Food Research International 55 (2014) 281–287

Contents lists available at ScienceDirect

Food Research International

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

Blood pressure lowering effects of Australian canola protein hydrolysates in spontaneously hypertensive rats

Adeola M. Alashi [a,b,d,*], Christopher L. Blanchard [a,c], Rodney J. Mailer [a], Samson O. Agboola [a,b], Andrew J. Mawson [a,b], Rong He [d,e], Sunday A. Malomo [d], Abraham T. Girgih [d], Rotimi E. Aluko [d]

[a] E.H. Graham Centre for Agricultural Innovation, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[b] School of Agricultural and Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[c] School of Biomedical Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
[d] Department of Human Nutritional Sciences and The Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2
[e] College of Food Science and Engineering, Nanjing University of Finance and Economics, Nanjing 210046, Peoples Republic of China

ARTICLE INFO

Article history:
Received 8 July 2013
Accepted 10 November 2013

Keywords:
Canola protein isolate
Protein hydrolysates
Hydrophobicity
Antihypertensive properties
Angiotensin converting enzyme
Renin
Spontaneously hypertensive rats

ABSTRACT

The *in vitro* and *in vivo* antihypertensive activities of canola protein hydrolysates and ultrafiltration membrane fractions (<1, 1–3, 3–5, & 5–10 kDa) were examined in this study. The hydrolysates were obtained after 4 h enzyme hydrolysis of canola protein isolate (CPI) using each of Alcalase, chymotrypsin, pepsin, trypsin and pancreatin. The hydrolysates had significantly ($p < 0.05$) reduced (35–70%) surface hydrophobicity when compared to the CPI. Alcalase hydrolysate contained the highest level of low molecular weight peptides and produced highest ($p < 0.05$) *in vitro* inhibition of angiotensin converting enzyme (ACE) activity. However, pancreatin hydrolysate was the most effective (63.2%) *in vitro* renin inhibitor. Membrane fractionation of pancreatin hydrolysate led to a 15% reduction in renin inhibition by the 1–3 kDa peptide fraction. In contrast, ACE and renin inhibitions were significantly ($p < 0.05$) increased by 10–20% after membrane ultrafiltration fractionation of the trypsin hydrolysate. Trypsin hydrolysate was ineffective at reducing hypertension in spontaneously hypertensive rats after oral administration (200 mg/kg body weight). However, Alcalase and pepsin hydrolysates showed appreciable antihypertensive effects, with Alcalase hydrolysate producing the greatest (−34 mm Hg) and fastest (4 h) decrease in systolic blood pressure (SBP). CPI had the most prolonged (24 h) SBP-reducing effect, which is attributable to the extensive protein hydrolysis in the GIT. We conclude that the Alcalase and pepsin hydrolysates may serve as useful ingredients to formulate antihypertensive functional foods and nutraceuticals.

1. Introduction

Plant peptides with bioactive properties obtained *via* enzymatic hydrolysis have continued to gain attention in food and nutraceutical research. Studies on plant protein hydrolysates obtained from canola, soybean, sunflower and peas have led to various findings involving the use of their bioactive peptides for short or long term treatment of hypertension (Barbana & Boye, 2011; Chen, Yang, Suetsuna, & Chao, 2004; Li et al., 2011; Megías et al., 2009; Wu, Aluko, & Muir, 2008). Dual health benefits of bioactive peptides have also been proposed because peptides may have the ability to modulate more than one physiological pathway. A lot of attention has also been recently placed on the potential of alternative or complementary approaches to regulating the renin–angiotensin-system (RAS) through renin inhibition, in addition to angiotensin converting enzyme (ACE) inhibition, as a means of regulating blood pressure (Segall, Covic, & Goldsmith, 2007).

