LWT - Food Science and Technology 43 (2010) 987–991

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Research Note

Angiotensin I-converting enzyme inhibitory properties and SDS-PAGE of red lentil protein hydrolysates

Joyce Irene Boye [a,*], Samira Roufik [b], Noemie Pesta [c], Chockry Barbana [a]

[a] Food Research and Development Centre, Agriculture and Agri-Food Canada, Casavant Blvd. West, St Hyacinthe, QC J2S 8E3, Canada
[b] Health Canada, Ottawa, Canada
[c] Université de Reims Champagne-Ardenne, 9 Boulevard de la Paix, Reims, Champagne-Ardenne, France

ARTICLE INFO

Article history:
Received 26 May 2009
Received in revised form
14 January 2010
Accepted 15 January 2010

Keywords:
ACE-inhibitory activity
Bioactivity
Protein hydrolysate
Pulse
Red lentils

ABSTRACT

Several research studies have shown that protein hydrolysates from milk and soy contain peptides that possess angiotensin I converting enzyme (ACE) inhibitory properties and may help to prevent hypertension. To date, no studies have been conducted to determine if red lentil (*Lens culinaris*) proteins contain peptides with ACE-inhibitory properties. The objective of the present work was to characterize the proteins present in red lentils and determine if tryptic hydrolysis could liberate peptides with ACE-inhibitory properties. Red lentil protein extracts were prepared and fractionated to obtain enriched albumin, legumin and vicilin fractions. Protein/peptide profiles were studied by electrophoresis and ACE-inhibitory activity was measured using the HPLC hippuryl-His-Leu (HHL) substrate method. Our results revealed that red lentil protein hydrolysates posses ACE-inhibitory properties. Furthermore, we demonstrated that the ACE-inhibitory property of the hydrolysates varied as a function of the protein fraction with the total lentil protein hydrolysate having the lowest half maximal inhibitory concentration (IC_{50}) (111 ± 1 μmol/L) (i.e., highest ACE-inhibitory activity), followed by the enriched legumin (119 ± 0.5 μmol/L), albumin (127 ± 2 μmol/L) and vicilin (135 ± 2 μmol/L) fractions, respectively.

1. Introduction

A major risk factor for developing cardiovascular disease, one of the leading causes of death in North America, is elevated blood pressure. Angiotensin II, a potent vasoconstrictor, is a major contributor to high blood pressure. Vasoconstriction occurs when rennin, an enzyme liberated by the kidneys, proteolytically acts on circulating angiotensinogen and converts it to angiotensin I (a decapeptide). In the presence of angiotensin converting enzyme (ACE), angiotensin I is cleaved to the octapeptide, angiotensin II resulting in arterial constriction and blood pressure elevation. ACE also breaks down bradykinin, a vasodilator, further contributing to the elevation in blood pressure. Inhibition of ACE is, therefore, important for the lowering of blood pressure as this results in a decrease in the concentration of angiotensin II and an increase in the levels of bradykinin (Erdos, 1975; Yang, Erdos, & Levin, 1970). ACE is a dipeptidyl carboxypeptidase (EC3.4.15.1) and many studies in recent years have focused on identifying compounds that can inhibit its activity (Quiros et al., 2007; Tsai, Chen, Pan, Gong, & Chung, 2008; Vermeirssen, Van Camp, & Verstraete, 2002; Wu & Ding, 2002). Various high blood pressure medications available on the market today are targeted towards ACE inhibition. Examples of such drugs include Captopril, Enalapril, Ramipril, and Quinapril (Accupril). Due to potential side effects of pharmaceutical drugs, there is increased interest to identify foods that naturally contain peptides with hypotensive properties. Several research studies have shown that protein hydrolysates from certain plant and animal sources contain peptides that demonstrate ACE-inhibitory properties and may help to prevent hypertension (Hartmann & Meisel, 2007; Wu & Muir, 2008; Yang, Yang, Chen, & Chen, 2008). Recently, some studies reported novel antihypertensive peptides in fermented milk products (Chen, Tsai, & Pan, 2007; Quiros et al., 2007; Tsai et al., 2008). In other studies, ACE-inhibitory activity after hydrolysis of different milk protein products with a variety of enzymes has been reported (Otte, Shalaby, Zakora, Pripp, & El-Shabrawy, 2007). Plant proteins such as soy, chickpea and pea, have also been shown to contain ACE-inhibitory peptides (Aluko, 2008; Chiang, Tsou, Tsai, & Tsai, 2006; Kuba, Tanaka, Tawata, Takeda, & Yasuda, 2003; Wu & Ding, 2002). To date, however, no studies have been conducted to determine if red lentil proteins contain peptides with ACE-inhibitory properties. IC_{50} values of 0.78–0.83, 0.15–0.69 and 0.008–0.89 mg protein/mL for common

* Corresponding author. Tel.: +1 450 768 3232; fax: + 1 450 773 8461.
E-mail address: joyce.boye@agr.gc.ca (J.I. Boye).

