Food Chemistry 141 (2013) 153–159

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Antihypertensive and free radical scavenging properties of enzymatic rapeseed protein hydrolysates

Rong He [a,b,*], Adeola Alashi [b,e], Sunday A. Malomo [b,c], Abraham T. Girgih [b,c], Dongfang Chao [b,c], Xingrong Ju [d], Rotimi E. Aluko [b,c,*]

[a] College of Food Science, Jiangnan University, Wuxi 214122, People's Republic of China
[b] The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
[c] Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2
[d] College of Food Science and Engineering, Nanjing University of Finance and Economics, Nanjing 210003, People's Republic of China
[e] School of Agriculture and Wine Sciences, Charles Sturt University, PB 588, Wagga Wagga, NSW, Australia

ARTICLE INFO

Article history:
Received 5 November 2012
Received in revised form 13 February 2013
Accepted 20 February 2013
Available online 7 March 2013

Keywords:
Rapeseed protein isolate
Protein hydrolysate
Antihypertensive properties
Angiotensin converting enzyme
Renin
Oxygen radical absorbance capacity
Spontaneously hypertensive rat
Membrane ultrafiltration

ABSTRACT

In this study, rapeseed protein isolate (RPI) was digested with various proteases to produce rapeseed protein hydrolysates (RPHs), which were then separated into different peptide fractions (<1, 1–3, 3–5, and 5–10 kDa) by membrane ultrafiltration. Membrane fractionation showed that peptides with sizes <3 kDa had significantly ($p < 0.05$) reduced surface hydrophobicity when compared to the RPHs and peptide fractions with sizes >3 kDa. In contrast, the <3 kDa peptides showed significantly ($p < 0.05$) higher oxygen radical scavenging ability when compared to the >3 kDa peptides and RPHs. *In vitro* inhibition of angiotensin I-converting enzyme (ACE) was significantly ($p < 0.05$) higher for the Thermolysin, Proteinase K and Alcalase RPHs when compared to the pepsin + pancreatin (PP) and Flavourzyme RPHs. The Alcalase RPH had significantly ($p < 0.05$) higher renin inhibition among the RPHs, while with the exception of Thermolysin, the 5–10 kDa peptide fraction had the least renin-inhibitory ability when compared to the <5 kDa peptide fractions. Oral administration (100 mg/kg body weight) of the RPHs and RPI to spontaneously hypertensive rats (SHR) showed the Alcalase RPH to be the most effective in blood pressure (BP) reduction (~24 mm Hg) while Proteinase K RPH was the least effective (~5 mm Hg) after 8 h. However, the PP RPH had the most prolonged effect with BP reduction of ~20 mm Hg after 24 h of oral administration. We conclude that the strong BP-lowering ability of Alcalase and PP RPHs could be due to high resistance of the peptides to structural degradation coupled with high absorption rate within the gastrointestinal tract.

1. Introduction

Hypertension or high blood pressure, which is estimated to affect one third of the worldwide population, is one of the primary risk factors associated with cardiovascular disease events including myocardial infarction, heart failure, stroke, and vascular dementia (Sharp et al., 2011). The renin angiotensin system (RAS) plays a vital role in blood pressure regulation with renin and angiotensin I-converting enzyme (ACE) as the main regulators that control the RAS pathway (Daien et al., 2012). Blood pressure (BP) regulation is based on the fact that renin can convert angiotensinogen to angiotensin I (AT-I), which in turn is converted by ACE to angiotensin II (Ang-II). Ang-II is a potent vasoconstrictor that also induces the release of aldosterone and therefore, increases sodium concentration and blood pressure. Besides, ACE is also known to hydrolyze bradykinin (Yang, Erdos, & Levin, 1970), which is a potent vasodilator, thus leading to the inability of the blood vessels to relax following contraction. Therefore, by inhibiting ACE activity, formation of angiotensin II and destruction of bradykinin will be reduced, which can contribute to lowering of blood pressure. Another strategy is to inhibit renin activity directly, which could provide a more complete blockade of the RAS, since renin catalyzes the rate-determining step in RAS (Fitzgerald, 2011). In addition, oxidative stress is both a cause and a consequence of hypertension, which induces cardiovascular and renal damage with associated increase in blood pressure. For example, it has been shown that Ang II-dependent hypertension is particularly sensitive to NAD(P)H oxidase derived reactive oxygen species (ROS) (Uzuner, Tokay, Cetin, & Yesilkaya, 2010). Therefore, some therapies based on scavenging of ROS could have potential effects in the management of diseases associated with vascular damage, including hypertension.

