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Antioxidant activities of enzymatic rapeseed protein hydrolysates and the membrane ultrafiltration fractions

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ABSTRACT

In this study, rapeseed protein isolate was hydrolyzed with various proteases to obtain hydrolysates that were separated by membrane ultrafiltration into four molecular size fractions (<1, 1–3, 3–5, and 5–10 kDa). Alcalase hydrolysis significantly ($p < 0.05$) produced the highest yield of protein hydrolysate while Flavourzyme produced the least. The <1 kDa fraction was the most abundant after the membrane ultrafiltration of the protein hydrolysates, which indicates that the proteases were efficient at reducing the native rapeseed proteins into low molecular weight peptides. Antioxidant properties of the resulting hydrolysates and membrane fractions were characterized and results showed the Pepsin + Pancreatin (P + P) protein hydrolysate had significantly highest ($p < 0.05$) scavenging activity against DPPH radical among the unfractionated enzymatic hydrolysates. But the P + P hydrolysate was not as effective as other hydrolysates during long-term inhibition of linoleic acid oxidation. For most of the samples, fractionation into the <1 kDa peptides significantly ($p < 0.05$) improved DPPH and superoxide scavenging properties when compared to the unfractionated protein hydrolysates. Only the <1 kDa fraction showed ferric reducing antioxidant power and the effect was dose-dependent. Overall, Alcalase and Proteinase K seem to be more efficient proteases to release antioxidant peptides from rapeseed proteins when compared to P + P, Flavourzyme and Thermolysin.

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1. Introduction

Oxidative stress occurs as a result of an imbalance between the productions of reactive oxygen species (ROS) and availability of antioxidant endogenous compounds. Depletion of endogenous antioxidant compounds and/or excessive production of ROS can damage membranes, proteins, enzymes, and DNA resulting in the development of chronic disease conditions (Ray, Huang, & Tsuji, 2012; Yongvanit, Pinlaor, & Bartsch, 2012). In an effort to design preventive and curative strategies, reduction in the degree of oxidative stress has been identified as a key factor in the therapeutic management of brain disorders, diabetes, cardiac hypertrophy and cardiovascular disease (Bains & Hall, 2012; Lepping et al., 2011; Maulik & Kumar, 2012). Hence there is the need for development of antioxidants from natural sources that can prevent the deleterious effects of ROS. Food-derived antioxidant peptides that commonly contain 2–20 amino acid residues are considered natural antioxidant resources in comparison to synthetic compounds such as butylated hydroxyanisole and butylated

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hydroxytoluene (Haenen et al., 1996; Sarmadi & Ismail, 2010). Peptides derived from natural sources have been the focus of growing interest because of their potential health benefits associated with low molecular weight, low cost, high activity, easy absorption, and little or no negative side effects (Sarmadi & Ismail, 2010). Enzymatic hydrolysis of proteins is one effective approach that can be used to release antioxidant peptides without affecting nutritive value. Various studies have been conducted to investigate antioxidant properties of food protein-derived peptides and hydrolysates, especially from animal protein sources like milk, egg, fish, and blood plasma (Lin, Guo, You, Yin, & Liu, 2012; Najafian & Babji, 2012; Qian et al., 2011; Sun, Luo, Shen, Li, & Yao, 2012), as well as some plant proteins like algae, soy, corn, and sunflower (Li et al., 2010; Park, Lee, Baek, & Lee, 2010; Ren, Zheng, Liu, & Liu, 2010; Sheih, Fang, Wu, & Lin, 2010). The antioxidant properties of these peptides largely depend on enzyme specificity, degree of hydrolysis, and the nature of the peptides released including molecular weight, amino acid composition, and hydrophobicity (Sarmadi & Ismail, 2010).

Rapeseed protein isolate (RPI), which is obtained from rapeseed meal, is considered a suitable source of dietary protein due to its excellent balance of essential amino acid composition and high bioavailability (Barbin, Natsch, & Muller, 2011; Dong et al., 2011; Yoshie-Stark, Wada, Schott, & Wasche, 2006). The inclusion of RPI as edible films to maintain quality of Seolhyang strawberries (Shin, Jang, Song, Song, & Bin Song, 2011), and as substitute for milk protein to reduce vascular and oxidative disturbances have been recently reported (Magne et al., 2009). Moreover, several studies have reported that enzymatic hydrolysis of RPI yielded peptides and hydrolysates that possess antioxidant (Makinen, Johannson, Gerd, Pihlava, & Pihlanto, 2012; Pan, Jiang, & Pan, 2011) and antitumor properties in Hela cells (Xue, Liu, Wu, Zhuang, & Yu, 2010) as well as *in vitro* inhibition of angiotensin converting enzyme, a causative agent of hypertension (Makinen et al., 2012; Yamada et al., 2010). In regards to antioxidant activity, previous works have limited information on relationships between antioxidant activities and molecular size of peptides obtained from different rapeseed protein hydrolysates.

