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Electrophoretic Profiles and Angiotensin I-Converting Enzyme Inhibitory Activities of Nine Varieties of *Phaseolus Vulgaris* Protein Hydrolysates

Xin Rui[1,2], Joyce I. Boye[1]*, Chockry Barbana[1], Benjamin K. Simpson[3] and Shiv O. Prasher[2]

[1]*Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd. W., St-Hyacinthe, QC, J2S 8E3, Canada*
[2]*Department of Bioresource Engineering, Macdonald Campus, McGill University, 21,111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC, H9X 3V9, Canada*
[3]*Department of Food Science and Agricultural Chemistry, Macdonald Campus, McGill University, 21,111 Lakeshore Rd., Ste-Anne-de-Bellevue, QC, H9X 3V9, Canada*

Abstract

Nine dry bean (*Phaseolus vulgaris*) varieties largely grown in Canada were subjected to digestion using trypsin and *in vitro* gastrointestinal simulation (GIS) followed by a study of their *in vitro* ACE inhibitor properties and digestibility. GIS hydrolysates of all varieties presented significantly higher ACE inhibitory activities and degree of hydrolysis (DH) compared to those of trypsin hydrolysates ($P < 0.05$). Cranberry and light red kidney bean protein isolates contained 'T' type phaseolin and had higher DH values during both digestions, with average ACE inhibitory activities of 281.7–281.8 µg/mL and 141.6–185.1 µg/mL, respectively, for tryptic and GIS hydrolysates. The other seven bean varieties contained 'S' type phaseolin, and of these small red bean showed the lowest ACE inhibitory activities for both trypsin (IC_{50} of 170 µg/mL) and GIS (IC_{50} of 118 µg/mL) digestion, followed by navy bean, with IC_{50} of 200 µg/mL (trypsin digestion) and 137 µg/mL (GIS digestion). The results demonstrated that both methods of digestions yielded bioactive peptides, however, differing peptide profiles of the bean protein hydrolysates affected their *in vitro* ACE inhibitory property.

Keywords: Bean protein hydrolysates; Angiotensin I-converting enzyme; Digestibility; *in vitro* gastrointestinal digestion simulation; Trypsin

Introduction

Peptides with hypotensive properties have received increasing attention in recent times. Hypertension is ranked as one of the world's most common high-risk diseases, affecting about 22% of the world's population [1]. Angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is the key enzyme responsible for elevation of blood pressure as it is capable of converting the decapeptide, angiotensin I, to the octapeptide, angiotensin II, a potent vasoconstrictor, and also degrading bradykinin, a vasodilator, into inactive peptides [2]. Adverse effects resulting from the use of synthetic ACE inhibitors has enhanced research aimed at investigating and identifying natural sources of ACE inhibitory peptides, which may be freer of side effects.

Pulses, including pea, lentil, chickpea, and beans contain high levels of protein, fiber, as well as valuable minerals and vitamins which enhances their health-benefitting attributes. ACE inhibitory studies have been conducted on a few pulses, including chickpea [3-5], pea [5-7], and lentil [8]. However, very few studies have investigated the antihypertensive properties of beans [9,10]. Canada is one of the most important global producer and exporter of dry beans and several dry bean varieties are grown in the country. Unlike other pulses, there is a dearth of fundamental studies on the ACE inhibitory activities of beans and more importantly how this activity is affected by varieties. Data on bean bioactive properties could enable dry bean proteins to be explored as value added components in functional foods which will provide benefit to consumers, growers as well as producers.

An essential step for the generation of bioactive peptides from foods is enzymatic hydrolysis. Bean proteins have lower digestibility compared to animal proteins which can limit their nutritional value [11], and further affect the potential of generating ACE inhibitory peptides during hydrolysis. In the current study, the digestibility of proteins from nine varieties of dry beans (*Phaseolus vulgaris*) that are largely cultivated in Canada, namely navy, pink, pinto, cranberry, black, great northern, light red kidney, dark red kidney, and small red, were studied using trypsin and *in vitro* gastrointestinal digestion simulation (GIS). Subsequently, the *in vitro* ACE inhibitory properties of the protein digests were analyzed and compared to determine the bean variety with highest potential for ACE inhibition under the conditions studied.

