



## Effect of phenolic compounds in combination with modified atmospheric packaging on inhibition of quality losses of refrigerated Eastern little tuna slices

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### ABSTRACT

Quality changes of Eastern little tuna (*Euthynnus affinis*) slices treated with caffeic or tannic acid ( $0.2 \text{ g kg}^{-1}$ ) and the control during 15 days of refrigerated storage in air or under modified atmospheric packaging (MAP: 60%  $\text{CO}_2$ , 35%  $\text{N}_2$ , 5%  $\text{O}_2$ ) were studied. Tannic acid exhibited a greater preventive effect on metmyoglobin (metMb) formation and lipid oxidation than did caffeic acid ( $P < 0.05$ ). Samples treated with tannic acid and kept under MAP (MT) had the lowest lipid oxidation ( $P < 0.05$ ). After 12 days of storage, changes in unsaturated fatty acids, especially n-3 fatty acids, were lower in MT, compared with tuna slices stored in air. Based on microbiological acceptability, the shelf-life of tuna kept in air and MAP was estimated to be 6 and 12 days, respectively, irrespective of phenolic compounds treatment. Therefore, tannic acid exhibited a combined effect with MAP on inhibition of metMb formation, lipid oxidation and microbial growth, thereby improving the acceptance and increasing the shelf-life of tuna slices during refrigerated storage.

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### 1. Introduction

Antioxidants have been used to reduce lipid oxidation and off-odor development. Phenolic compounds, the secondary metabolites present typically in plants, have been reported for their antioxidative activity by donating electrons and chelating metals (Medina, Gallardo, González, Lois, & Hedges, 2007). Tang, Sheehan, Buckley, Morrissey, and Kerry (2001) reported the higher antioxidant effect of tea catechin in meat, poultry and fish than  $\alpha$ -tocopherol at the same concentration ( $0.3 \text{ g kg}^{-1}$ ) used. Corresponding to the ability of electron donating, caffeic acid showed the most preventive effect on lipid oxidation in minced horse mackerel, compared with other hydroxycinnamic acids and catechins (Medina et al., 2007).

Modified atmospheric packaging (MAP) has been proved to be an effective preservation method, in which the shelf-life extension and quality retention of fish and fish products can be achieved (Masniyom, Benjakul, & Visessanguan, 2002). Thiansilakul, Benjakul, and Richards (2011) found that high concentration of oxygen likely converted the purified oxymyoglobin (oxyMb) to

metmyoglobin (metMb) and induced a higher discoloration of Eastern little tuna fillet, compared with vacuum condition. MAP under a high level of  $\text{CO}_2$  (40–100%) is used to inhibit bacterial growth and extend shelf-life of fish and fishery products (Sivertsvik, Jeksrud, & Rosnes, 2002). Fresh seabass (*Lates calcarifer*) slices stored under MAP enriched with  $\text{CO}_2$  (80–100%) exhibited better odor, flavor and acceptability score in comparison with fish slices kept in air (Masniyom et al., 2002). In addition, using MAP (60%  $\text{CO}_2$ , 35%  $\text{N}_2$ , 5%  $\text{O}_2$ ) in combination with tannic acid treatment ( $0.2 \text{ g kg}^{-1}$ ) lowered lipid oxidation, heme protein changes and microbial growth as well as improved the sensory acceptance of refrigerated striped catfish slices (Maqsood & Benjakul, 2010).

Eastern little tuna (*Euthynnus affinis*) is a species available in the Gulf of Thailand and the Indian Ocean with the volume of 22,220 metric tons and a value of 18 million US dollars in 2008 which provide the high global economic value for canning and sashimi (Fisheries Foreign Affairs Division, 2008). To prolong the shelf-life of fish with minimized quality losses during storage, suppression of lipid oxidation and heme proteins changes are required. The objective of this investigation was to study the effect of phenolic compounds, caffeic acid or tannic acid, on quality changes of Eastern little tuna slices kept under different packaging atmospheres during refrigerated storage.

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## 2. Materials and methods

### 2.1. Chemicals

2-Thiobarbituric acid and 1,1,3,3-tetramethoxypropane were purchased from Sigma (St. Louis, MO, USA). Cumene hydroperoxide was procured from Fluka (Buchs, Switzerland). Ammonium thiocyanate and ferrous chloride were obtained from Riedel (Seelze, Germany). Sodium chloride, trichloroacetic acid, chloroform and methanol were procured from Merck (Darmstadt, Germany). Plate count agar (PCA) and Man Rogosa Sharpe (MRS) broth were obtained from Hi-media (Mumbai, India). All chemicals used were of analytical grade.

### 2.2. Fish samples

Eastern little tuna (*E. affinis*) with an average weight of 0.4–0.45 kg off-loaded after 24–36 h of capture were obtained from the dock in Songkhla province, Thailand. The fish were placed in ice with a fish/ice ratio of 1:2 (kg/kg) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within 1 h. Upon arrival, fish were washed with cold tap water, filleted, de-skinned and cut into slices with a weight of 50 g (thickness ~1.5–2 cm, length ~6–7 cm). The slices were placed on ice until use but not longer than 2 h.

