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Effect of pressure or temperature pretreatment of isolated pea protein on properties of the enzymatic hydrolysates

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ABSTRACT

Commercial isolated pea protein dispersion (IPP, 1%, w/v) was pretreated with high pressure (200–600 MPa, 5 min at 24 °C) or heat (100 °C, 30 min) prior to hydrolysis using 1–4% (w/w) alcalase concentrations. Fluorescence spectroscopy showed that heat pretreated IPP had a 35% higher level of exposed hydrophobic groups (measured as fluorescence intensity, FI) than the untreated protein. In contrast, the 200 MPa pressure pretreatment produced a 15% increase in FI while 400 and 600 MPa pretreatments, respectively, caused 5 and 60% decreases in FI. Heat pretreatment of IPP enhanced hydrolysis into smaller peptide sizes when compared to peptides from the 24 °C pretreated protein. The 200 MPa pretreatment enhanced IPP hydrolysis into smaller peptides, especially at lower (1–2%) alcalase concentrations. Protein hydrolysates produced from heat-pretreated IPP were less active against angiotensin converting enzyme (ACE) when compared to those from the 24 °C pretreated protein. In general, heat or high pressure pretreatment of IPP favored production of ACE- and renin-inhibitory enhanced protein hydrolysates at a lower (1%) alcalase concentration.

1. Introduction

The food industry is using high-pressure technology in the ranges of 100 to 600 MPa for many applications including inactivation of microorganisms and texture changes to produce food with high quality attributes and enhanced food safety (Gould, 1995; Jung, Tonello-Samson, & de Lamballerie-Anton, 2011; Knorr, 1999). During pressurization, the protein rearrangement will occur at an extent related to process conditions including pressure level, but covalent bonds are almost unaffected. However, the changes in protein structure during high pressure treatment can be used to enhance efficiency of protein hydrolysis. For example, several reports have indicated increased susceptibility to proteolysis following high pressure pre-treatments of soybean whey proteins (Penas, Prestamo, & Gomez, 2004), chickpea (Zhang, Jiang, Miao, Mu, & Li, 2012), red kidney bean (Yin, Tang, Wen, Yang, & Li, 2008), egg white (van der Plancken, Delattre, Indrawati, van Loey, & Hendrickx, 2004) and dairy whey (Chicon, Belloque, Alonso, & Lopez-Fandino, 2008) proteins.

In the food industry, heat processing is usually carried out to build or enhance texture, flavor, digestibility, microbiological safety, and inactivate toxins (Boye, Ma, Ismail, Harwalkar, & Kalab, 1997). But heat pretreatment of proteins before enzymatic hydrolysis can also lead to rearrangements of inter- and intra-molecular linkages, especially disulfide and hydrophobic bonds with concomitant changes in protein conformation and digestibility (Davis & Williams, 1998; Lee, Morr, & Ha, 1992; Tang, Chen, & Ma, 2009). Hydrophobic groups that had been inwardly-oriented are turned outwards, exposing them to unfavorable interaction with water and encourage increased protein–protein interactions. The extent of protein unfolding and exposure of hydrophobic patches would increase with increase in extent of heating (Boye et al., 1997; Tang et al., 2009). Privalov et al. (1989) estimated that proteins lose almost all secondary and tertiary structures when the temperature exceeds 80 °C, and would adopt a configuration that approaches a fully unfolded, random coil conformation. Thus, these heat-induced changes in protein conformation and polypeptide interactions could dictate degree of exposure of susceptible peptide bonds and influence the type of peptides generated during enzymatic hydrolysis.

