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Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates

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ABSTRACT

ACE inhibitory activity was studied for different hydrolysates obtained from protein concentrates of chickpea (*kabuli* and *desi*) and yellow pea (*Golden*) using *in vitro* gastrointestinal simulation, alcalase/flavourzyme, and papain. Protein/peptide profiles studied by SDS–PAGE and SE-HPLC, showed a rich composition of the hydrolysates in small peptides having MWs under 4 kDa. Papain hydrolysed yellow pea proteins showed the highest ACE inhibitory activity. In addition, chickpea desi proteins hydrolysed by *in vitro* gastrointestinal simulation showed higher ACE inhibition (IC_{50} of 140 ± 1 μg/ml) compared to its digests obtained by alcalase/flavourzyme (IC_{50} of 228 ± 3 μg/ml) or papain (IC_{50} of 180 ± 1 μg/ml) and to chickpea kabuli hydrolysed by gastrointestinal simulation (IC_{50} of 229 ± 1 μg/ml). The results demonstrate that enzymatic hydrolysates of chickpea and pea proteins contain bioactive ACE inhibitory peptides; furthermore, the type of enzyme used for hydrolysis affects the ACE inhibitory activity.

1. Introduction

Hypertension (defined as high systolic and diastolic blood pressures) is one of the major independent risk factors for cardiovascular disease (CVD) (FitzGerald & Murray, 2006) and is controlled by the renin–angiotensin system (Chen et al., 2009; Erdmann, Cheung, & Schröder, 2008). Angiotensin I-converting enzyme (ACE; peptidyldipeptide hydrolase, EC 3.4.15.1) is an enzyme associated with the renin–angiotensin system which hydrolyses angiotensin I (a decapeptide) to the octapeptide angiotensin II, a potent vasoconstrictor, resulting in arterial constriction and blood pressure elevation. In addition, ACE also breaks down bradykinin, a vasodilator, further contributing to blood pressure elevation. Inhibition of ACE is therefore important for lowering blood pressure (Chen et al., 2009; Erdmann et al., 2008; FitzGerald & Murray, 2006).

Hypertension may be controlled by dietary modifications, exercise, calcium channel agonists, angiotensin II receptor blockers, diuretics and ACE inhibitors (FitzGerald & Murray, 2006). Owing to the potential side effects of pharmaceutical drugs, such as cough, skin rashes and angioedema, there is increased interest in identifying foods that naturally contain peptides with hypotensive properties (Roy, Boye, & Simpson, 2010). Several research studies have showed that enzymatic hydrolysates of food proteins contain peptides with ACE inhibitory properties. Some of these peptides are present within the parent protein structure and could be released through proteolysis (Abubakar, Saito, Kitazawa, Kawai, & Itoch, 1998; Aluko, 2008; FitzGerald & Murray, 2006; Kawamura, 1997; Maeno, Yamamoto, & Takano, 1996; Okamoto, Hanagata, Kawamura, & Yanagida, 1995; Qian, Je, & Kim, 2007; Suh & Whang, 1999; Ukeda et al., 1992; Wu & Muir, 2008).

Recently, we have shown that the tryptic digest of red lentil proteins posses ACE inhibitory properties. Furthermore, we demonstrated that the IC_{50} values of the hydrolysates varied significantly as a function of the protein fraction with the total lentil protein isolate having the lowest IC_{50} (440 ± 4 μg/ml), followed by the enriched legumin (476 ± 2 μg/ml), albumin (509 ± 8 μg/