Blood pressure is physiologically controlled by two major pathways, the RAS and the kinin–nitric oxide system (KNOS) (Martínez-Maqueda, Miralles, Recio, & Hernández-Ledesma, 2012). The RAS involves hydrolysis of angiotensinogen by the proteolytic action of renin (an aspartyl protease), which converts it to angiotensin I (AT I). AT I is then cleaved at the histidyl residue from the C-terminus by the activity of ACE (a glycoprotein peptidyl dipeptide hydrolase) to produce angiotensin II (AT II), which is the major end product of the RAS (Segall et al., 2007). AT II is a powerful vasoconstrictor that functions by binding to receptors located in tissues all over the body, eliciting reaction flows that initiate blood vessel contractions to maintain blood pressure (Montani & Vliet, 2004). However, excessive production of AT II may result in severe blood vessel contractions and limited relaxation, which can lead to high blood pressure (hypertension) development (Udenigwe & Aluko, 2012). The KNOS is involved in the production of bradykinin which exerts its antihypertensive effects by eliciting reactions that increase intracellular Ca^{2+} concentration, leading to the activation of nitric oxide synthase (NOS)-dependent production of nitric oxide, a powerful vasodilator (Griendling, Garret, & FitzGerald, 2003). However, ACE is also able to degrade bradykinin to inactive peptide fragments and thus increased levels of ACE lead to the prevention of vasodilation and the

* Corresponding author at: School of Agricultural and Wine Sciences, Charles Sturt University Locked Bag 588, Wagga Wagga, NSW 2678, Australia. Tel./Fax: +61 269332539.
E-mail address: aalashi@csu.edu.au (A.M. Alashi).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.11.015

promotion of vasoconstriction. Hence the inhibition of ACE and more importantly, renin could have a profound effect on blood pressure and provide for better management of hypertension (Segall et al., 2007).

Canola meal is a by-product of canola oil production and is generally used as an animal feed because it contains up to 38% protein and a well balanced amino acid profile, which is better than the FAO/WHO reference values (Dong et al., 2011; Tan, Mailer, Blanchard, & Agboola, 2011). Utilizing canola meal protein in human health applications could increase meal value and improve profitability of the canola processing industry. Antihypertensive properties of canola meal and rapeseed protein hydrolysates have been studied, with remarkable outcomes, based on the inhibition of ACE activity (Marczak et al., 2003; Wu et al., 2008; Yoshie-Stark, Wada, & Wäsche, 2008). However, studies on canola hydrolysates which focus on both the inhibition of ACE and renin and *in vivo* antihypertensive activities are limited. Recent work on rapeseed protein hydrolysates showed the effect of both the inhibition of renin and ACE in reducing blood pressure in hypertensive rats (He, Alashi, et al., 2013). Therefore, the objective of this study was to determine the ACE- and renin-inhibitory activities of protein hydrolysates prepared through enzymatic hydrolysis of Australian canola protein isolate using five proteases (Alcalase, chymotrypsin, trypsin, pepsin and pancreatin). The blood pressure-lowering ability of the protein hydrolysates was evaluated using spontaneously hypertensive rats (SHRs).

2. Materials and methods

2.1. Materials

Canola meal was provided by Cootamundra Oilseeds Pty (Cootamundra, NSW, Australia), pepsin was purchased from Worthington Biochemical Corp. (Lakewood, NJ), while human recombinant renin inhibitor screening assay kit was purchased from Cayman (Cayman Chemical, Ann Arbor, MI, USA). Rabbit lung ACE, Alcalase 2.4 L (protease from *Bacillus licheniformis*), Pancreatin, Trypsin S II, Chymotrypsin, and N-[3-(2-furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) were purchased from Sigma Chemicals (St. Louis, MO) Ultrafiltration membranes with 1, 3, 5 and 10 kDa molecular weight cut-off (MWCO) sizes were purchased from Fisher Scientific (Oakville, ON, Canada).

2.2. Preparation of canola proteins, hydrolysates, and fractions

2.2.1. Extraction of canola protein isolate (CPI)

Defatted canola meal (20 g) was suspended in 200 ml of 0.1 M NaOH pH 12.0, stirred for 1 h and then centrifuged at 18 °C and 3000 *g* for 10 min. Two additional extractions of the residue from the centrifugation process were carried out with the same volume of 0.1 M NaOH and the supernatants pooled. The pH of the supernatant was adjusted to the isoelectric point (pH 4.0) using 0.1 M HCl solution; the precipitate formed was recovered by centrifugation as described above. The precipitate was washed with distilled water, adjusted to pH 7.0 using 0.1 M NaOH, freeze-dried and the protein isolate was stored at −20 °C until required for further analysis.

