Available at www.sciencedirect.com

ScienceDirect

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

Antioxidative activities of hydrolysates from seabass skin prepared using protease from hepatopancreas of Pacific white shrimp

*Theeraphol Senphan, Soottawat Benjakul**

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

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form
2 October 2013
Accepted 3 October 2013
Available online 26 October 2013

Keywords:
Fish skins
Pacific white shrimp
Seabass
Alcalase
Antioxidant activity

ABSTRACT

Antioxidative activities of hydrolysates from skin of seabass (*Lates calcarifer*) with different degrees of hydrolysis (DH: 10–40%) prepared using an ammonium sulphate precipitated fraction (ASPF) from Pacific white shrimp hepatopancreas and commercial Alcalase were compared. The hydrolysate prepared using ASPF or Alcalase had increases in 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activities and ferric reducing antioxidative power (FRAP) as DH increased ($P < 0.05$). When the hydrolysate prepared using ASPF with 40% DH was subjected to a gastrointestinal model system (GIMs), ABTS radical scavenging activity and chelating activity increased, especially in the duodenal condition. The hydrolysate (500–2000 mg/L) could inhibit lipid oxidation in a lecithin liposome system in a dose dependent manner. Based on gel filtration using a Sephadex™ G-15 column, peptide with a molecular weight of 364 Da showed the strongest ABTS radical scavenging activity. Therefore, the extract from hepatopancreas could be used to increase DH of hydrolysate from seabass skin.

1. Introduction

Pacific white shrimp and its products have become economically important for Thailand. By the year 2010, the total amount of frozen Pacific white shrimp and products was 408,000 metric tonnes and the products were mainly exported to the USA and Japan (Senphan & Benjakul, 2012). During shrimp processing, a large amount of by-products including cephalothorax, shell, etc. are generated (Senphan & Benjakul, 2012). Amongst all shrimp products, whole shrimp without hepatopancreas are currently showing increased demand. The hepatopancreas is removed using a vacuum sucking machine (Senphan & Benjakul, 2012). Shrimp hepatopancreas can be a major source of proteases, especially trypsin and chymotrypsin (Sriket et al., 2012). Proteases in the hepatopancreas from freshwater prawn actively hydrolysed various proteinaceous substrates (Sriket et al., 2012).

Seabass (*Lates calcarifer*) are very popular in South-East Asia owing to their white flesh and delicacy (Sinthusamran, Benjakul, & Kishimura, 2013). During fillet production, skin is generated and can serve as a potential source for gelatin (Sinthusamran et al., 2013). Additionally, gelatin from fish skin has been used for production of gelatin hydrolysate with bioactivities (Ngo, Qian, Ryu, Park, & Kim, 2010). The beneficial effects of bioactive peptides are well known in scavenging free radicals and reactive oxygen species or in preventing the oxidative damage by interrupting the radical chain reaction of lipid peroxidation (Harnedy & FitzGerald, 2012; Kim & Wijesekara, 2010). Gelatin hydrolysate from bigeye snapper skin, possessing antioxidative activity including DPPH and ABTS radicals scavenging activity and ferric reducing antioxidant

* *Corresponding author.* Tel.: +66 7428 6334; fax: +66 7455 8866.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).
1756-4646/$ - see front matter
http://dx.doi.org/10.1016/j.jff.2013.10.001

power, could be prepared with the aid of protease from fish pyloric caeca (Khantaphant & Benjakul, 2008; Phanturat, Benjakul, Visessanguan, & Roytrakul, 2010). To reduce the cost of commercial protease used for gelatin hydrolysate production, the cheap proteases from shrimp processing byproducts, especially from hepatopancreas, could be an alternative. The direct hydrolysis of pretreated skin, a major source of collagen and gelatin, without prior gelatin extraction, can shorten the processing time and lower the operation cost. Furthermore, new peptides could be generated due to varying specificity towards substrates amongst different proteases. Those peptides can serve as the functional supplement in foods or drinks. Therefore, the present study aimed to investigate the impact of protease from hepatopancreas in comparison with commercial protease on hydrolysis of skin of seabass and to study the antioxidative activities and characteristics of the resulting hydrolysate.

2. Materials and methods

2.1. Chemicals

Alcalase was obtained from Novozyme (Bagsvaerd, Denmark). The compounds 2,4,6-trinitrobenzenesulphonic acid (TNBS), 2,2′-azinobis (3-thylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyltriazine (TPTZ), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulphonic acid sodium salt (ferrozine), ethylenediaminetetraacetic acid (EDTA), pepsin from porcine gastric mucosa (EC 3.4.23.1), pancreatin from porcine pancreas, trypsin from bovine pancreas (EC 3.4.21.4) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were procured from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Sephadex™ G-15 and gel filtration calibration kit (vitamin B12, flavin adenine dinucleotide and glycine-tryrosine) were obtained from GE Healthcare (Uppsala, Sweden).

