ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 3, 14 SECTIO DDD 2010

Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Poland

MONIKA KARAŚ, ANNA JAKUBCZYK, BARBARA BARANIAK

Antiradical and antihypertensive activity of peptides obtained from proteins pea sprouts (Pisum sativum) by enzymatic hydrolysis

Antyrodnikowa i antynadciśnieniowa aktywność peptydów otrzymanych w wyniku enzymatycznej hydrolizy białek kiełków grochu *(Pisum sativum)*

Legume protein may be useful in human food nutrition as a source of bioactive peptides with antiradical and antihypertensive properties. Numerous scientific studies have shown that sprouts are a group of plant products with important disease preventive and health protective properties. Therefore, in recent years sprouts have become increasingly significant for consumers who value a healthy lifestyle [6]. Lipids, carbohydrates and storage proteins during germination are broken down to smaller molecules and become more digestible and consequently more accessible nutrients [16]. Sprouts include a lot of bioactive compounds such as vitamins B_1 and B_2, proteins and fiber content [3].

Angiotensin I-converting enzyme (ACE, dipeptidyl carboxpeptidase, EC 3.4.15.1) is an important enzyme involved in blood pressure regulation and in the electrolyte and fluid balance. ACE converts the inactive form of angiotensin I (decapeptide) into potent angiotensin II (octapeptide) vasoconstricting, but on the other hand, it also inactivates the antihypertensive vasodilator bradykinin [11]. As a consequence, ACE-inhibitory substances are often used in the treatment of blood pressure and hypertensive decrease. ACE inhibitor drugs, such as captopril, benazepril, and enalapril, are the first-line therapy for hypertension but they can cause serious side effects such as cough, angioedema, taste disturbances and skin rashes [12]. Therefore, scientists have carried out extensive research in the area of methods for the separation, purification and characterization of natural sources of ACE inhibitors. Many ACE inhibitory peptides are released *in vitro* from animal or plant proteins such as casein [14], fish proteins [10] and mung bean protein [7]. On the other hand, a common source of many diseases can be free radicals. These are atoms or groups of atoms with released electrons aiming at stability, which may be achieved by their coupling. They may react with proteins, lipids or DNA and consequently cause metabolism disorder. Antioxidants are chemical or natural compounds, capable of inhibiting oxidative reactions and therefore protecting human organism and food ingredients from the destructive activity of free radicals [17]. Interest in identifying foods as natural sources of antiradical compounds has increased. Several studies have proved that certain

cereal products also have antiradical activity [2]. Many of these compounds, in particular peptides, were released after gastrointestinal enzyme digestion or by *in vitro* enzymatic hydrolysis.

The aim of this work was to determine antiradical and antihypertensive activity of peptides derived from pea sprouts.

MATERIAL AND METHODS

The materials were 5-days pea sprouts (*Pisum sativum* var. Bajka). Raw pea sprouts were selected and cleaned to remove contaminants. The enzymes of pea sprouts were inactivated by heating for 15 min at 100 °C.

Preparation of protein isolate (PSPI). The pea sprouts protein isolate was prepared according to the process described by Magias with minor modifications [8].

Preparation of pea sprouts protein hydrolysate (PSPH). The 4 % (w/v) protein isolate solution was prepared and hydrolyzed with trypsin for 2 h (in optimal condition). Hydrolysis was carried out using the following hydrolysis parameters: enzyme–substrate ratio 1:20; temperature at 37 °C; pH 8.0. Hydrolysis was stopped by heat treatment at 100 °C for 10 min. Hydrolysate was clarified by centrifuging at 8000 rpm for 20 min at 4 °C to remove insoluble substrate fragments and residual enzyme. The supernatant containing the peptides was collected for further fractionation. The hydrolysate was then frozen and stored at -20 °C before further analysis.

The peptides concentration was measured by TNBS (trinitrobenzene sulfonic acid) method by Adler-Nissen [1]. The protein hydrolysate was used for purification and determination of ACE inhibitory and antiradical activity of peptides.

Fractionation of PSPH with DEAE - cellulose and Sephadx G – 15 gel filtration. The PSPH was separated by ion exchange chromatography on DEAE cellulose column (linear gradient of NaCl from 0 to 0.5 M). Fractions (2 ml each) were collected at a flow rate of 0.8 ml/h, and absorbance was measured at 220 nm to determine the elution profile of the sample. Fractions associated with each peak showing antioxidant and ACE inhibitory activity were pooled and evaporated under vacuum. The fraction exhibiting the highest antiradical and ACE inhibitory activity was purified by gel filtration using Sephadex G-15 with 0.1 mM borate buffer solution (pH 8.3) (flow rate: 0.8 ml/min). In the fractions the antiradical and ACE inhibitory activity of peptides was determined.