2.2.2. Enzymatic hydrolysis of CPI

CPI was hydrolyzed for 4 h using each of five food grade enzymes (Alcalase, chymotrypsin, pepsin trypsin and pancreatin) at an enzyme: substrate ratio of 1:20 to obtain canola protein hydrolysates (CPHs). The optimum conditions used for each enzyme are outlined in Table 1. The pH was maintained for each hydrolysis process accordingly using either 1 M NaOH or 1 M HCl. The enzymes were inactivated by heating at 85 °C for 10 min. The resulting hydrolysates were then centrifuged and the supernatant lyophilized as the CPHs were stored at −20 °C for further analysis.

Table 1
Hydrolysis conditions for canola protein hydrolysates.

Enzyme	Abbreviation	Hydrolysis conditions (4 h duration)		Degree of hydrolysis (free amino group, mg/g of hydrolysate protein)*
		pH	Temp (°C)	
Alcalase	AH	8.0	60	65.9 ± 0.3[a]
Chymotrypsin	CH	8.0	37	48.5 ± 0.2[b]
Pepsin	PH	3.0	37	45.2 ± 0.3[b]
Trypsin	TH	8.0	37	46.0 ± 0.4[b]
Pancreatin	PcH	8.0	40	44.8 ± 0.2[b]

*CPI: 2.3 ± 0.2 [c] free amino group (mg/g of protein).

2.2.3. Determination of degree of hydrolysis

The degree of hydrolysis (DH) of canola protein hydrolysates was estimated using the trichloroacetic acid (TCA) soluble free amino group detection method described by McKellar (1981), with slight modifications. Briefly, 4 mL of 12% TCA solution was added to 0.5 g of canola protein hydrolysate samples in a test tube, mixed and incubated for 20 min at room temperature. This solution was filtered and 0.2 mL of the aliquot was added to 2 mL of 1 M potassium tetraborate buffer (pH 9.2) and 0.8 mL of 5 mM picrylsulfonic acid solution. The resulting solution was incubated in the dark at room temperature. After 30 min, 0.8 mL of 2 M dihydrogen orthophosphate solution, containing 18 mM sodium hydrosulfide was added and the absorbance read at 420 nm. Glycine (1–10 mM) and blank (0.5 mL deionized water) were treated the same as the samples above and the absorbance obtained from glycine was used to plot a standard curve. The absorbance of samples was converted to mg free amino groups (glycine) per gram of protein/hydrolysate from the standard curve.

2.2.4. Membrane fractionation

Each CPH was fractionated by membrane ultrafiltration stirred cell Amicon® 8400 (Millipore Corp. Billerica, MA, USA), using a sequence of membranes with molecular weight cut-off (MWCO) values of 1, 3, 5 and 10 kDa respectively. The retentate from 1 kDa was passed through a 3 kDa membrane whose retentate was passed through a 5 kDa membrane. Finally the retentate from 5 kDa membrane was then passed through a 10 kDa membrane. Permeate collected from each membrane (<1, 1–3, 3–5, or 5–10, respectively) was lyophilized and stored at −20 °C for further use. Percentage yield was calculated as the ratio of the hydrolysate and ultrafiltration processed fractions on a protein equivalent basis to that of the unhydrolyzed CPI and CPHs, respectively. Protein content of samples was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.3. Determination of surface hydrophobicity (H_o)

H_o was determined using a hydrophobicity fluorescence probe, 1-anilino-8-naphtalene-sulfonate (ANS) as described by Kato and Nakai (1980) with some modifications. Hydrolysates were serially diluted to a final concentration of 0.005–0.025% (w/v) in 0.01 M phosphate buffer, pH 7.0. A 20 μL aliquot of ANS (8.0 mM in phosphate buffer) was added to 4 mL of each sample concentration and fluorescence intensity (FI) of the mixture was measured with a JASCO FP-6300 spectrofluorimeter (JASCO Corp., Tokyo, Japan) at excitation and emission wavelengths of 390 and 470 nm, respectively. The initial slope of the FI *versus* hydrolysate concentration (μg/mL) plot (calculated by linear regression analysis) was used as an index of H_o.