### 2.3. Effect of caffeic acid or tannic acid in combination with MAP on quality changes of Eastern little tuna slices during refrigerated storage

Caffeic acid and tannic acid were dissolved separately in distilled water to gain a concentration of 10 g L<sup>-1</sup>. The mixtures were adjusted to pH 7.0 using 2 mol L<sup>-1</sup> NaOH. One mL of caffeic acid or tannic acid stock solution (10 mg mL<sup>-1</sup>) was pipetted onto Eastern little tuna slice (50 g) to obtain a final concentration of 0.2 g kg<sup>-1</sup>. The mixture was kneaded manually for 1 min. This concentration showed the high efficacy in prevention lipid oxidation in Eastern little tuna slices without causing the off-odor or discoloration (data not shown). Fish slices were placed on the 20 × 12 cm<sup>2</sup>-polystyrene trays (2 slices per tray) and inserted in 17.78 × 27.94 cm<sup>2</sup>-nylon/polyethylene bag with the selected gas permeability (O<sub>2</sub> transmission rate of 0.66 g m<sup>-2</sup> day<sup>-1</sup> at 23 °C, 101.33 kPa). The samples were packaged under MAP (60% CO<sub>2</sub>, 35% N<sub>2</sub>, 5% O<sub>2</sub>) with a gas/sample ratio of 2:1 (mL/g) using a Henko-vac type 1000 machine (Technovac, Italy). Fish slices treated without and with caffeic acid or tannic acid were packaged in air and designated as A, AC and AT, respectively, while those packaged under MAP were referred to M, MC and MT, respectively. For the control samples (A and M), distilled water (1 mL) was added to the slices. All samples were stored at 4 °C and taken for analyses at day 0, 3, 6, 9, 12 and 15 days. The fish slices were ground to uniformity before analyses, except for fishy-odor assessment, in which the slices were used without grinding.

### 2.4. Determination of metMb formation

Mb in ground samples was extracted following the method of Benjakul and Bauer (2001). Ground sample (2 g) was added with 20 mL of cold 40 mmol L<sup>-1</sup> Na-phosphate buffer (pH 6.8). The mixture was homogenized at 13,500 rpm for 10 s using an IKA Labortechnik homogenizer (Selangor, Malaysia), followed by centrifugation at 3000g for 30 min at 4 °C using an Allegra 25R centrifuge (Beckman Coulter, Palo Alto, CA, USA). The supernatant was filtered through Whatman No. 1 filter paper. The absorbances of Mb solutions were measured at 503, 525, 557 and 582 nm.

MetMb formation was calculated following a modified Krzywicki's equation (Tang, Faustman, & Hoagland, 2004) as follows:

$$[\text{metMb}] = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$$

where  $R_1 = A_{582}/A_{525}$ ,  $R_2 = A_{557}/A_{525}$  and  $R_3 = A_{503}/A_{525}$ .

### 2.5. Measurement of redness

Colorimetric values of ground samples with different treatments were determined using a colorimeter (JP7100F, Juki Corp., Tokyo, Japan). The instrument was calibrated using a white and black standard. All samples were determined for  $a^*$ -value (redness).

### 2.6. Measurement of lipid peroxides and thiobarbituric acid reactive substances (TBARS)

Peroxide value (PV) was determined as described by Richards and Hultin (2002) with some modifications. A standard curve was prepared using cumene hydroperoxide at concentrations ranging from 0 to 20 μmol L<sup>-1</sup>. PV was calculated and expressed as μmol cumene hydroperoxide/kg sample. TBARS were determined following the method of Buege and Aust (1978). A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 40 μmol L<sup>-1</sup>. TBARS values were calculated and expressed as μmol malonaldehyde kg<sup>-1</sup> sample.

### 2.7. Lipid extraction and determination of fatty acid profile

Lipids of fresh tuna, A and MT samples stored for 12 days were extracted following the method of Bligh and Dyer (1959) prior to analysis of fatty acid profile. The fatty acid methyl esters (FAMES) were prepared according to the method of AOAC (2000). The prepared methyl ester was injected into the gas chromatography (Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) at a split ratio of 10:1. A model of J&W 122-2362 DB-23 capillary column (50%-Cyanopropyl-methylpolysiloxane, 60 m × 0.25 mm) (Chrom Tech, Inc., Apple Valley, MN, USA) was used. The analytical conditions were: injection port temperature of 250 °C and detector temperature of 270 °C. The oven was programmed from 170 to 220 °C at a rate of 1 °C min<sup>-1</sup>. Retention times of FAME standards were used to identify chromatographic peaks of samples. Fatty acid content was calculated based on the peak area ratio and expressed as g fatty acid/100 g lipids.

### 2.8. Microbiological analysis

Fish slices with different treatments (25 g) were collected aseptically in a stomacher bag. After adding with 10 volumes of 8.5 g L<sup>-1</sup> sterile saline solution, the samples were mixed using a Stomacher blender (Stomacher M400, Seward Ltd., Worthington, England) for 1 min and a series of 10-fold dilutions was made by the same diluent. Total viable count (TVC) and psychrophilic bacterial count (PBC) were determined by plate count agar (PCA) with the incubation at 35 °C for 2 days (Hasegawa, 1987) and 7 °C for 7 days (Cousin, Jay, & Vasavada, 1992), respectively. Lactic acid bacteria (LAB) count was also determined using MRS agar after 3 days of incubation at 37 °C (Ordóñez, De Pablo, Perez de Castro, Asensio, & Sanz, 1991). Microbial counts were expressed as log cfu g<sup>-1</sup>.

### 2.9. Determination of fishy odor

Fishy-odor intensity was evaluated by 6 trained panelists who had the extensive experience in the evaluation of off-odors of raw fish. Panelists were trained for 3 sessions with 1.5 h each by sniffing

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