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Effects of skipjack roe protein hydrolysate on properties and oxidative stability of fish emulsion sausage

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ABSTRACT

Effects of skipjack roe protein hydrolysate (SRPH) at various levels (0–3 g/100 g) on properties and oxidative stability of emulsion sausage from broadhead catfish (*Clarias macrocephalus*) fortified with skipjack tuna roe lipids were investigated. The addition of SRPH increased hardness, cohesiveness and resilience of sausage ($p < 0.05$). Finer fat globules were visualised in the sample added with SRPH at higher amounts. Nevertheless, the incorporation of SRPH at all levels had no impact on likeness of sausages. SRPH was shown to retard lipid oxidation of sausage during extended storage of 12 days as evidenced by the lower peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS), in comparison with the control. After 12 days, the sausage with 3 g/100 g SRPH had the retained docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), accounting more than 80%. Addition of SRPH had no effect on the organoleptic properties but could prevent the development of rancidity. Nevertheless, it showed no pronounced impact on microbial growth. SRPH could therefore be used as a natural antioxidative emulsifier in cooked fish emulsion sausage.

1. Introduction

Emulsion sausage is commercially prepared by poultry, pork or beef emulsified with backfat from several sources. During comminution, bundles of fibres, myofibrils and filaments are disrupted and the size of fatty tissues is continuously reduced. In the presence of salt, myofibrillar proteins become soluble and migrate to the fat globule surface, concentrate and form protein matrix at the fat/water interface (Heinz, 1991; Youssef & Barbut, 2010). During comminuting, the temperature is usually controlled to prevent the mechanical overheating, which could lower the emulsifying property of proteins. This is associated with the migration of unemulsified fat to the product surface (Heinz, 1991; Liu, Callahan, & Solomon, 2009). Collapse of emulsion in sausage negatively affects the texture, mouthfeel and overall acceptance (Youssef & Barbut, 2009). As a result, the application of emulsifier is essential to improve the stability of emulsion product.

Recently, meat or fishery products fortified with *n*-3 fatty acids have considerable market growth due to health concern. Recommended daily intake of total *n*-3 polyunsaturated fatty acids (PUFAs) around 3–5.5 g has been made by British Nutrition Foundation (1992) but should not exceed 3.0 g/day in the form of fish oil, food and dietary supplements sources (FDA, 2000). The substitution of pork or beef backfat, mainly comprising saturated fatty acids, with fish oil possessing a high amount of PUFAs particularly DHA and EPA can enhance the nutrition quality of meat product. However, those PUFAs are susceptible to oxidation, in which undesirable off-odour can be developed. Emulsion sausages prepared from African walking catfish (*Clarias gariepinus*) and rohu (*Labeo rohita*) exhibited the lower oxidative stability when increasing amount of refined tuna oil was incorporated (Panpipat & Yongsawatdigul, 2008). Cáceres, García, and Selgas (2008) reported that conventional and low-fat cooked sausages added with 60 g/kg fish oil were prepared with sensorial acceptability. Although oils enriched with EPA and DHA are of nutritive value, the use of oil at high proportion commonly generates product with soft texture and the resulting product is susceptible to oxidation (Cáceres et al., 2008). Therefore the addition of antioxidant is required to prevent lipid oxidation. Previous *in vitro* study indicated that SRPH having 5% degree of hydrolysis (DH) prepared by Alcalase had good emulsifying properties and antioxidative activities, both radical scavenging activities and metal chelating activity (Intarasirisawat, Benjakul, Visessanguan, & Wu, 2012). Additionally, different fish roe hydrolysates have been

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known to have antioxidative activities. DPPH radical scavenging activity and reducing power were found for protein hydrolysate from roe of channa (*Channa striatus*), rohu (*L. rohita*) (Galla, Pamidighantam, Akula, & Karakala, 2012) and herring (*Clupea harengus*) (Sathivel et al., 2003). Therefore, SRPH could be used as antioxidative emulsifier in oil–water-emulsion meat system. Nevertheless, there is a little information regarding the use of fish protein hydrolysate in fish emulsion sausage supplemented with fish oil containing high PUFA. Therefore, the aim of this study was to determine the effect of skipjack roe hydrolysate on properties and oxidative stability of broadhead catfish emulsion sausage fortified with tuna roe lipids during the refrigerated storage.

2. Materials and methods

2.1. Chemicals

Cumene hydroperoxide and osmium tetraoxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid and glutaraldehyde were obtained from Merck (Damstadt, Germany). Thiobarbituric acid, ammonium thiocyanate and ferrous chloride were purchased from Fluka Chemical Co. (Buchs, Switzerland).