ABTS radical – scavenging activity assay. The scavenging activity against the ABTS radical [2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was determined by the decolourisation assay [9]. The ABTS scavenging activity was expressed as the scavenging percent.

ACE – inhibitory activity assay. The method used for determining ACE-inhibitory activity was that described by Hollenberg with minor modifications [4]. The ACE-inhibitory activity was expressed as IC_{50} value, which was defined as the peptides concentration (mg/ml) required for half-scavenging.

RESULTS

The concentration of peptides in the PSPI was 4.2 mg/ml, after 2 h of digestion increased to 48.88 mg/ml. The results of ACE inhibitory and antiradical activity of peptides are shown in Table 1. The sprouts pea protein hydrolysate obtained by trypsin digestion was separated by ion exchange chromatography. The hydrolysate was fractionated into eight individual fractions (Fig.1). The values of ACE inhibitory and antiradical activity of peptides in separated fractions are presented in Table 2. The highest activity was determined in the first fraction (IC_{50} = 0.24 mg/ml for ACE inhibitory and 100 % free radical scavenging activity – 0.2 mg/ml peptides concentration). This fraction was further purified by gel filtration chromatography using Sephadex G-15. After separation, twenty-one individual fractions were collected (Fig.2c). In all the fractions ACE inhibitory and antiradical activity of peptides were measured (Fig.2a, b). The highest ACE inhibitory activity was noted in the twelfth fraction and antiradical in the fourth fraction (IC_{50} = 0.007 mg/ml and 28.68 %, respectively).

Tab. 1. Peptides concentration, IC_{50} value of ACE inhibitory activity (mg/ml) and antiradical activity (%) in PSPH and PSPI

Pea sprouts proteins	Peptides concentration (mg/ml)	IC_{50} value of ACE inhibitory activity (mg/ml)	Antiradical activity (%) peptides concentration 0.2mg/ml
PSPI	4.20	10.72	20.37
PSPH	48.88	3.44	98.29

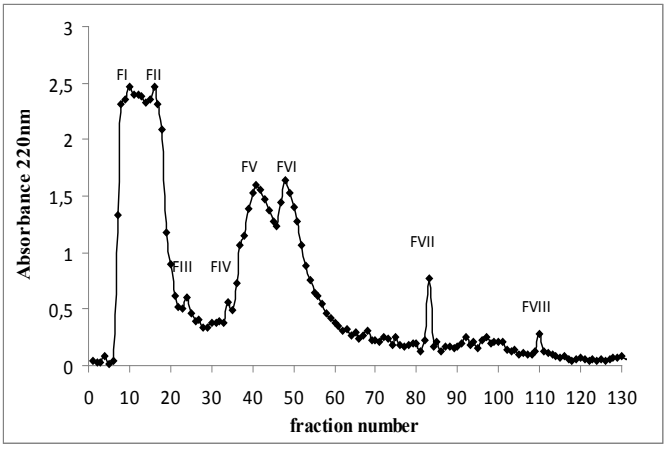

Fig. 1. Ion exchange gel chromatography profile of the trypsin hydrolysate of pea sprouts protein on DEAE-cellulose column

Tab. 2. Peptides concentration, IC_{50} value of ACE inhibitory activity (mg/ml) and antiradical activity (%) in fractions obtained after separated PSPH using DEAE cellulose

Fraction number	Peptides concentration (mg/ml)	IC_{50} value of ACE inhibitory activity (mg/ml)	Antiradical activity (%) peptides concentration 0.2mg/ml
I (7-14)	1.82	0.24	100
II (15-22)	9.40	0.85	53.98
III (23-26)	4.76	0.37	54.12
IV (33-35)	2.36	not noted	49.93
V (36-45)	1.05	not noted	51.99
VI (46-58)	2.14	0.53	51.35
VII (81-86)	1.40	0.25	50.14
VIII (109-111)	0.58	not noted	51.14

DISCUSSION

RADICAL-SCAVENGING ACTIVITY

In this experiment the obtained values of free radicals scavenging activities were 20.37 %, 98.29 % for protein isolate and hydrolysates, respectively. This result is compared with Pihlanto et al. a study by where antiradical activity increased from 5.6 % to 89.4 % for potato products after 5 h hydrolysis [9]. According to Yokomizo et al., the antioxidative activity of the hydrolysates with seven different proteases was measured. In each experiment the antiradical activity of all hydrolysates increased with hydrolysis time [17]. These results suggest that enzyme hydrolysis improves biological activity.

