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Antioxidant properties of peptide fractions from tuna dark muscle protein by-product hydrolysate produced by membrane fractionation process

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ABSTRACT

Tuna dark muscle by-product was hydrolyzed using Alcalase and fractionated by ultrafiltration (UF) and nanofiltration (NF) membrane processes into three fractions. The antioxidant activities of tuna dark muscle by-product hydrolysate (TPH) and its peptide fractions were evaluated. The TPH showed the highest iron chelating activity (75%) compared to other peptide fraction hydrolysates. The NF permeate showed the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activities (75% and 65% respectively). The NF retentate (1–4 kDa) that contained antioxidant amino acids such as Tyr, Phe, Pro, Ala, His, and Leu, which accounted 30.3% of the total amino acids showed the highest superoxide radical and reducing power activities. The effect of the membrane fractionation on the molecular weight distribution of the peptide fractions and their bioactivities was underlined. In conclusion, the TPH and their peptide fractions may serve as useful ingredients in food industry for various applications and for the formulation of nutritional products.

1. Introduction

The formation of free radicals such as superoxide anion and hydroxyl radical in foods leads to the oxidation of the qualitative decay of foods, which lead to rancidity, toxicity and destruction of significant biomolecules for physiologic metabolism (Qian, Jung, Byun, & Kim, 2008; Wang et al., 2013). Free radicals are believed to play a significant role in the occurrence of diseases, such as cardiovascular diseases, diabetes mellitus, neurological disorders, cancer and even the Alzheimer disease (Diaz, Frei, Vita, & Keaney, 1997; Peng, Xiong, & Kong, 2009). Therefore, the consumption of dietary antioxidant from natural sources is essential for the inhibition of the oxidation and the formation of free radicals occurring in food and preventing their deterioration. The natural antioxidant is potentially effective for promoting human health by increasing the body antioxidant content. Thus, antioxidants are useful in food systems for retarding lipid peroxidation which affects their nutritive value (Samaranayaka & Li-Chan, 2011).

Recent studies have reported that marine protein hydrolysates are abundant natural sources of antioxidants with good inhibition activities of free radicals and reactive oxygen species (Qian et al., 2008; Rajapakse, Mendis, Jung, Je, & Kim, 2005). Actually, large quantities of fish protein processing by-products are discarded without any attempt to recover and valorisation. Considering the limited biological resources, there is a great need to find a solution for better management and use of generated by-products (Guérard, Sumaya-Martinez, Laroque, Chabeaud, & Dufossé, 2007). Each year, the global tuna fishing is about 3 billion tons (FAO, 2008). However, the processing industry and canned tuna generate large amounts of solid waste that can reach 50 to 70% of the raw material. These fish protein by-products are generally inactive within the crude protein sequence. Thus, the bioactive peptides responsible of biological activity are released upon endogenous and/or exogenous enzyme hydrolysis of fish protein under controlled conditions (Hsu, 2010; Sarmadi & Ismail, 2010). Therefore, to recover the bioactive components from fish protein by-products for further utilization and valorisation, the enzymatic hydrolysis of fish by-products is the most efficient technology to recover added-value peptides from fish by-products with functional, biological and nutritional properties (Kristinsson & Rasco, 2000; Shahidi, Han, & Synowiecki, 1995). In recent years, many studies have isolated a great number of bioactive peptides with high antioxidant activity such as the ability to scavenge hydroxyl radicals, superoxide anion radicals, hydrogen peroxide, reducing power, metal ion chelating and high effects on prevention lipid oxidation. These peptides have been produced by enzymatic hydrolysis of different fish proteins such as protein of conger eel muscle (Ranathunga, Rajapakse, & Kim, 2006), tuna dark muscle by-product (Hsu, 2010; Je, Qian, Lee, Byun, & Kim, 2008), croaker (Da Rosa Zavareze et al., 2014; Nazeer, Sampath Kumar, & Jai Ganesh, 2012), *Sphyrna* muscle

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(Luo et al., 2013; Wang, Li, Chi, Zhang, & Luo, 2012), blue mussel muscle (Wang et al., 2013), lanternfish (Chai, Chan, Li, Shiau, & Wu, 2013), salmon by-product (Ahn, Je, & Cho, 2012; Ahn, Kim, & Je, 2014; Je et al., 2013), monkfish (Chi et al., 2014) and cod (Halldorsdottir, Sveinsdottir, Freysdottir, & Kristinsson, 2014; Sabeena Farvin et al., 2014).

The bioactivity of peptides with 2–20 amino acids could be affected by different parameters such as source of protein, degree of hydrolysis, peptide structure, amino acid composition, type of protease used and molecular weight (MW) (Li et al., 2013; Memarpoor-Yazdi, Mahaki, & Zare-Zardini, 2013; Sarmadi & Ismail, 2010). Therefore, several study reported that the biological activity of peptides was related to the MW (Jeon, Byun, & Kim, 1999). In particular, fractions with MW between 100–500 Da and 1000–3500 Da would be the most interesting bioactive peptides for nutritional and pharmaceutical uses (Vandanjon, Grignon, Courois, Bourseau, & Jaouen, 2009). The extraction and recovery of these fractions from the hydrolysate are the key issues. Furthermore, many studies used gel permeation chromatography for purification peptide fragments with the highest bioactivities. However, the membrane separation is a useful technology for the fractionation of molecules and offers a good alternative separation for achieving an effective process (Drioli, Stankiewicz, & Macedonio, 2011). Compared to other bioseparation methods such as gel chromatography, membrane technology offers several advantages such as higher productivity, lower capital investment, high throughput of products and while maintaining product purity under ambient conditions, ease of translation to large-scale commercial production and easy equipment cleaning.