0023-6438/$ – see front matter
doi:10.1016/j.lwt.2010.01.014

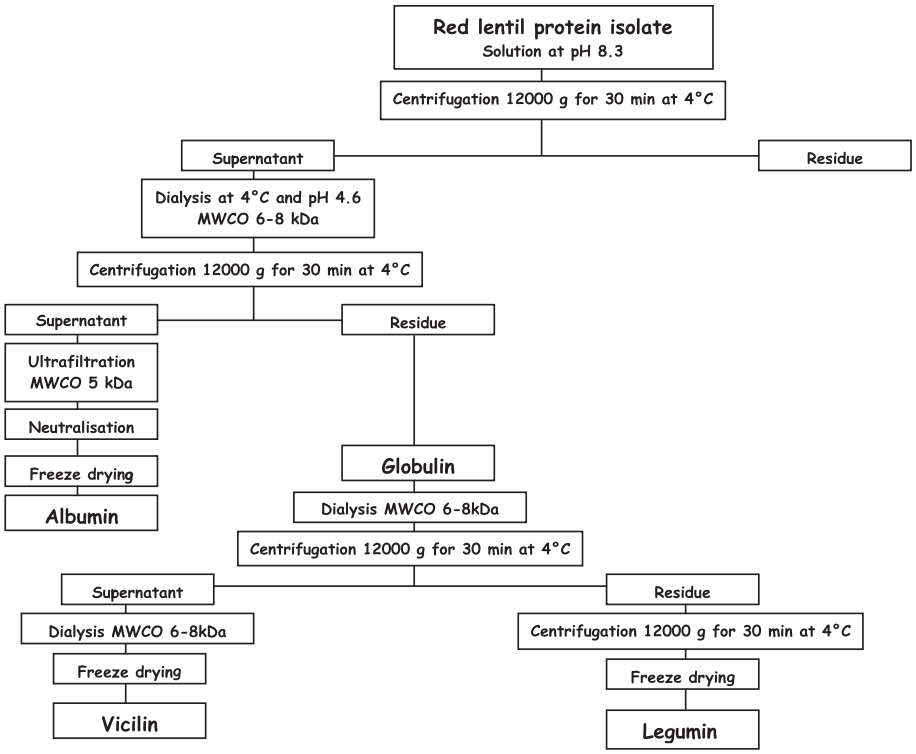

Fig. 1. Schematic representation of the process used for the fractionation of the albumin, vicilin and legumin fractions from red lentil protein isolate. (Based on the method described by Gupta and Dhillon (1993)).

beans, pinto beans and green lentils, respectively, after *in vitro* gastrointestinal digestion have been very recently reported (Akıllıoğlu & Karakaya, 2009). The major proteins found in lentils are albumins and globulins (Bhatty, 1988; Gupta & Dhillon, 1993). Legumins (molecular weight (MW) 350–400 kDa) and vicilins (MW 150–200 kDa) are the two main globulins. Other minor proteins present in lentils are prolamins and glutelins. Hydrolysis of lentil proteins could potentially liberate peptide sequences with ACE-inhibitory properties. The aim of the proposed work, therefore, was to extract the proteins present in red lentils and determine if tryptic hydrolysis could liberate peptides with ACE-inhibitory properties. Additionally, the polypeptide profiles of the different red lentil protein fractions and their tryptic hydrolysates were characterized by SDS-PAGE.

2. Materials and methods

2.1. Materials

The red lentil used for this study was the *Common Blaze* certified variety which was provided by Simpson Seeds Inc. (Saskatchewan, Canada). All other chemicals used were of analytical grade.

2.2. Protein extraction and fractionation

Protein isolates were prepared from red lentils using alkaline extraction followed by ultrafiltration. Briefly, whole lentil seeds were frozen in liquid nitrogen and ground using a Brinkmann centrifugal mill (Brinkmann Instruments Canada, Mississauga, ON, Canada) equipped with a 5-mesh sieve with 1.5 mm pore size. Ground seeds (10 g) were suspended in water (100 g/L) and the pH was adjusted to 9 using 1 mol equiv/L NaOH. The dispersions were stirred for 60 min at 25 ± 1 °C to facilitate protein solubilization, while maintaining the pH at 9, and then centrifuged at room temperature at 12,000 g for 30 min. The supernatant was subjected to ultrafiltration using a 50 kDa MW Cut-Off membrane and a volume concentration ratio of 5 followed by diafiltration using a volume dilution factor of 4. The retentate was neutralized to pH 7.0 using 1 mol equiv/L HCl, frozen and then freeze-dried (red lentil protein isolate). The protein isolate was further fractionated to the respective albumin, globulin and glutelin fractions using a combination of salt extraction, isoelectric precipitation and membrane separation techniques as described previously (Gupta & Dhillon, 1993) (Fig. 1). The pH of solutions was adjusted using 1 mol equiv/L NaOH or HCl. The enriched fractions were hydrolyzed as described below and freeze-dried for ACE-inhibitory studies.

2.3. Protein analysis

Protein content of the red lentil protein isolate and the enriched fractions were analysed by Leco (Leco FP-428, Leco Corp., St. Joseph, Mich., U.S.A.), using the combustion method (AOAC, 1995) and a nitrogen conversion factor of 6.25.

2.4. Protein hydrolysis

Trypsin was used to hydrolyze the red lentil protein fractions into peptide fragments under saturated substrate conditions. The tryptic digestion (E/S: 1/25) was conducted for 24 h at 37 °C and pH 6.5. The reaction was stopped by heating at 90 °C for 10 min, and all samples were centrifuged at 12,000 g for 20 min at 4 °C, and the supernatant was filtered and lyophilized.

2.5. SDS-polyacrylamide gel electrophoresis

SDS-PAGE analysis of the lentil protein isolate, enriched fractions and their tryptic digests was performed on precast 10–20% gradient polyacrylamide Tris/HCl and Tris/Tricine gels using the