* Corresponding authors. Address: College of Food Science, Jiangnan University, Wuxi 214122, People's Republic of China (R. He), The Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2. Tel.: +1 204 474 9555; fax: +1 204 474 7593 (R.E. Aluko).
E-mail addresses: ronghe.nufe@hotmail.com (R. He), rotimi.aluko@ad.umanitoba.ca (R.E. Aluko).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.02.087

It has been recognized that nutritional factors play a significant role in the prevention or treatment of hypertension, and food derived bioactive peptides are attracting increasing interest because of their safety and multi-functionality (Udenigwe & Aluko, 2012). Recently, various ACE−inhibitory peptides were obtained from rice, soybean and peanut (Jimsheena & Gowda, 2010; Kang, Kim, Ahn, & Lee, 2012; Rho, Lee, Chung, Kim, & Lee, 2009), showing that plant proteins are good sources of ACE-inhibitory peptides. In addition to ACE inhibition, some studies have also demonstrated that food-derived peptides could inhibit the activity of renin (Girgih, Udenigwe, Li, Adebiyi, & Aluko, 2011; Li & Aluko, 2010), and possess free radical scavenging activities (Huang, Majumder, & Wu, 2010). Accordingly, it is believed that simultaneous inhibition of ACE and renin during antihypertensive therapy by food-derived peptides could potentially provide better BP-lowering effects than inhibiting ACE activity alone (Udenigwe & Aluko, 2012).

Rapeseed is an important global commodity, and is a good source of edible oil preceded only by soybean in the world. Rapeseed meal, which is a byproduct of oil extraction, is considered to be a potential alternative source of plant protein containing up to 50% protein on a dry weight basis. Rapeseed protein has a well-balanced amino acid composition (including high levels of lysine, cysteine and methionine) that is comparable to that of other commonly consumed legumes, and can be used in the development of numerous products for the therapeutic, functional foods, and nutraceutical industries (Yoshie-Stark, Wada, Schott, & Wasche, 2006). Recently, the nutritional and functional properties of rapeseed protein isolate (RPI) was evaluated and compared with those of milk protein isolate and soy protein isolate. The results emphasize the ability of RPI to induce particular metabolic effects at the tissue level in healthy individuals during the postprandial period (Dong et al., 2011). Another study also explained the properties of RPI as an edible film ingredient in food packaging (Jang, Lim, & Bin Song, 2011). In addition, rapeseed protein hydrolysates (RPHs) produced by Alcalase has been reported to possess ACE-inhibitory activity (Mäkinen, Johannson, Gerd, Pihlava, & Pihlanto, 2012), but the work did not provide information on the relationships between ACE inhibition and molecular weight of peptides obtained from different proteinase hydrolysates, or ability of the RPHs to inhibit renin activity. Other previous works have also shown that rapeseed protein hydrolysates possess ACE-inhibitory (Marczak et al., 2003) and antioxidant properties (Cumby, Zhong, Naczk, & Shahidi, 2008; He, Girgih, Malomo, Ju, & Aluko, 2013).

Since the structure and activity of antihypertensive peptides could be affected by the method of production, there is need to evaluate the efficiency of proteases in releasing antihypertensive peptides from RPI. Therefore, the first objective of the study was to determine the ability of several proteases to produce antihypertensive RPHs from RPI, evaluated as *in vitro* inhibitions of both ACE and renin activities. Since high oxidative stress may also contribute to blood pressure elevation, the antioxidant activity of the RPHs was determined using the oxygen radical absorption capacity (ORAC) method. The second objective was to determine actual BP-lowering effects of the RPHs in spontaneously hypertensive rats (SHRs) and relate the observed *in vivo* effects to *in vitro* ACE and renin inhibitions.

