Food Chemistry 119 (2010) 133–140

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chemical composition and antioxidative activity of Thai traditional fermented shrimp and krill products

Nandhsha Faithong, Soottawat Benjakul *, Suttirug Phatcharat, Wanwisa Binsan

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

ARTICLE INFO

Article history:
Received 16 February 2009
Received in revised form 25 April 2009
Accepted 5 June 2009

Keywords:
Antioxidant
Fermented products
Shrimp
Krill
Kapi
Koong-Som
Jaloo
Proteolysis

ABSTRACT

Chemical composition and antioxidative activities of some Thai traditional fermented shrimp and krill products including Jaloo, Koong-Som and Kapi were studied. All products did not contain myosin heavy chain or actin, but contained a large amount of small peptides. Kapi had the highest protein content, whereas carbohydrate content varied with products. Water-soluble fraction from all products possessed DPPH and ABTS radical-scavenging activity, as well as ferric reducing antioxidant power (FRAP) in a concentration-dependent manner. At the same concentration tested, the water-soluble fraction from Kapi exhibited the highest antioxidative activity. Soluble fraction of all products showed high stability over a wide pH range (2–11) and was stable after heating at 40–100 °C for 15–60 min. Fractions from all products heated at 100 °C had increases in FRAP, suggesting the enhancement of antioxidant activity. Therefore, fermented shrimp and krill products could be used as a potential source of nutrients and natural antioxidants.

1. Introduction

Oxidation of biomolecules, including lipid peroxidation, involves a series of free radical-mediated chain reactions and is associated with several types of biological damage (Haliwell, Aeschbach, Löliger, & Aruoma, 1995). Much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit lipid peroxidation and to protect biomolecules from damage by free radicals (Haliwell et al., 1995). Proteins and peptides from food have been found to be physiologically active or bioactive. Many peptides that are released *in vitro* or *in vivo* from animal or plant proteins have regulatory functions in the human body, apart from serving as important nutrients. Food-derived peptides exhibit antimicrobial properties, blood-pressure lowering effects, cholesterol-lowering ability, antithrombotic and antioxidant activities (Hartmann & Meisel, 2007).

Fermentation, a common practice in food preservation, plays an important role in improvement of nutritional and functional properties of foods. Cleavage of food proteins by microbial or indigenous proteases yields the bioactive peptides, leading to substantial increases in the biological properties of the food (Steinkraus, 2002). Moreover, fermented food products are a good source of peptides and amino acids (Rajapakse, Mendis, Jung, Je, & Kim, 2005; Sathivel et al., 2003). Among indigenous fermented products, fermented fishery products have been widely consumed in Southeast Asia as main dishes or condiments, due to their delicacy and high nutritional properties. Enzymatic fermentation of small fish and shrimp mediated by indigenous proteases yields short chain peptides and free amino acids, rendering the typical flavour and taste. To prevent putrefaction and food poisoning as well as to yield meaty-savoury flavour, the addition of salt in the range of 2–13% or higher to protein-rich substrates is common practice (Steinkraus, 2002).

Kapi is a typical traditional fermented shrimp paste, generally prepared from the planktonous shrimp or krill (*Acetes vulgaris* or *Mesopodopsis orientalis*), mixed with salt at a ratio of 3–5:1 and sun-dried to decrease the moisture content, then blended thoroughly. The paste is compacted and allowed to ferment for at least 2 months until the desired aroma has developed (Phithakpol, 1993). Jaloo, an indigenous salted krill (*M. orientalis*), is usually produced in communities near the mangrove coastal shore in the south of Thailand. Jaloo is prepared in the same manner as Kapi except that the drying is not required. Fermentation of Jaloo under anaerobic conditions generally takes 2–3 days. Koong-Som is another shrimp product produced by mixing small shrimp (*Acetes* sp.) with salt and palm-sap-sugar concentrate as a source

* Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.06.056

of carbohydrate. The mixtures are then left in anaerobic jars at room temperature to develop the fermentation by lactic acid bacteria until a sour taste and the typical flavour of fermented shrimp have developed (TCPS 1032, 2005).

Antioxidative activities associated with enzymatic hydrolysis or fermentation of marine animals have been reported. Fermentation of blue mussel could produce an antioxidative peptide with MW of 962 kDa, exhibiting strong scavenging effects towards superoxide, hydroxyl and DPPH radicals (Rajapakse et al., 2005). Tuna backbone proteins hydrolysed by pepsin contained antioxidant peptide (1519 Da), which had a capability of quenching free radicals, including superoxide, hydroxyl and DPPH radicals (Je, Qian, Byun, & Kim, 2007). Round scad meat hydrolysed by Alcalase or Flavourzyme had DPPH and ABTS radical activity and metal chelating activity; however the activities depended on the degree of hydrolysis and the amount of hydrolysate (Thiansilakul, Benjakul, & Shahidi, 2007). Hydrolysate from silver carp derived using Alcalase or Flavourzyme was reported to show hydroxyl radical-scavenging activity and metal chelating activity (Dong et al., 2008). Antioxidative activity of shrimp (*Acetes chinensis*) products was enhanced when hydrolysed by crude protease from *Bacillus* sp. (He, Chen, Sun, Zhang, & Gao, 2006). Additionally, Mungoong, a paste prepared from the cephalothorax of white shrimp, contained peptides which acted as an effective antioxidant with high stability over a wide pH and temperature range (Binsan et al., 2008).