Thus, the membrane separation processes are used for the fractionation of high value molecules from by-product protein hydrolysate with the objective of enhancing their functional properties. However, the influence of factors on membrane fractionation including the concentration factor, transmembrane pressure, pH of solution, final retention factors and mode of fractionation, on the outcome of this process has been studied (Bourseau et al., 2009; Saidi, Deratani, Belleville, & Amar, 2014; Saidi, Deratani, Ben Amar, & Belleville, 2013). Saidi et al. (2013) have reported that the optimized conditions for a two-step membrane fractionation process (for UF step: 25 °C, 2 bar, 60 g/L, 3 m/s, pH 8 and for NF step: 25 °C, 10 bar, 1.25 m/s, pH 8) lead to the recovery of the higher rate of interest peptide fractions with MW of 1–4 kDa.

Therefore, in this study, we utilized tuna dark muscle hydrolysate produced using Alcalase and fractionated by two membrane fractionation process (ultrafiltration (UF) step and nanofiltration (NF) step) (Saidi et al., 2014) to evaluate the antioxidant activities of different peptide fractions obtained using various assays and the inhibition of linoleic acid autoxidation. Thus, the aim was to indentify the most antioxidant peptide fraction hydrolysate for application as a functional ingredient in food product production and to investigate the influence of membrane fractionation process on the peptide MW, antioxidant properties and amino acid composition.

2. Materials and methods

2.1. Reagents

Chemicals required for the assays including 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), phenazine methosulfate (PMS), nitrotetrazolium blue chloride (NBT), butylated hydroxyanisole (BHA), α-tocopherol, linoleic acid, salicylic acid, ammonium thiocyanate, potassium ferricyanide and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfide acid sodium salt (ferrozine) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other employed chemicals and reagents were of analytical grade.

2.2. Substrate

The tuna dark muscle by-product was obtained from TUNA EL SULTON Foods Industry Ltd. (Sfax, Tunisia). During the processing of canned tuna, the dark muscle was recovered and it was placed in polyethylene bag (4000 mL) and then transported to the laboratory in iced condition where they were stored at −20 °C until being used. The protein, lipid, ash and moisture contents of the dark muscle by-product were 26, 2.4, 1.35 and 70.5%, respectively. The chemical composition was determined according to the AOAC methods (AOAC, 2005). Indeed, moisture content was determined by placing approximately 2 g of sample into a pre-weighted aluminum dish. Samples were then dried in an oven at 105 °C until a constant weight. The total crude protein (N*6.25) in raw materials was determined using the Kjeldahl method (NF-EN-25663). Total lipid was determined by Soxhlet extraction. Ash content was estimated by heating a pre-dried sample in a crucible at 600 °C for 2 h. The soluble protein content of the fish hydrolysate was measured after centrifugation by the Biuret method, using bovine serum albumin as a standard protein.

2.3. Enzyme

Alcalase PROLYVE 1000 is an alkaline serine endopeptidase produced by fermentation using a selected strain of *Bacillus licheniformis* (provided by Lyven France). The optimal conditions of use determined by the producer were: a temperature between 55 and 60 °C and a pH between 9.0 and 10.5. A declared minimal activity is 2.2 Anson (One Anson unit is defined as the amount of enzyme which, under specified conditions, digests denatured hemoglobin and liberates Folin-positive amino acids and peptides, corresponding to 1 μmol of tyrosine in 1 min). Alcalase can be inactivated by heating at 90 °C for 10 to 15 min.

2.4. Production of tuna dark muscle by-product hydrolysates

The hydrolysis of tuna dark muscle by-product was conducted under optimal conditions for production of interest peptide fractions with MW of 1–4 kDa (temperature 55 °C, pH 8.5, enzyme substrate ratio (E/S) 1% and duration of hydrolysis 60 min). These conditions were determined by Saidi et al. in previous study using Alcalase (Saidi et al., 2014). In the first step of hydrolysis, the tuna dark muscle was suspended in deionized water in a reaction vessel equipped with a stirrer (stirrer velocity: 300 rpm), heated to the appropriate temperature and adjusted to the appropriate pH. In the second step, the enzyme was added to the mixture at an E/S of 1%. The enzymatic hydrolysis reactions were performed for 60 min to achieve an optimum of production of interest peptide fractions (1–4 kDa) and 20% of degree of hydrolysis (DH). The DH was determined according to the procedure described by Saidi et al. (2014).

After the reaction, the mixture was placed in a boiling water bath for 20 min to inactivate the enzyme. Afterward, the tuna dark muscle by-product hydrolysate (TPH) was centrifuged (Centrifuge SIGMA) at 9500 rpm for 30 min to remove the unhydrolyzed residue (Fig. 1). A portion of the supernatant was taken to determine the protein content and the other part was freeze-dried and stored at −20 °C until use.

2.5. Preparation of tuna protein hydrolysate fractions by membrane process

The obtained TPH was subjected to fractionation by membrane fractionation process developed by Saidi et al. (2014) using UF and NF membranes. The UF experiments were carried out using three channel tubular ceramic membrane (hydraulic diameter: 3.5 mm, membrane length and surface area: 23 cm and 155 cm^2, Molecular Weight Cut-off (MWCO): 8 kDa) purchased from Tami Industry (Nyon, France). A flat membrane (polyethersulfone membrane NP010 with MWCO of 1 kDa, Micrody Nadir) was used in NF experiments. The filtration experiments were carried out in tangential filtration mode with a very versatile lab-scale pilot equipped with tubular or flat sheet membranes. The used membranes were characterized according to the procedure described by Saidi et al. (2013). Indeed, the membrane process includes three successive steps of membrane separation (UF-concentration, UF-diafiltration and NF concentration) carried out in batch mode. It can