Materials and Methods

Materials

Protein isolates of nine varieties of beans (*Phaseolus vulgaris*), namely navy, pink, pinto, cranberry, black, great northern, dark red kidney, light red kidney, and small red beans, were prepared as described in Rui et al. [12]. Low molecular weight calibration kits were from Amersham Pharmacia Biotech (Uppsala, Sweden). Precast 10-20% gradient tris/tricine gels, precast 16.5% tris/tricine gels, tricine sample buffer, and coomassie brilliant blue G-250 were from Bio-Rad Laboratories (Hercules, CA). α-Amylase (A 6380), pepsin (P 6887), trypsin (T 0303), α-chymotrypsin (C 4129), ACE regent (A 6778), Hippuryl-His-Leu (HHL) (H 1635) and 2,4,6-trinitrobenzenesulphonic acid (TNBS) (P 2297) were purchased from Sigma-Aldrich Co. (Oakville, ON). All other regents used were of analytical grade.

Protein analysis

Protein/peptide contents in protein hydrolysates were determined

***Corresponding author:** Joyce Irene Boye, Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Blvd. W., St-Hyacinthe, QC, J2S 8E3, Canada, Tel: +1 450 768 3232; Fax: +1 450 773 8461; E-mail: Joyce.Boye@agr.gc.ca

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according to previous studies [5,13] with the Dumas combustion method using a LECO FP-428 apparatus (LECO Corp., St. Joseph, MI, USA) [14]. A factor of 6.25 was used for the conversion of nitrogen to protein based on the average nitrogen content (16%) of amino acids in protein mixtures.

***In vitro* protein digestibility:** *In vitro* protein digestibility of bean protein isolates were determined according to a previously published method based on measuring pH drop after 10 min of *in vitro* digestion [15].

Protein hydrolysis

Protein hydrolyses using trypsin and *in vitro* gastrointestinal simulation (GIS) digestions were carried out separately in duplicate. Digestions were conducted using a pH-stat apparatus (TIM 865, Radiometer Analytical SAS, Villeurbanne, France) with substrate (concentration of 2.5% w/v, based on protein content) dispersed in phosphate buffer (0.01 M, pH 8.0) (Trypsin hydrolysis) or glycine buffer (0.01 M, pH 7.0) (GIS hydrolysis). Trypsin digestions were performed with an enzyme to substrate ratio (E/S) of 1:25 (w/w, based on protein content) for 2 h at 37°C. GIS digestions were started by pre-treatment of the bean protein isolates with α-amylase solution (1 mg/mL, 0.01 M glycine buffer, pH 7.0) at a ratio at 1:12.5 (v/w) at 37°C for 3 min and then followed by sequential digestions of pepsin, trypsin and α-chymotrypsin with E/S: 1/250 (w/w, based on protein content) as described previously in Barbana and Boye [5]. For both trypsin and GIS digestion, enzyme inactivation after sampling was achieved by heating in boiling water for 10 min followed by centrifugation at 12,000 g, 4°C for 20 min. The supernatants were collected and freeze dried for further electrophoresis and ACE inhibition studies. To follow the time course of hydrolysis, aliquots of the hydrolysates were withdrawn at different time intervals during digestion for electrophoretic analysis. The samples were diluted 10 fold with Laemmli buffer, heated in boiling water for 10 min and stored at -20°C until analysis.

Degree of Hydrolysis

Degree of hydrolysis (DH, %) was determined based on the reaction of free amino groups with TNBS [16]. Total number of amino acid groups was determined by hydrolyzing the samples in 6 M HCl at 110°C for 24 h. A series concentration of L-Leucine was used to generate the standard curve.

In vitro angiotensin I-converting enzyme inhibitory activity determination

ACE inhibitory activities were determined by using the HPLC method described previously in Barbana and Boye [5]. A 4.60 x 250 mm Aqua C18 reverse-phase HPLC column (5 µm particle size, 125 Å, Phenomenex) was used. Hippuryl-His-Leu (HHL) was used as substrate. IC_{50} values (concentration inhibiting half maximal ACE activity) were determined by graphed ACE inhibition percentages versus semi-logarithmic values of sample concentrations.

Electrophoresis

SDS-PAGE analyses of the bean trypsin hydrolysates were carried out using a Bio-Rad Criterion Cell (Bio-Rad Laboratories, Inc., Mississauga, ON) with 16.5% tris-tricine gels. GIS hydrolysates were analyzed using 10-20% gradient tris-HCl gels. For studies under denaturing conditions, 5% (v/v) β-mercaptoethanol (β-Me) was added to the electrophoresis reagents during sample preparation prior to sample loading. Electrophoreses were conducted under constant voltage of 100 V, using Bio-Rad polypeptide standards (1.423–26.625 kDa) and low-molecular mass standard markers (14.4–97 kDa) calibration kits from Amersham Pharmacia Biotech for 16.5% tris-tricine gels and 10-20% gradient tris-HCl gels, respectively. Gels were scanned with a Bio-Rad GS-690 calibrated imaging densitometer and analyzed using a Multi-Analyst/PC Analysis software (Bio-Rad Laboratories, Inc., Mississauga, ON).

Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to determine the significant differences between means ($P < 0.05$) using SAS Server Interface (version 2.0.3, SAS Institute, Cary, NC).

Results

In vitro protein digestibility of the protein isolates extracted from the nine bean varieties ranged from 83.67% (black bean) to 88.19% (great northern bean). The highest values were found for navy bean and great northern bean, at 88.01% and 88.19%, respectively ($P < 0.05$) (Table 1). Trypsin digestion of the bean protein isolates gave low degree of hydrolysis (DH) values, ranging from 1.72% (dark red kidney bean) to 4.59% (cranberry bean), whereas higher DH values were obtained after *in vitro* GIS digestion, ranging from 7.22% (dark red kidney bean) to 16.12% (pinto bean) (Table 1). Among the nine varieties, hydrolysates of cranberry, light red kidney and pinto bean for both methods of digestion exhibited significantly higher DH values than the other six varieties ($P < 0.05$).

Under both reducing and non-reducing conditions, SDS-PAGE profiles of the trypsin hydrolysates of all investigated varieties showed predominant bands with estimated molecular mass (MM) of 24 kDa (Figure 1a and b). The intensity of the 24 kDa band was much less for the hydrolysates of cranberry and light red kidney, but there were more intense bands at lower MM between 3–6 kDa, and also additional bands around 16 kDa (lane 4 and 7, Figure 1a and b). The hydrolysate of black bean had a large amount of a high MM fraction which remained at the top of the gel (lane 5, Figure 1a and b); this band was absent for navy and great northern bean hydrolysates (lane 1 and 6, Figure 1a and b).

Unlike the dry bean trypsin digests, the majority of the bands observed in the SDS-PAGE after *in vitro* GIS digestions had a MM around 47 kDa (Figure 1c and d), and no protein/protein aggregates were observed at the top of the gel. Additionally, there was not much of a difference between the samples treated with and without β-Me. Again,

	Navy	Pink	Pinto	Cranberry	Black	Great northern	Light red kidney	Dark red kidney	Small red
Invitro digestibility	88.01±0.13^{a}	84.21±0.90^{c}	85.84±0.13^{b}	85.03±1.02^{bc}	83.67±0.64^{c}	88.19±0.64^{a}	84.39±0.13^{bc}	84.30±0.51^{c}	84.21±0.64^{c}
Trypsin DH	3.29±0.03^{d}	2.72±0.36^{f}	3.97±0.20^{c}	4.59±0.02^{a}	1.91±0.04^{g}	2.64±0.03^{f}	4.32±0.04^{b}	1.72±0.05^{g}	3.03±0.08^{e}
GIS DH	8.25±0.83^{cd}	11.92±0.18^{b}	16.12±1.73^{a}	15.16±0.22^{a}	10.28±0.51^{bc}	11.12±2.78^{b}	15.27±0.15^{a}	7.22±0.06^{d}	9.84±0.08^{bc}

In vitro digestibility data was measured in duplicate. Degree of hydrolysis data was measured in triplicate. Values after ± show the standard deviations.
$^{a\text{-}g}$ Values followed by different letters in the same row are significantly different (P<0.05).

Table 1: *In vitro* digestibility (%) of bean protein isolates and degree of hydrolysis (DH, %) of bean proteins hydrolysed using trypsin and *in vitro* gastrointestinal simulation (GIS) digestion.