2.2. Preparation of ammonium sulphate fraction from hepatopancreas

Hepatopancreas of Pacific white shrimp was collected from Sea wealth frozen food Co., Ltd., Songkhla province, Thailand. Hepatopancreas was packaged in polyethylene bag, stored in ice using a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 2 h. Upon arrival, hepatopancreas was powdered in liquid nitrogen and homogenised in three volumes of acetone at −20 °C for 30 min according to the method of Kishimura & Hayashi, (2002). The homogenate was filtered in vacuo on Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, UK). The residue obtained was then homogenised in two volumes of acetone at −20 °C for 30 min, and then the residue was left at room temperature until dried and free of acetone odour.

To prepare the crude extract, acetone powder was suspended in 10 mM Tris–HCl, pH 8.0 containing 1 mM $CaCl_2$ (an extraction buffer) at a ratio of 1:50 (w/v) and stirred continuously using a magnetic stirrer model BIG SQUID (IKA®-Werke GmbH & CO.KG, Staufen, Germany) at 4 °C for 3 h. The suspension was centrifuged for 10 min at 4 °C at 10,000*g* to remove the tissue debris using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). The solution was referred to as “crude extract”.

Crude extract was subjected to ammonium sulphate precipitation at 40–60% saturation as described by Khantaphant and Benjakul (2008). After the addition of ammonium sulphate, the mixture was stirred gradually at 4 °C for 30 min. Thereafter, the mixture was centrifuged at 8000*g* for 30 min at 4 °C and the pellet obtained was dissolved in the minimum volume of 50 mM Tris–HCl buffer, pH 8.0. The solution was dialysed against 20 volumes of the extraction buffer overnight at 4 °C with three changes. The dialysate was kept in ice and referred to as ‘ammonium sulphate precipitated fraction, ASPF’. The supernatant was lyophilised using a SCANVAC CoolSafe™ freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The lyophilised sample (10 g) was dissolved with 50 mL of cold distilled water (4 °C). The solution was determined for protease activity and further used.

2.3. Protease activity assay

Both ASPF and Alcalase were determined for protease activity using casein as substrate as per the method of An, Seymour, Wu, and Morrissey (1994). The assay was performed at pH 8 and 60 °C. One unit of activity was defined as that releasing 1 μmol of tyrosine per min (μmol Tyr/min).

2.4. Preparation of hydrolysate from skins of seabass

Firstly, skins ($1 \times 1\ cm^2$) were soaked in 0.1 M NaOH with a skin/solution ratio of 1:10 (w/v) at room temperature (25 °C) with a gentle stirring using an overhead stirrer equipped with a propeller (RW 20.n, IKA Labortechnik, Staufen, Germany) at a speed of 150 rpm. The solution was changed every 30 min for totally three times to remove non-collagenous proteins and pigments. Alkaline-treated skins were washed with tap water until the neutral or faintly basic pH wash water was obtained. The skins were then soaked in 0.05 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 2 h at room temperature with a gentle stirring to swell the collagenous material in fish skin. Acid-treated skins were washed as previously described. Swollen skins were added with distilled water at a ratio of 1:10 (w/v). The pH of the mixture was adjusted to pH 8.0 using 2 M NaOH. The mixture was incubated at 60 °C for 15 min.

To compare hydrolysis efficacy towards swollen skins, ASPF or Alcalase was added into pre-incubated mixture at levels of 5 and 10 units/g swollen skin dry matter. At hydrolysis time designated (5, 10, 15, 20, 30, 40, 60, 90, 120 and 180 min), 1 mL of sample was taken and mixed with 1 mL of 2% SDS solution (90 °C) before placing in a temperature controlled water bath (Memmert, Bavaria, Germany) at 90 °C for 10 min to inactivate the enzyme and solubilise proteins. The sample was used for DH determination. The hydrolysis time rendering the initial velocity was selected for further study.

To prepare hydrolysates with different DHs (10%, 20%, 30% and 40%), ASPF or Alcalase with different amounts were added as described by Benjakul and Morrissey (1997). The hydrolysis was carried out for 60 min at 60 °C, prior to termination as previously described. The obtained hydrolysates were centrifuged at 8000*g* for 10 min. The supernatants were lyophilised and further subjected to analyses.