Abbreviations: 2-ME, β-mercaptoethanol; ACE, angiotensin I-converting enzyme; AF, alcalase/flavourzyme; ANOVA, analysis of variance; AU, Anson units; CVD, cardiovascular disease; DCPC, desi chickpea protein concentrate; DCPH, desi chickpea protein hydrolysate; $DCPH_{AF}$, desi chickpea protein hydrolysate obtained by alcalase/flavourzyme hydrolysis; $DCPH_{GIS}$, desi chickpea protein hydrolysate obtained by gastrointestinal simulation hydrolysis; $DCPH_{P}$, desi chickpea protein hydrolysate obtained by papain hydrolysis; DH, degree of hydrolysis; GIS, gastrointestinal simulation; HHL, Hippuryl-His-Leu; HPLC, high-performance liquid chromatography; IC_{50}, half maximal inhibitory concentration; KCPC, kabuli chickpea protein concentrate; KCPH, kabuli chickpea protein hydrolysate; $KCPH_{AF}$, kabuli chickpea protein hydrolysate obtained by alcalase/flavourzyme hydrolysis; $KCPH_{GIS}$, kabuli chickpea protein hydrolysate obtained by gastrointestinal simulation hydrolysis; $KCPH_{P}$, kabuli chickpea protein hydrolysate obtained by papain hydrolysis; LAPU, leucine aminopeptidase unit; MW, molecular weight; P, papain; SDS, sodium dodecyl sulphate; SDS–PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; SE-HPLC, size-exclusion high-performance liquid chromatography; TFA, trifluoroacetic acid; TNBS, 2,4,6-trinitrobenzenesulphonic acid; YPPC, yellow pea protein concentrate; YPPH, yellow pea protein hydrolysate; $YPPH_{AF}$, yellow pea protein hydrolysate obtained by alcalase/flavourzyme hydrolysis; $YPPH_{GIS}$, yellow pea protein hydrolysate obtained by gastrointestinal simulation hydrolysis; $YPPH_{P}$, yellow pea protein hydrolysate obtained by papain hydrolysis.

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ml) and vicilin (539 ± 9 μg/ml) fractions, respectively (Boye, Roufik, Pesta, & Barbana, in press).

Pulses (pea, lentil, chickpea, lupin, beans) are considered as good foods for health and they are nutritionally good sources of carbohydrates (e.g. fibre, resistant starch and oligosaccharides), protein, vitamins and minerals (Boye et al., 2010). Studies have suggested that regular dietary intake of pulses may be associated with reduced risk of some diseases, particularly diabetes, cancer and CVD (Roy et al., 2010).

Canada produces approximately 5 million tonnes of pulses annually (Agriculture and Agri-Food Canada, 2008). With growing consumer awareness of the health benefits of pulses, there is need for scientific research to identify the specific components in pulses that may have health benefits. More specifically, there is increasing interest by the pulse industry to understand the properties of pulse foods and their potential use as functional foods without the need for extensive processing.

The specific objective of the present work, therefore, was to determine if protein hydrolysates obtained using gastrointestinal enzymes, alcalase/flavourzyme (AF) and papain (P) from two varieties of chickpea (CDC Xena kabuli and Myles desi) and one variety of yellow pea (CDC Golden) possessed ACE inhibitory properties *in vitro*.

2. Materials and methods

2.1. Materials

The two varieties of chickpea used for this study were CDC Xena kabuli and Myles desi, and the yellow pea variety was CDC Golden. All the pulses were provided by Simpson Seeds Inc. (Moose Jaw, SK, Canada). Alcalase 2.4 Anson units (AU) g^{-1} and flavourzyme 1000 MG were obtained from Novo Nordisk A/S (Bagsværd, Denmark). Alcalase is an endopeptidase from *Bacillus licheniformis*, whereas flavourzyme, with activity of 1.0 leucine aminopeptidase units (LAPU) g^{-1}, is a protease complex from *Aspergillus oryzae* with endo- and exoprotease activities. Pepsin (1310 U), trypsin (11600 U), α-chymotrypsin (55 U), Papain (10 U), ACE (5 U), Hippuryl-His-Leu (HHL) and 2,4,6-trinitrobenzenesulphonic acid (TNBS) were obtained from Sigma–Aldrich Co. (Oakville, ON, Canada). All other chemicals used were of analytical grade.