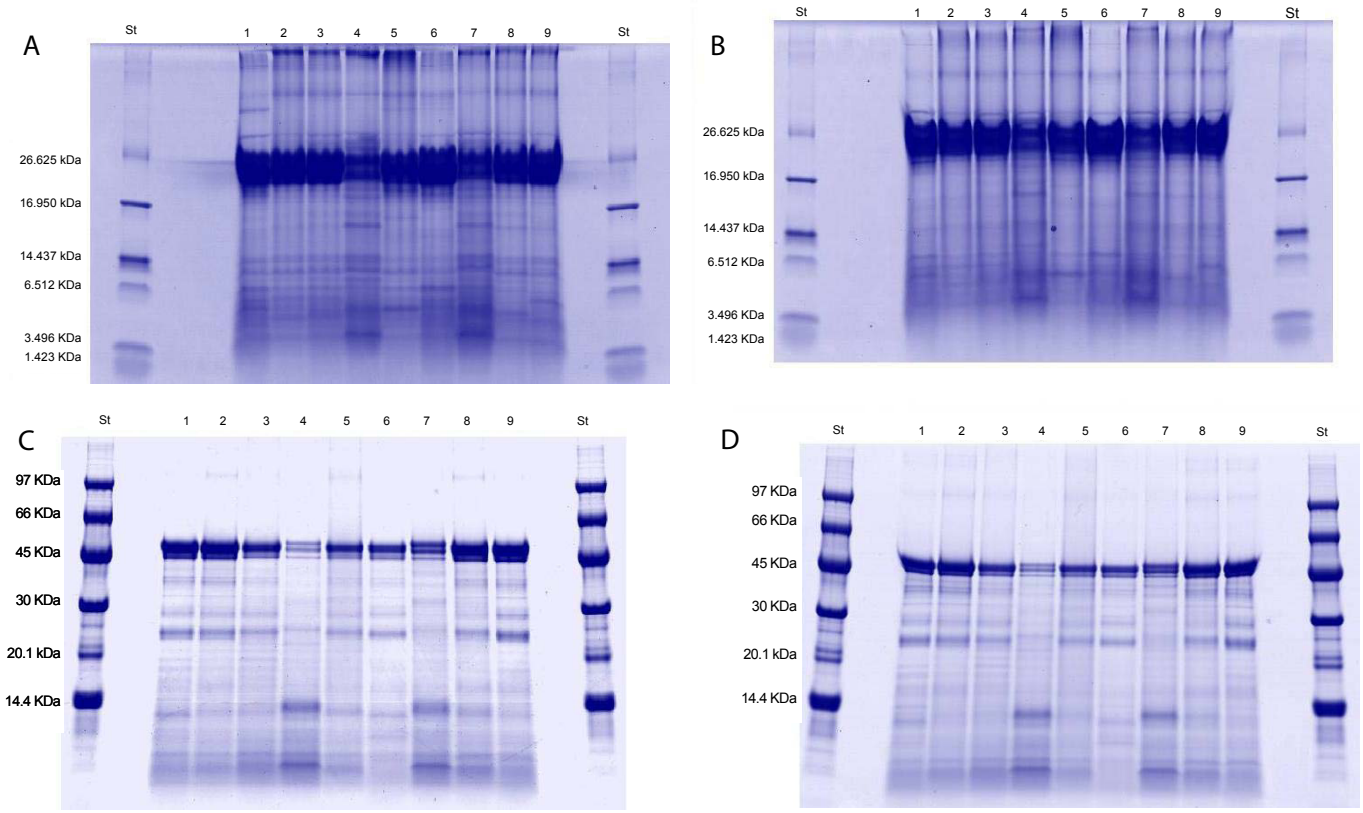

Figure 1: SDS-PAGE of bean protein trypsin hydrolysates (a, b) and *in vitro* gastrointestinal simulation (GIS) hydrolysates (c, d): (a, c) non-reducing conditions, (b, d) reducing conditions (5% β-ME), Lane 1-9 are 1- navy; 2- pink; 3- pinto; 4- cranberry; 5- black; 6- great northern; 7- light red kidney; 8- dark red kidney; 9- small red.

cranberry and light red kidney bean hydrolysates showed different profiles compared to the seven other varieties (lane 4 and 7, Figure 1c and d), with the presence of three subunits having MMs of 47, 50, and 53 kDa, whereas the other seven varieties presented only two subunits profiles with MMs of 46 and 50 kDa. Cranberry and light red kidney bean hydrolysates also contained smaller proteins having MMs less than 14 kDa compared to other varieties, and there were fewer bands between the 24 to 38 kDa range.

All investigated bean varieties showed ACE inhibitory activities after trypsin and *in vitro* GIS digestions. The latter method of hydrolysis yielded significantly lower IC_{50} values compared to the former ($P < 0.05$) (Figure 2). Lower IC_{50} values represent higher ACE inhibitory property. Thus, small red bean hydrolysates from both trypsin and GIS digestions, had significantly higher ($P < 0.05$) ACE inhibitory activities compared to other varieties undergoing similar digestion, with IC_{50} values of 170 µg/mL and 118 µg/mL, respectively. Navy bean hydrolysates had the next highest ACE inhibitory activity with IC_{50} values of 200 µg/mL (trypsin digestion) and 137 µg/mL (GIS digestion). Varieties presenting the least ACE inhibitory activities for both digestions were black bean trypsin hydrolysates (IC_{50} of 406 µg/mL) and pinto and dark red kidney GIS hydrolysates (IC_{50} of 198 µg/mL and 199 µg/mL, respectively).

