4	8.6	3.9
CC	21.5	17.5	21.4	19.9	7.2	3.5
CD	18.6	18.1	24.4	19.8	6.3	1.0

*amount of sample used expressed as dry weight calculated from their moisture content. The moisture content was determined by oven method at 105 °C, **shrimp paste sautéed, packed in bottled and pasteurized.

Spices and ingredients *e.g.* garlic are known to exhibit antioxidant activity. However, in the present study, product CA exhibited lower activity than did product CD where no ingredients or oil was added. It was possible that the added ingredients did not contribute to the observed increase in scavenging activity. Extended heating (30 min) showed slight decreased in activity. However, when samples were bottled and heat-pasteurized, activity between samples significantly increased and was relatively similar with each other.

Brown color development in shrimp paste. Optical density of shrimp paste during heat processing as an index of brown color formation was variable. A marked increase in optical density was recorded when samples were further processed into bottled product. The observed increase in DPPH radical scavenging activity after bottle-processing (Table 4) could probably be due to the development of reaction products induced by heat.

Table 4

Optical density (brown color indicator) of extracts (15 mg*)

Sample code	Brown color (O.D.)		
	Time of heating (min)		
	10	30	90**
CA	013	016	022
CB	014	016	022
CC	018	015	017
CD	024	017	032

*amount of sample used expressed as dry weight calculated from their moisture content. The moisture content was determined by oven method at 105 °C, **shrimp paste sautéed, packed in bottled and pasteurized.

Discussion. Naturally occurring antioxidants are found in most plants and animal tissues. Majority of natural antioxidants are phenolic compounds and the most important groups are the tocopherols, flavonoids and phenolic acids. Shrimps are rich in protein and other essential nutrients as well as natural antioxidants (Rosenzweig & Babbit 1991; Seymour et al 1996).

Antioxidants are substances which significantly inhibit or delay oxidative processes such as lipid peroxidation even at low concentrations. For instance, polyunsaturated fatty acids (PUFAs) between salted (1:3) and unsalted ferments are similar in shrimp paste produced in Bolinao, Pangasinan, Philippines (Montano et al 2001). Salt-fermented

shrimp paste is also observed to maintain large amounts of EPA and DHA, which are not greatly affected during prolonged fermentation, regardless of the level of salt and temperature of fermentation (Peralta 2008; Peralta et al 2007). These observations could be attributed to the presence of naturally occurring antioxidants as well as substances with antioxidant properties produced during fermentation.

Fermentation involves the transformation of organic substances to simpler compounds such as peptides, amino acids and other nitrogenous compounds by bacterial or endogenous enzymes. While they are important contributors to the flavor and aroma of fermented products (Mackie et al 1971; Raksakulthai & Haard 1992), some exhibit antioxidant capacity (Kitts & Weiler 2003). Hydrolysate of shrimp processing byproduct contain high amount of hydrophobic amino acids (40.4 %) that may contribute to the high antioxidant activity (Zhao et al 2011). The amino acid content in salt-fermented shrimp paste (mostly taurine, alanine and lysine) increases from 886 to 1392 mg 100g⁻¹ after 10 day of fermentation (Peralta et al 2005). Amino acids such as tryptophan and histidine (Houlihan & Ho 1985), glycine and alanine (Hui-Chun et al 2003) exhibit antioxidative property. Tyrosine and lysine are generally accepted to be antioxidants (Wang & Gonzalez de Mejia 2005).

Amino compounds such as amino acids and peptides can function as primary antioxidants and can also interact with other substances to form Maillard reaction products (MRPs) (Kitts & Weiler 2003). They are non-enzymatic browning reactions that occur in foods. Lysine, as one of the major amino acid in salt-fermented shrimp paste, could have reacted with other substance thus reflecting an increase in activity. Sugar – lysine (Jing & Kitts 2004; Wijewickreme et al 1999), glucose-glycine (Yoshimura et al 1997) and sugar-protein (Benjakul et al 2005) model systems have been shown to exhibit antioxidant activity.

Heating results in oxidation reactions occurring rapidly (Pokorny & Schmidt 2001). In some cases, oxidation reactions show opposite effects on the antioxidant properties of foods. Partially oxidized polyphenols, for instance, exhibit higher antioxidant activity than that of non-oxidized phenols. Jeong et al (2004) observe that antioxidant activities in citrus peel extracts increase as heating temperature increases. Antioxidant activity in shrimp hydrolysate is stable when heated up to 100 °C (Zhao et al 2011). In the present study, heat treatment for 10 min significantly enhanced antioxidant activity. However, extending heating to 30 min resulted in a slight decrease in activity. During boiling, antioxidant activity of peptides and amino acids probably was affected by way of denaturation, chemical interaction with other substances or evaporation of some volatile antioxidants (Pokorny & Schmidt 2001). It is a commonly accepted observation that to minimize natural antioxidant degradation due to prolonged heating and evaporation, it is necessary to reduce residence time of food at high temperature and employ optimal evaporation methods such as rapid rate of heat transfer or low temperature operations.

Thermal processing at elevated temperature, *e.g.* pasteurization, probably influence the transformation of antioxidants into a more active and resistant compound such as MRPs. Antioxidant efficiency of MRPs is influenced by factors such as ratio and type of amino acid compounds and sugar involved, temperature, pH and water activity (Manzocco et al 2001). MRPs from sugar-tuna stomach hydrolysate heated at 95 °C and 115 °C increases the DPPH radical scavenging activity (Martinez-Sumaya et al 2005). MRPs from glucose-glycine heated for 1 h inhibits more than 90 % of active oxygen species existing in the form of hydroxyl radicals in the sample (Yoshimura et al 1997). Although the concentration of natural antioxidant is significantly reduced as a consequence of thermal treatments, the overall antioxidant properties of tomato derivatives and coffee are maintained or even enhanced by the development of MRPs (Nicoli et al 1997).

Conclusions. The present study showed that antioxidant activity in low-salt fermented shrimp paste was enhanced when subjected to thermal treatments probably due to either the increased resistance of natural antioxidants or its transformation into a more active compound or the formation of novel compounds such as MRPs or any combination of these three occurrences. Thus, the overall effect of thermal processing of low-salt

fermented shrimp was favorable where it converted the product into a shelf-stable commodity as well as increased its antioxidant capabilities which is known to have health benefits.

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Authors:

Ernestina Mateo Peralta, University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Fish Processing Technology, Philippines, Miagao, Iloilo 5023, e-mail: le_peralta03@yahoo.com

Augusto Erum Serrano Jr., University of the Philippines Visayas, College of Fisheries and Ocean Sciences, Institute of Aquaculture, Philippines, Miagao, Iloilo 5023, e-mail: serrano.gus@gmail.com

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