The application of enzymatic hydrolysis to modify proteins can produce a wide range of peptides for food and nutrition applications. According to the report of Balti, Bougatef, et al. (2010), enzyme hydrolysis can be used to improve the functional properties of proteins. Moreover, enzyme hydrolysis could generate bioactive peptides with various activities, such as angiotensin converting enzyme (ACE) inhibition (Balti, Nedjar-Arroume, Adje, Guillochon, & Nasri, 2010; Kim et al., 2001), free radical scavenging or renin inhibition (Girgih, Udenigwe, Li, Adebiyi, & Aluko, 2011; Udenigwe, Lin, Hou, & Aluko, 2009). Bioactive peptides have been shown to impart several health benefits, including improving cardiovascular system, immune system, gastrointestinal system and nervous system. Among the most common chronic diseases, hypertension is the number one killer in North America. Within the

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renin–angiotensin system that regulates human blood pressure, angiotensin-converting enzyme (ACE) and renin play important roles. Renin is an enzyme responsible for converting angiotensinogen to angiotensin I by cleaving angiotensinogen between its 10th and 11th amino acids (Udenigwe, Li, & Aluko, 2012). ACE is a membrane-bound dipeptidyl carboxypeptidase that catalyzes conversion of inactive angiotensin I to the active form, angiotensin II by removing a dipeptide from the carboxyl-terminal (Peach, 1997). Excessive plasma level of angiotensin II can lead to severe blood vessel contractions and cause development of hypertension. Thus, inhibitions of ACE and renin activities have been used for blood pressure control in hypertensive people.

A large number of bioactive peptides from natural food sources have been reported to have ACE-inhibitory activities. Mung bean protein subjected to 2 h alcalase hydrolysis showed good ACE-inhibitory activity (Li, Wan, Le, & Shi, 2006). Peanut flour subjected to 6 h alcalase hydrolysis also has been reported to have ACE-inhibitory activity of up to 45% (Guang & Phillips, 2009a). Corn gluten meal has shown ACE inhibition bioactivity after 5 h of alcalase hydrolysis (Yang, Tao, Liu, & Liu, 2007). Moreover, the ACE-inhibitory activity of enzymatic hydrolysates from soybean, wheat and rice has also been reported (Guang & Phillips, 2009b). Li and Aluko (2010) reported the ACE- and renin-inhibitory activities of pea protein hydrolysates and purified peptides. Impact of pressure has been investigated only in few studies but there is scanty information on the use of high pressure and temperature pretreatments of pea proteins to influence the release of bioactive peptides. Therefore, this work was carried out to determine the effect of high pressure or temperature pretreatment of isolated pea protein on peptide size distribution as well as ACE- and renin-inhibitory properties of the enzymatic protein hydrolysates.

2. Materials and methods

2.1. Materials

Commercial isolated pea protein (IPP) also called Propulse™ was a gift from Nutri-Pea Limited (Portage la Prairie, Manitoba, Canada) and was produced using alkaline extraction followed by isoelectric pH precipitation (Nickel, 1981). The IPP was spray-dried to give a protein concentrate that contains 82% protein content (as is basis). Alcalase (protease from *Bacillus licheniformis*), ACE from rabbit lung (E.C.3. 14.15.1) and N-(3-[2-furyl]acryloyl)-phenylalanylglycylglycine (FAP GG) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Human recombinant renin inhibitor screening assay kit was purchased from Cayman Chemical Company, Inc. (Ann Arbor, MI, USA).

2.2. Pressure and heat pretreatments

The IPP was solubilized (1%, w/v) in 0.05 M phosphate buffer pH 7.0, with stirring at 4 °C overnight before being subjected to 200, 400, and 600 MPa pressure pretreatments, for a dwell time of 5 min and an initial temperature of 23 °C using a Stansted ISO-Lab high pressure food processor (Stansted Fluid Power Ltd., Stansted, UK). The pressure transmitting medium consisted of a 50/50 propylene glycol/water mixture. A control maintained at atmospheric pressure (0.1 MPa) was also prepared. For thermal pretreatment, a 5% (w/v) aqueous slurry of IPP was heated to and held at 100 °C for 30 min in a water bath and then cooled to room temperature; a sample that was prepared at 24 °C was used as the control. The pressure- and heat-treated samples were then freeze-dried and stored at −20 °C until required for analysis.