To further understand changes occurring during the hydrolysis treatment, samples from the navy, black, and small red bean digests were collected at different time intervals during the digestion and subjected to electrophoresis (Figure 3a, b, c, d, e and f). The three varieties were chosen based on the ACE inhibitory results, which showed navy and small red bean hydrolysates to have high ACE inhibitory activity, and marked improvement in ACE inhibitory activity of black bean hydrolysates after GIS treatment compared to the trypsin digestion.

Minor differences were observed in the electrophoretic profiles with black bean protein isolates having intense bands in the high MM range and missing bands in the 60 to 97 kDa MM range (lane 0 min, Figure 3b). After addition of trypsin, proteins having MM higher than 30 kDa from all the investigated samples degraded within 5 min (Figure 3a, b and c) leaving only one persistent faint band with MM of 45 kDa. Two new bands, with MM of around 24 kDa (major) and 28 kDa (minor) appeared which did not degrade any further even after 120 min of hydrolysis (Figure 3a, b and c).

The electrophoretic profiles of the samples subjected to GIS treatment were very similar (Figure 3d, e and f). As expected, pre-treatment with α-amylase did not change the SDS-PAGE profiles (lane 1–2, Figure 3d, e and f). Addition of pepsin had little impact on the 47 kDa protein (lane 3–8, Figure 3d, e and f). However, it led to the degradation of the proteins with MMs of 20 kDa, 33 kDa, and higher (>50 kDa). New bands with estimated MM of 22 kDa were generated for navy and small red bean hydrolysates (lane 3–8, Figure 3d and f). Subsequent treatments with trypsin and α-chymotrypsin increased the intensity of the 24 kDa and 28 kDa proteins for all varieties (lane 9–14, Figure 3d, e and f), whereas the newly formed proteins with MM around 22 kDa generated after the addition of pepsin were gradually degraded to smaller peptides with the addition of trypsin and α-chymotrypsin (lane 9–14, Figure 3d and f).

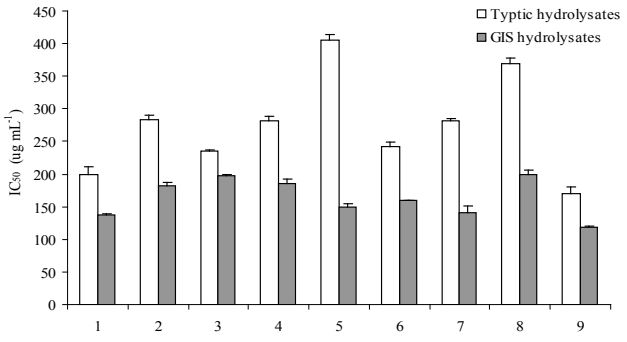

Figure 2: IC_{50} values for ACE inhibition properties of bean protein hydrolysates: 1- navy; 2- pink; 3- pinto; 4- cranberry; 5- black; 6- great northern; 7- light red kidney; 8- dark red kidney; 9- small red.

Discussion

Dry bean proteins are known to be resistant to proteolysis [17]. Various workers have indicated that the predominant protein in dry beans, i.e. phaseolin, which has a MM of around 47 kDa, has a compact structure and high β-sheet conformation which makes it resistant to peptic digestion and partially resistant to trypsin and α-chymotrypsin digestion [12,18,19]. This is consistent with our findings. The degree of hydrolysis (DH) value, which represents the extent of hydrolysis, was very low for all investigated varieties using both methods of digestion. Previous researchers [5,20] have reported almost three times higher DH values i.e. 27.08% – 40.78% for other pulses such as chickpea, pea and lentil subjected to similar GIS digestion. The results, therefore, suggest that the bean samples analyzed in this study are more resistant to digestion than some of these other pulses. The limited degree of hydrolysis did not, however, prevent the enzymatic release of ACE