The close interrelationships of protein hydrolysate antioxidant activities with amino acid composition and sequence as well as peptide molecular weight has generated increased interest in evaluating efficiency of proteases in releasing antioxidant peptides from RPI. Therefore, this study was aimed at determining the ability of several proteases to convert RPI into antioxidant peptides followed by evaluating the relationships of measured antioxidant activities with peptide size and amino acid composition of the protein hydrolysates.

2. Materials and methods

2.1. Materials

The defatted rapeseed meal (DRM) was supplied by COFCO Eastocean Oil & Grains Industries Co., Ltd., (Zhang Jiagang, China). The meal was grounded to pass through a 15 mm screen sieve. Alcalase, Proteinase K, Pepsin, Pancreatin, Thermolysin, Flavourzyme, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Triton X-100, pyrogallol, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide, 1,10-phenanthroline, ferrous sulfate, linoleic acid, ammonia thiocyanate, ferrous chloride, and reduced L-glutathione (GSH) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the other analytical grade reagents were obtained from Fisher Scientific (Oakville, ON, Canada).

2.2. Preparation of rapeseed protein isolates (RPI)

RPI was produced from DRM according to the method described by Yoshie-Stark, Wada, and Wasche (2008) with slight modifications. Briefly, DRM was dispersed in deionized water (1:15 w/v), adjusted to pH 10.0 with 1 M NaOH, and then mixed at 45 °C for 2 h. The slurry was centrifuged at 10,000*g* for 30 min, the supernatant recovered, adjusted to pH 4.5 with 1 M HCl and centrifuged again. The precipitated proteins were recovered and re-dispersed in deionized water, adjusted to pH 7.0 with 1 M NaOH and freeze–dried to produce RPI powder. Protein content of RPI was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.3. Preparation of rapeseed protein hydrolysates and membrane fractions

Hydrolysis of RPI was conducted with Alcalase, Proteinase K, Pepsin + Pancreatin (P + P), Thermolysin and Flavourzyme under different conditions using a pH-stat method (Chabanon, Chevalot, Framboisier, Chenu, & Marc, 2007). RPI (5% w/v, protein basis) was suspended in deionized water in a reaction vessel equipped with a stirrer, heated to the appropriate temperature and adjusted to the appropriate pH prior to the addition of the proteolytic enzyme; the reaction conditions are shown in Table 1. Each enzyme was added to the slurry at an enzyme/substrate ratio (E/S) of 1:25 (based on the protein content of the protein isolate). Digestion was performed at the above conditions for 4 h; pH of the reaction mixture was kept constant by the pH-stat with 2 M NaOH except for the Pepsin reaction. After digestion, the enzyme was inactivated by adjusting the reaction mixture to pH 4.0 with 2 M HCl followed by immersing the reaction vessel in boiling water bath for 10 min and undigested proteins were precipitated by centrifugation at 8000*g* for 60 min. A portion of the supernatant containing target peptides was freeze–dried as the rapeseed protein hydrolysate (RPH) while the remaining portion was passed through ultrafiltration membranes with molecular weight cut-off (MWCO) of 1, 3, 5, and 10 kDa using an Amicon stirred ultrafiltration cell. Ultrafiltration was performed sequentially: first through the 1 kDa and retentate passed through 3 kDa; retentate from 3 kDa was passed through the 5 kDa whose retentate was passed through the 10 kDa membrane. The permeate from each MWCO membrane was collected as <1, 1–3, 3–5, and 5–10 kDa peptide fractions, respectively. All the permeates were freeze–dried and stored at −20 °C until needed for further analysis. The protein contents of the freeze–dried RPH and peptide fractions were determined using the modified Lowry method (Markwell et al., 1978). The above digestion and fractionation protocols were performed in triplicate. The percent yield of RPH was

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