2.3. Intrinsic fluorescence spectroscopy

The fluorescence measurements were determined at 25 °C using an FP-6300 spectrofluorimeter (JASCO, Tokyo, Japan). Tryptophan was used to determine structural changes of IPP because the emission signals for tyrosine and phenylalanine were weak. Stock protein solutions were prepared by mixing the IPP in sodium phosphate buffer (0.01 M, pH 7.0) for 30 min followed by centrifugation (10000 ×*g* for 30 min); protein content of the supernatant was determined using the modified Lowry method (Markwell, Hass, Bieber, & Tolbert, 1978). Each stock sample supernatant solution was then diluted to obtain 20 μg/ml protein concentration. The intrinsic fluorescence of each protein solution was measured using excitation wavelength of 295 nm and fluorescence emission spectra were recorded between 300 and 500 nm (Schmid, 1989).

2.4. Enzymatic hydrolysis

Slurries of pressure or heat pretreated IPP (or non-pretreated controls) containing 5% (w/v) protein content were prepared with distilled water and adjusted to pH 9.0 using 2 M NaOH. Each slurry was heated to 50 °C followed by addition of alcalase (1%, 2%, 3% and 4% by weight of protein in the IPP). After 4 h of hydrolysis, the reaction was terminated because there was no more evidence of proteolysis (pH remained constant). The reaction beaker was immersed in boiling water for 15 min to inactivate the enzyme, cooled to room temperature and adjusted to pH 4.0 with 1 M HCl solution. The cooled slurries were centrifuged at 10000 ×*g* and 20 °C for 30 min and the supernatant collected and freeze-dried. The protein concentration of the hydrolysates was determined by modified Lowry method (Markwell et al., 1978).

2.5. Peptide size distribution

The peptide size distribution of protein hydrolysates was determined using the AKTA system, with Superdex Peptide12 10/300 GL 10 × 300 mm column (GE Healthcare, Montreal, Canada). Peptide elution was carried out at 25 °C using 50 mM phosphate buffer containing 0.15 M NaCl, pH 7.0, at a flow rate of 0.5 ml/min; eluted peptides were detected at 215 nm.

2.6. ACE inhibition assay

The ACE-inhibitory activity was determined by the method of Holmquist, Bunning, and Riordan (1979). FAPGG was used as the substrate at a concentration of 0.5 mM dissolved in 50 mM pH 7.5 Tris–HCl buffer that contained 0.3 mM NaCl. A 1 ml aliquot of this solution was mixed with 20 μl of ACE (1 U/ml; final activity of 20 mU) and 200 μl of peptide solutions (final concentration of 1 mg protein/ml) in 50 mM Tris–HCl buffer. Absorbance readings were taken at 345 nm for 2 min at room temperature. For the blank (uninhibited reaction), Tris–HCl was used instead of peptide solution. ACE activity was expressed as rate of reaction (ΔA/min) and inhibitory activity was calculated as:

$$\text{ACE inhibition } (\%) = 1 - \left[\Delta A \cdot \min^{-1}{}_{(\text{sample})} / \Delta A \cdot \min^{-1}{}_{(\text{blank})}\right] \times 100.$$

2.7. Renin inhibition assay

Renin inhibition assay was performed according to the instructions provided with the renin inhibitor screening assay kit (Cayman Chemical Company, Inc., Ann Arbor, MI, USA). The renin assay buffer was diluted 10 times with MilliQ water to give a final assay buffer of 50 mM Tris–HCl, pH 8.0 containing 100 mM NaCl. Renin solution was diluted 20 times before use. First, 20 μl of renin substrate, 160 μl of assay buffer and 20 μl of MilliQ water were added to the background well, while 20 μl of substrate, 160 μl of assay buffer and 10 μl of water were added to the blank (uninhibited) well. Lastly, 20 μl of substrate, 160 μl of assay buffer and 10 μl of peptide fractions were added to the corresponding sample (inhibitor) wells. Final sample concentration was 1 mg protein/ml. To initiate the reaction, 10 μl of renin was added to