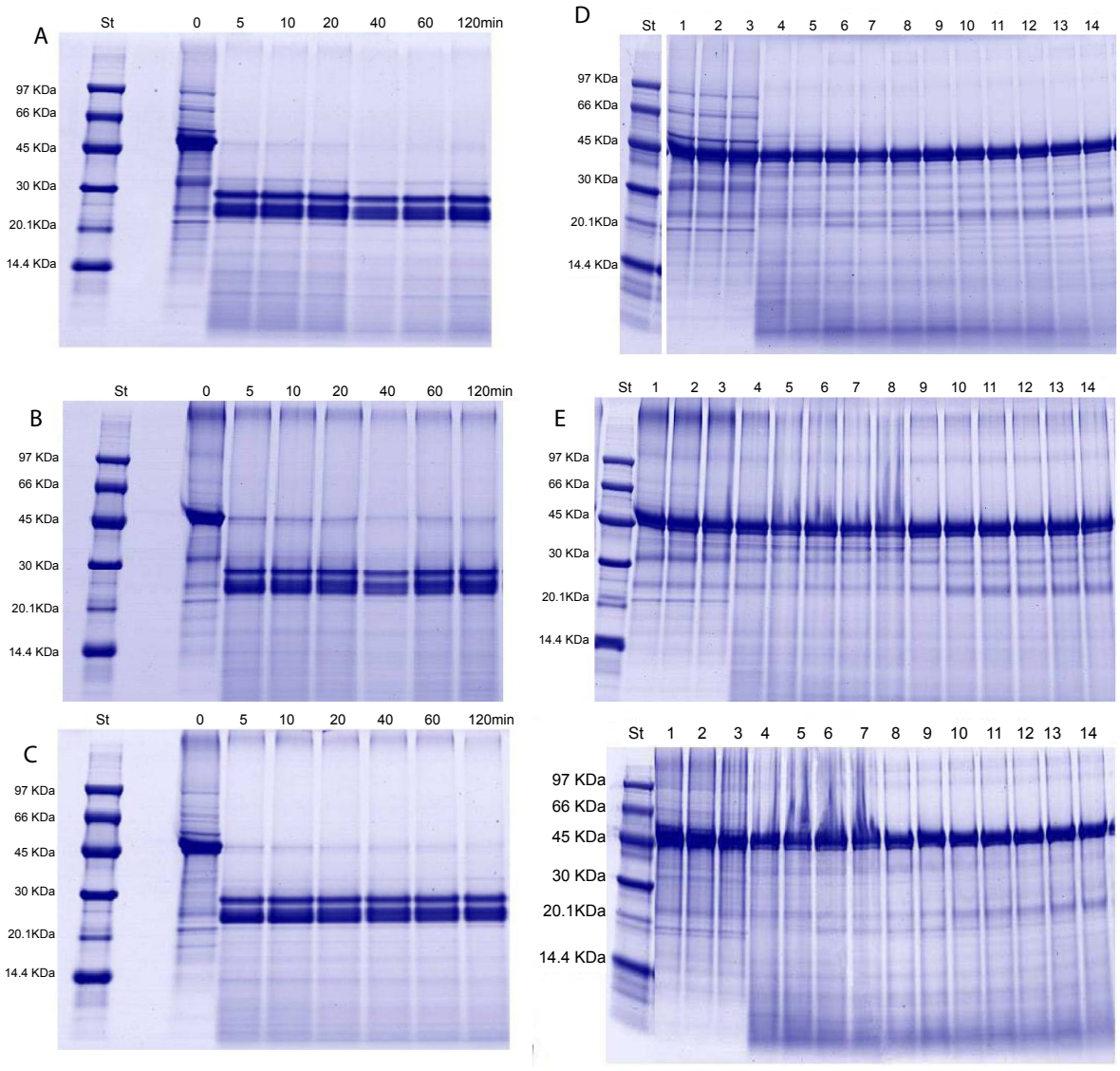

Figure 3: SDS-PAGE of bean protein hydrolysates after different time intervals of trypsin hydrolysis (a-navy, b-black, and c-small red); and *in vitro* gastrointestinal simulation (GIS) digestion hydrolysates (e-navy, f-black, and g-small red) under reducing conditions (5% β-ME): lane 1: 0 min; lane 2: 3 min after α-amylase digestion; lanes 3 to 8: 0 min, 5 min, 10 min, 30 min, 60 min, 120 min of peptic digestion, respectively; lanes 9 to 14: 0 min, 5 min, 10 min, 30 min, 60 min, 150 min of trypsin/α-chymotrypsin digestion, respectively.

inhibitory peptides from the intact proteins. All investigated dry beans from both digestion treatments demonstrated comparable *in vitro* ACE inhibitory activities to some pulses hydrolysates, such as chickpea protein GIS digests (IC_{50}:140 – 229 μg/mL) [5], and pea protein GIS digests (IC_{50}: 70 – 159 μg/mL) [5,7]. The ACE inhibitory activities were, on the other hand, less than those reported for lentil protein trypsin digests (IC_{50}: 111 μg/mL) and GIS digests (IC_{50}: 53 – 90 μg/mL) [8,20].