Fermented shrimp/krill products have been consumed widely in southeastern Asian countries as a condiment or main dish. Apart from serving as a good source of proteins, those products might possess bioactivities, especially natural antioxidants, which provide health benefits. Accordingly, these products could be marketed as health foods with high market value. However, no information regarding the bioactivity of fermented fishery products, especially from shrimps or krill have been reported. Therefore, the objective of this study was to determine the chemical composition and antioxidative activities of fermented shrimp and krill products produced in Thailand, including Kapi, Koong-Som and Jaloo.

2. Materials and methods

2.1. Chemicals

Ethanol, methanol and trichloroacetic acid were obtained from Merck (Darmstadt, Germany). 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-trinitrobenzenesulphonic acid (TNBS) were purchased from Sigma Chemical Co. (St. Louis, MO). 2,4,6-Tripyridyl-*s*-triazine (TPTZ), ferric chloride hexahydrate, potassium persulphate, acrylamide, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and bis-acrylamide were procured from Fluka Chemical Co. (Buchs, Swizerland). Sodium sulphite and ammonium thiocyanate were obtained from Riedel-de Haen (Seelze, Germany).

2.2. Samples

Five samples of Kapi (KP1, KP2, KP3, KP4, KP5) produced from planktonous shrimp (*A. vulgaris*), 2 samples of Koong-Som (KS1, KS2) produced from small shrimps (*Acetes* sp.) (180–200 shrimps/kg) and 2 samples of Jaloo (JL1, JL2) produced from krill (*M. orientalis*) were purchased from different producers in local markets in Songkhla and Trang provinces, Thailand. For each sample (JL1, JL2, KS1, KS2, KP1, KP2, KP3, KP4 and KP5), three different lots were used. For each lot, three samples were purchased and pooled as the composite sample. All samples were packaged in polyethylene bags, stored at 4 °C until use and the storage time was not greater than 1 month.

2.3. Proximate analysis and pH determination of fermented shrimp/krill

Moisture, ash, fat, protein and salt contents of Kapi, Koong-Som and Jaloo were determined according to AOAC methods (1999) with the analytical No. of 35.1.13, 35.1.14, 35.1.25, 35.1.15 and 35.1.18, respectively. Sample pH was determined by a pH meter (Sartorius, Goetingen, Germany), as described by Benjakul, Seymour, Morrissey, and An (1997).

2.4. Determination of free amino group content and degree of hydrolysis (DH) of fermented shrimp/krill

DH of sample was determined according to the method of Benjakul and Morrissey (1997). The sample (1 g) was mixed with 9 ml of 70 mM SDS. The mixture was homogenised at a speed of 11,000 rpm for 1 min and was heated at 85 °C for 30 min. The mixture was then subjected to centrifugation at 10,000*g* for 15 min at room temperature using a Sorvall Model RC-5B Plus refrigerated centrifuge (Newtown, CT). To the supernatant obtained (125 μl), 2.0 ml of 0.2 M phosphate buffer (pH 8.2) and 1.0 ml of 3.41 mM TNBS solution were added. The solution was mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Schwabach, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulphite. The mixture was then cooled at room temperature for 15 min. The absorbance was read at 420 nm and free amino group content was expressed in terms of L-leucine. Degree of hydrolysis (*DH*) was calculated following the method of Benjakul and Morrissey (1997) with a slight modification:

$$\%DH = (L/L_{max}) \times 100$$

where L is the amount of free amino group in the product and L_{max} is the total free amino group after acid hydrolysis (6 M HCl at 100 °C for 24 h).

2.5. SDS–polyacrylamide gel electrophoresis of fermented shrimp/krill

Protein patterns of Kapi, Koong-Som and Jaloo were determined by SDS–PAGE using 4% stacking gel and 12.5% running gel, according to the method of Laemmli (1970). Samples (3 g) were solubilised in 27 ml of 0.17 M SDS (85 °C). The mixture was homogenised for 1 min at a speed of 13,000 rpm using an IKA homogeniser and incubated at 85 °C for 1 h to dissolve total proteins. Proteins (15 μg) determined by the Biuret method (Robinson & Hodgen, 1940) were loaded onto the gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini-Protean II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.25 mM Coomassie Brilliant Blue R-250 in 12.3 M methanol and 1.25 M acetic acid and destained with 12.3 M methanol and 1.25 M acetic acid for 15 min, followed by 1.23 M methanol and 1.25 M acetic acid for 3 h.

2.6. Preparation of soluble fraction from fermented shrimp/krill

Jaloo (2 g) or Koong-Som (5 g) or Kapi (2 g) was mixed with distilled water (100 ml) and the mixture was homogenised at a speed of 10,000 rpm for 3 min. The homogenate was stirred at room temperature for 30 min. The mixture was then centrifuged at 3000*g* for 10 min at room temperature using a Sorvall Model RC-5B Plus refrigerated centrifuge to remove undissolved debris. The supernatant was used for determination of antioxidative activity.