2.2. Protein extraction

Protein concentrates were prepared at a pilot-plant scale from chickpea and pea using alkaline extraction followed by isoprecipitation at pH 4.5 as described by Boye et al. (2010). Briefly, ground seeds were suspended in water and the pH was adjusted to 9. The dispersions were stirred for 60 min at approximately 25 °C to facilitate protein solubilization, while maintaining the pH at 9. The solution was filtered using a Kason vibrating sieve (Kason Corporation, Millburn, New Jersey, USA) with apertures of 195, 160 and 65 μm. The cake recovered from the tops of the different apertures was pressed using a Prefora-6510 vertical cheese press (Gram Equipment, Denmark) with a pressure of 100 psi, to extract the liquid. The liquid phase, recovered from the outlet of the apertures and the press, was combined and filtered twice using a filter press (Tiho 6SS4-812 Filter Press, Star Systems, Timmonsville, South Carolina, USA), with 2 μm and subsequently 0.8 μm filters. The pH of the supernatant was adjusted to 4.5 using HCl 2 M to precipitate the proteins and the precipitate formed was recovered by centrifugation as described above. Finally, the precipitate was washed with distilled water and the pH was neutralised to 7 using NaOH 2 M followed by spray-drying (Boye et al., 2010). Due to the high fat content of the chickpea protein concentrates (*kabuli* [KCPC] and *desi* [DCPC]) they were defatted with hexane (1:3 (w/v) powder:solvent ratio) then rehydrated with distilled water and lyophilized to obtain a final fat content in both concentrates of <1%. The yellow pea protein concentrate (YPPC) was not defatted, because the total fat content expressed on a dry basis was less than 1%.

2.3. Protein analysis

Protein contents in the different pulse protein concentrates were analyzed with a LECO FP-428 apparatus (LECO Corp., St. Joseph, MI, USA) using the combustion method and a nitrogen conversion factor of 6.25 (AOAC, 1995).

2.4. Protein hydrolysis

Suspensions of the chickpea and pea protein concentrates (5% w/w) were hydrolyzed in a pH-stat apparatus (TIM 865, Radiometer Analytical SAS, Villeurbanne, France) under controlled hydrolysis conditions for pH, temperature, enzyme concentration and stirring speed using alcalase, flavourzyme, pepsin, trypsin, α-chymotrypsin and papain. The conditions used for the hydrolysis were tailored for each of the enzymes in order to optimize their activity. The hydrolysis parameters used are shown in Table 1. Additionally, sequential digestion with the microbial enzymes alcalase and flavourzyme was performed. To simulate human gastrointestinal digestion (gastrointestinal simulation, GIS), sequential hydrolysis with pepsin (gastric digestion) followed by trypsin and α-chymotrypsin (duodenal digestion) was conducted as described previously by Vermeirssen, Augustijns, et al. (2005) and Vermeirssen, Van Camp, and Verstraete (2005). The enzymatic reactions were stopped either by heating at 90 °C for 15 min (papain hydrolysis) or by acidification to pH 5 (GIS hydrolysis) and to pH 4 (AF hydrolysis) with HCl 1 M. At the end of the hydrolysis, all the samples were centrifuged at 12,000*g* at 4 °C for 20 min, and the supernatant obtained was lyophilized and used for subsequent studies.

2.5. Degree of hydrolysis

The degree of hydrolysis (DH, %) was determined for each digestion by assaying the free amino groups with TNBS using the following equation (Adler-Nissen, 1979):

$$\mathrm{DH}\ (\%) = (h/h_{\mathrm{tot}}) \times 100 = 100 \times [(\mathrm{AN}_2 - \mathrm{AN}_1)/\mathrm{N}_{\mathrm{pb}}]$$

where h is the number of peptide bonds broken, h_{tot} is the total number of bonds per unit weight, AN_1 is the amino nitrogen content in the protein substrate before hydrolysis (mg g^{-1} protein), AN_2 is the amino nitrogen content in the protein substrate after hydrolysis (mg g^{-1} protein), and N_{pb} is the amino nitrogen content in the peptide bonds in the protein substrate (mg g^{-1} protein) as determined after total hydrolysis with HCl 6 M at 110 °C for 24 h. The AN_1 and AN_2 values were obtained from the standard curve of absorbance at 340 nm versus milligrams of amino nitrogen per litre generated with L-leucine (Spellman, McEvoy, O'Cuinn, & FitzGerald, 2003).

2.6. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) analysis of the chickpea and pea protein concentrates and their different enzymatic hydrolysates was performed on precast 10–20% gradient polyacrylamide Tris–HCl and 16.5% Tris–tricine gels, respectively, using the Bio-Rad Criterion Cell (Bio-Rad Laboratories, Inc., Mississauga, ON, Canada). For studies under reducing conditions, 5% (v/v) β-mercaptoethanol (2-ME) was