Different enzymatic treatments had varying impacts on the hydrolysate profiles and the ACE inhibitory properties of the dry beans. The SDS-PAGE patterns indicated trypsinolysis of phaseolin occurred very quickly and produced peptides with MM half of the original sizes. This phenomenon, which is in agreement with other previous studies [21,22], may be explained by the presence of a hydrophilic region located near the center of phaseolin which is easily accessible for proteases to attack [21]. Interestingly, phaseolin was hardly degraded during GIS digestion probably due to the lower enzyme to substrate (E/S) ratios used in the GIS digestion, i.e. 1:250 compared to 1:25 used for trypsinolysis. Previous studies demonstrated minor degradation of phaseolin during trypsinolysis using an E/S ratio of 1:100; at a higher E/S ratio of 1:10 phaseolin was halved in 3 min [21,23]. Compared to trypsin digestion, the significantly higher DH values ($P < 0.05$) of the GIS digestion suggests that a larger number of small peptides were released during the GIS digestion. This was reflected in the improvement of the ACE inhibitory activities for all investigated dry bean samples after GIS treatment.

Of all the dry bean varieties, distinctive peptide profiles were observed for the cranberry and light red kidney bean hydrolysates. The SDS-PAGE profiles suggested that these two bean varieties contained 'T' type phaseolin, as categorized by Brown et al. [24], who demonstrated three phaseolin types, namely, 'S', 'T', and 'C' after the cultivars Sanilac, Tendergreen, and Contender, respectively. The other seven investigated bean varieties showed 'S' type phaseolin. It appears, therefore, that dry bean proteins with 'T' type phaseolin had higher proteolytic susceptibility to both digestion treatments, based on the high DH values obtained for cranberry and light red kidney bean hydrolysates as well as the intensive bands observed on the SDS-PAGE profiles at the lower MM range. Digestibility differences between various types of phaseolin were reported recently [25]. The study showed that 'T' type phaseolin had higher DH values than 'S' type phaseolin. Interestingly, the cranberry and light red kidney bean hydrolysates obtained from both digestion treatments did not yield significantly higher ACE inhibitory activities compared to the other seven varieties, even though they seemed to contain more small peptides.

Black bean was particularly different among the samples. DH value of black bean hydrolysates from GIS digestion was 5.4 times higher than that of the trypsin digestion, which was the highest among all the samples. Moreover, the differences between IC_{50} values from the two digestions were also the highest for black bean, i.e. 2.7 times. This observation might be linked to the more intensive degradation of large protein aggregate in black bean after GIS digestion and the consequent liberation of larger quantities of small peptides as shown on the SDS-PAGE profiles.

A comparison between the digestibility and ACE inhibitory activity values of the dry bean varieties showed no obvious relationship. As an example, small red bean, which had the highest ACE inhibitory activities, had average DH values and similar SDS-PAGE profiles compared to the other varieties for both digestion treatments. Peptides must meet several criteria (e.g., shape, molecular mass, hydrophobicity, charge and electronic properties) in order to yield ACE inhibitory properties [26]. They are expected to be short, i.e. 2 – 12 amino acids [26] and are more likely to have hydrophobic amino acids as C-terminal residue, such as tryptophan, tyrosine, phenylalanine and proline [27]. Thus, the ACE inhibitory activities of the dry bean hydrolysates analysed in this study may have been influenced not only by the extent of hydrolysis but also other factors, such as parent protein composition, structure, sequence and enzymatic digestion mechanisms [5] which opens up avenues for further studies.

In conclusion, the current study investigated for the first time the *in vitro* ACE inhibitory properties of protein hydrolysates obtained from nine largely grown Canadian *Phaseolus vulgaris* bean varieties. We demonstrated that: a) all investigated bean varieties had ACE inhibitory activities when subjected to both trypsin and *in vitro* GIS digestion treatments; b) all investigated varieties showed significantly higher ACE inhibitory activities after GIS digestion than after trypsinolysis. Additionally, the interesting findings for cranberry bean and light red kidney bean hydrolysates opens up possibilities for future studies about the relationships between phaseolin types and their bio-functional properties. More specifically, navy bean and small red bean which exerted the highest ACE inhibitory activity would be ideal targets for future studies related to ACE inhibitory properties. Overall, this manuscript provided novel fundamental knowledge regarding the ACE inhibitory activity of dry bean and could prove useful for future applications (e.g., incorporation of dry bean protein hydrolysates from specific bean varieties in functional foods targeting hypertension).