2.2. Extraction of tuna roe lipids

Lipids from tuna roe were extracted following the Bligh and Dyer method (1959) with silght modification. Skipjack roe (75 g) was homogenised with 150 mL of the cold chloroform–methanol mixture (1:1, v/v) for 1 min. The homogenate was added with 75 mL of chloroform and homogenised for another 30 s. The mixture was filtered through a Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, UK.). The filtrate was transferred to a separating funnel and allowed to stand at 4 °C overnight. The bottom phase (chloroform phase) was drained off into Erlenmeyer flask containing sodium sulphate anhydrous (1–2 g). The mixture was shaken thoroughly to remove the residual water. Lipid in chloroform was decanted into a rounded-bottom flask through a filter paper (Whatman No. 4). The chloroform was evaporated at 25 °C using a rotary evaporator (Rotavapor, model R-14, Buchi, Tokyo, Japan). Lipids obtained were transferred into the amber bottle, flushed with N_2 gas, closed tightly and stored at −20 °C until use.

2.3. Preparation of SRPH

SRPH with 5% DH, the percent ratio of the number of peptide bonds cleaved to the total number of peptide bonds in the substrate, was prepared as per the method of Intarasirisawat et al. (2012). The defatted sample (2.5 g dry matter) was suspended in 95 mL of distilled water and pre-incubated at 50 °C for 20 min prior to enzymatic hydrolysis. The hydrolysis reaction was initiated by the addition of Alcalase and the hydrolysis was taken at 50 °C, pH 8.0 for 1 h. The mixture was then placed into a water bath at 85 °C for 15 min to terminate the enzymatic reaction. Hydrolysate was lyophilised using a freeze-dryer (Model Duratop™ lP/Dura Dry™ lP, FTS® System, Inc., Stone Ridge, NY, USA).

2.4. Preparation of fish sausage

Broadhead catfish (*Clarias macrocephalus*) with the size of 1.0–1.2 kg/fish were purchased from a market in Hat Yai, Songkhla, Thailand. Fish were placed in ice using a fish/ice ratio of 1:2 and transported to the Department of Food Technology, Prince of Songkla University, within 30 min. Fish were manually decapitated, eviscerated and washed with ice water (4 °C). Bone and skin were removed. Fish fillets were then minced using a meat grinder (MX-T2G National, Tokyo, Japan). Emulsion sausage was prepared following the method of Panpipat and Yongsawatdigul (2008) with a slight modification. Fish mince (60 g) was mixed with 20 g/kg NaCl at 4 °C for 5 min. Subsequently, 20 g of the mixture between sunflower oil, containing 200 mg/L vitamin E (Morakot Industries PCL, Samutprakan, Thailand) and tuna roe lipids (7:3, v/v) were added. Other ingredients including 2 g/kg tripolyphosphate, 24 g/kg tapioca starch and 10 g/kg sugar were added. SRPH was incorporated at varying concentrations (0.5, 1 and 3 g/100 g). Moisture content of all samples was adjusted to 75 g/100 g. The mixture was ground for 3 min. The paste was stuffed into a 2.2 cm diameter cellophane casing. Both ends were sealed tightly. The samples were pre-incubated at 55 °C for 40 min prior to cooking at 80 °C for 15 min. The samples were placed in a polyethylene bag, sealed and kept at 4 °C.

2.5. Study on microstructure of fish sausage containing SRPH at different levels

The microstructure of the sausages added with SRPH at different levels was determined using a scanning electron microscope (SEM). Samples with a thickness of 2–3 mm were fixed with 25 mL/L glutaraldehyde in 0.2 mol/L phosphate buffer (pH 7.2). Fixed samples were washed with 0.1 mol/L phosphate buffer (pH 7.2) for 10 min and then post-fixed in 0.2 mol/L phosphate buffer (pH 7.2) containing 10 g/L osmium tetraoxide for 1 h. The fixed samples were then rinsed in 0.1 mol/L phosphate buffer for 10 min and rinsed with distilled water for 10 min before being dehydrated in ethanol with serial concentrations of 500, 700, 800, 900 and 1000 mL/L. Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

2.6. Effect of SRPH on properties of emulsion sausage during refrigerated storage

Sausages fortified with tuna roe lipids and added with SRPH at different levels were monitored for chemical, textural and microbiological changes every 2 days for totally 12 days, except fatty acid profile, which was determined at day 0 and 12 of storage. Sensory evaluation was conducted at day 0, 6 and 12 of storage.

2.6.1. Measurement of peroxide value (PV)

PV of sausage samples was determined according to the method of Richards and Hultin (2002) and Sakanaka, Tachibana, Ishihara, and Raj Juneja (2004). PV was expressed as μmol cumene hydroperoxide/kg sample.

2.6.2. Measurement of thiobarbituric acid-reactive substances (TBARS)

TBARS value was determined as described by Buege and Aust (1978). TBARS value was expressed as mg MDA/kg sample.

2.6.3. Measurement of fatty acid profiles

Fatty acid compositions were determined as fatty acid methyl esters (FAMEs) using a gas chromatography (GC-14A, Shimadzu Co., Kyoto, Japan) equipped with fused silica capillary column Carbowax-30 m (30 m, 0.25 mm ID, Alltech Ltd., Deerfield, IL, USA) and flame ionisation detector (FID) (Alltech Ltd., Deerfield, IL, USA). Helium was used as the carrier gas at a flow rate of 30 cm/s. The initial temperature of the column was set at 170 °C and increased to 225 °C with a rate of 1 °C/min and then held at 220 °C for an