2. Materials and methods

2.1. Materials

The defatted rapeseed protein meal (RPM) was supplied by COFCO Eastocean Oil & grains industries Co., Ltd., (Zhangjiagang, China). It should be noted that this is a high erucic acid *Brassica napus* variety and is different from the low erucic acid canola variety. The meal was milled and passed through a 15 mm screen. Alcalase, Proteinase K, Pepsin, Pancreatin, Thermolysin, Flavourzyme, Angiotensin I-converting enzyme (ACE), *N*-(3-[2-furyl]acryloyl)-phenylalanylglycylglycine (FAPGG), 8-Anilino-1-naphthalenesulfonic acid ammonium salt (ANS), Captopril, L-glutathione reduced (GSH), and 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH), were purchased from Sigma-Aldrich (St. Louis, MO). Renin Inhibitor Screening Assay Kit was purchased from Cayman Chemicals (Ann Arbor, MI). Ultrafiltration membranes, fluorescein free acid, trolox and other analytical grade reagents were obtained from Fisher Scientific (Oakville, ON, Canada).

2.2. Preparation of rapeseed protein isolate

RPI was produced from RPM according to a previously reported method (Yoshie-Stark, Wada, & Wasche, 2008) with slight modifications. RPM was dispersed in deionized water (1:15, w/v), adjusted to pH 10.0 with 1 M NaOH, mixed at 45 °C for 2 h and the slurry was centrifuged at 10,000×*g* for 30 min. The supernatant was recovered, adjusted to pH 4.5 with 1 M HCl, allowed to stand for 1 h at room temperature followed by centrifugation at 10,000×*g* for 30 min. The recovered precipitated proteins were washed with anhydrous ethyl alcohol (to remove the polyphenolic components), re-dispersed in deionized water, adjusted to pH 7.0 with 1 M NaOH and freeze-dried to produce RPI. Protein content of the RPI was determined by the modified Lowry method (Markwell, Haas, Bieber, & Tolbert, 1978).

2.3. Preparation of enzymatic rapeseed protein hydrolysates and membrane fractions

Hydrolysis of the RPI was conducted using each of the following enzyme and reaction conditions as previously reported (He et al., 2013): Alcalase (50 °C, pH 8.0); Proteinase K (37 °C, pH 7.5); Thermolysin (50 °C, pH 8.0); Flavourzyme (50 °C, pH 6.5); and Pepsin (37 °C, pH 2.0) + Pancreatin (37 °C, pH 7.5). 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 value prior to addition of the proteolytic enzyme. Each protease was added to the RPI slurry at an enzyme to substrate ratio (*E*/*S*) of 4:100, based on the protein content of the RPI. Digestion was performed for 4 h (pH maintained constant by addition of NaOH) after which the enzymes were inactivated by adjusting to pH 4.0 with 2 M HCl followed by immersing the reaction vessel in boiling water bath for 10 min. The undigested proteins were precipitated by centrifugation at 8000×*g* for 60 min. A portion of the supernatant containing target peptides was freeze dried to make RPH, while the remaining portion was passed through ultrafiltration membranes with molecular weight cut-off (MWCO) of 1, 3, 5, 10 kDa in an Amicon stirred ultrafiltration cell. Supernatant was first passed through the 1 kDa membrane and the retentate passed through 3 kDa. The 3 kDa retentate was passed through a 5 kDa membrane whose retentate was then passed through a 10 kDa membrane (He et al., 2013). The permeate from each MWCO membrane (<1, 1–3, 3–5, and 5–10 kDa, respectively) were collected, lyophilized, and stored at −20 °C until needed for further analysis. The protein contents of the freeze dried rapeseed protein hydrolysates (RPH) and membrane fractions were also determined using the modified Lowry method (Markwell et al., 1978).

2.4. Analysis of molecular weight distribution

Molecular weight distribution of RPH peptides was determined using an AKTA FPLC system (GE Healthcare, Montreal, PQ) equipped with a Superdex Peptide12 10/300 GL column