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References

1. International Society of Hypertension (2010) Healthy weight, healthy blood pressure.
2. Yang HY, Erdös EG, Levin Y (1970) A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. Biochim Biophys Acta 214: 374–376.
3. Pedroche J, Yust MM, Girón-Calle J, Alaiz M, Millán F, et al. (2002) Utilisation of chickpea protein isolates for production of peptides with angiotensin I-converting enzyme (ACE)-inhibitory activity. J Sci Food Agri 82: 960-965.
4. Yust MM, Pedroche J, Girón-Calle J, Alaiz M, Millán F, et al. (2003) Production of ace inhibitory peptides by digestion of chickpea legumin with alcalase. Food Chem 81: 363-369.
5. Barbana C, Boye JI (2010) Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates. Food Res Int 43: 1642-1649.
6. Vermeirssen V, Van der Bent A, Van Camp J, Van Amerongen A, Verstraete W (2004) A quantitative in silico analysis calculates the angiotensin I converting enzyme (ACE) inhibitory activity in pea and whey protein digests. Biochimie 86: 231-239.
7. Vermeirssen V, Van Camp J, Verstraete W (2005) Fractionation of angiotensin I converting enzyme inhibitory activity from pea and whey protein in vitro gastrointestinal digests. J Sci Food Agri 85: 399-405.
8. Boye JI, Roufik S, Pesta N, Barbana C (2010) Angiotensin I-converting enzyme inhibitory properties and SDS-PAGE of red lentil protein hydrolysates. LWT - Food Sci Technol 43: 987-991.
9. Akıllıoğlu H, Karakaya S (2009) Effects of heat treatment and in vitro digestion on the Angiotensin converting enzyme inhibitory activity of some legume species. Eur Food Res and Technol 229: 915-921.
10. Torruco-Uco J, Chel-Guerrero L, Martínez-Ayala A, Dávila-Ortíz G, Betancur-Ancona D (2009) Angiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from *Phaseolus lunatus* and *Phaseolus vulgaris* seeds. LWT - Food Sci Technol 42: 1597-1604.
11. De Shpande SS, Nielsen SS (1987) In vitro enzymatic hydrolysis of phaseolin, the major storage protein of Phaseolus vulgaris L. J Food Sci 52: 1326-1329.

12. Rui X, Boye JI, Ribereau S, Simpson BK, Prasher SO (2011) Comparative study of the composition and thermal properties of protein isolates prepared from nine Phaseolus vulgaris legume varieties. Food Res Int 44: 2497-2504.

13. Hernández-Ledesma B, Quirós A, Amigo L, Recio I (2007) Identification of bioactive peptides after digestion of human milk and infant formula with pepsin and pancreatin. Int Dairy J 17: 42-49.

14. AOAC International (1995) Official methods of analysis. Washington, DC: AOAC.

15. Hsu HW, Vavak DL, Satterlee LD, Miller GA (1977) A multienzyme technique for estimating protein digestibility. J Food Sci 42: 1269-1273.

16. Adler-Nissen J (1979) Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. J Agri Food Chem 27: 1256-1262.

17. Sathe SK (2002) Dry bean protein functionality. Crit Rev Biotechnol 22: 175-223.

18. Liener IE, Thompson RM (1980) In vitro and in vivo studies on the digestibility of the major storage protein of the navy bean (Phaseolus vulgaris). Plant Foods for Hum Nutr 30: 13-25.

19. Momma M (2006) A pepsin-resistant 20 kDa protein found in red kidney bean (Phaseolus vulgaris L.) identified as basic subunit of legumin. Biosci Biotechnol Biochem 70: 3058-3061.

20. Barbana C, Boye JI (2011) Angiotensin I-converting enzyme inhibitory properties of lentil protein hydrolysates: Determination of the kinetics of inhibition. Food Chem 127: 94-101.

21. Nielsen SS, Deshpande SS, Hermodson MA, Scott MP (1988) Comparative digestibility of legume storage proteins. J Agri Food Chem 36: 896-902.

22. Romero J, Ryan DS (1978) Susceptibility of the major storage protein of the bean, Phaseolus vulgaris L., to in vitro enzymic hydrolysis. J Agri Food Chem 26: 784-788.

23. Deshpande SS, Damodaran S (1989) Structure-digestibility relationship of legume 7S proteins. J Food Sci 54: 108-113.

24. Brown JWS, Ma Y, Bliss FA, Hall TC (1981) Genetic variation in the subunits of globulin-1 storage protein of French bean. TAG-Theor Appl Genet 59: 83-88.

25. Montoya CA, Lallè JP, Beebe S, Souffrant WB, Leterme P (2005) Proceedings of the Fourth International Food Legumes Research Conference. Kamala Printn-publish, New Delhi, India.

26. Hernández-Ledesma B, del Mar Contreras M, Recio I (2011) Antihypertensive peptides: Production, bioavailability and incorporation into foods. Adv Colloid Interface Sci 165: 23-35.

27. Cheung HS, Wang FL, Ondetti MA, Sabo EF, Cushman DW (1980) Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. Importance of the COOH-terminal dipeptide sequence. J Biol Chem 255: 401-407.