ACE- INHIBITORY PEPTIDE ACTIVITY

In this experiment the IC_{50} value of ACE inhibitory activity for PSPI and PSHI was 10.72 and 3.44 mg/ml, respectively. ACE inhibitory activity was found in whey and pea hydrolysates by Vermeirssen et al. and the results show that IC_{50} was 0.048 and 0.076 mg/ml, respectively [15]. According to Li et al., the alcalase mung bean protein hydrolysate showed ACE inhibitory activity with the IC_{50} value of 0.62 mg/ml [7]. However, Kuba et al. obtained IC_{50} 0.34 mg/ml for soy protein hydrolysates [5]. Many *peptides* with ACE inhibitory activity *were isolated* from fish protein hydrolysates, milk and derived products such as cheese [10, 13]. The research carried out by Srinivas and Prakash demonstrate that the IC_{50} value for α-casein protein after hydrolysis with chymotrypsin was 0.1 mg/ml [14]. These results suggest that peptides can be used as a protective substance for hypertensive patients.

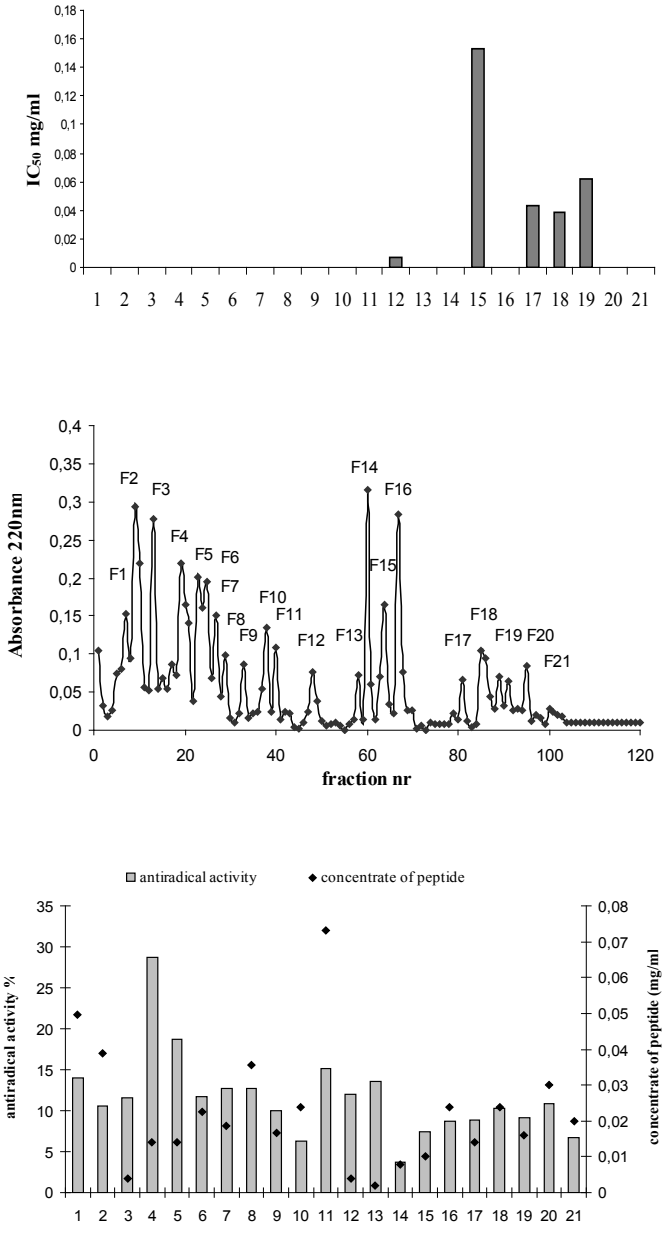

Fig. 2. Gel filtration profile of pea sprouts protein hydrolysates (c), peptides concentration and antiradical activity percent (a) and ACE inhibition activity (b) of all fractions

CONCLUSIONS

Results of this study imply that pea sprouts proteins hydrolysates may be a source of bioactive compounds with ACE-inhibitory and antioxidant activity. The bioactivities of protein hydrolysates were most likely related to peptides and/or free amino acids liberated during digestion. Therefore, pea sprouts proteins are a promising source for the production of bioactive compounds for functional foods and diet supplements that may be beneficial to human health.

REFERENCES

1. Adler-Nissen J.: Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenosulfonic acid. J. Agric. Food Chem., 27, 1256, 1979
2. Baublis A. et al.: Potential of wheat-based breakfast cereals as a source of dietary antioxidants. Journal of American College of Nutrition, 19, 308, 2000
3. Gopalan C. et al.: Nutritive Values of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad (India) 2004.
4. Hollenberg N.: Treatment of hypertension: the place of angiotensin-converting enzyme inhibitors in the nineties. J. Cardiovasc. Pharmacol., 20, Suppl. 10, 29, 1992
5. Kuba M. et al.: Production of angiotensin I-converting enzyme inhibitory peptides from soybean protein with *Monascus purpureus* acid proteinase. Process Biochemistry, 40, 2191, 2005
6. Kurtzweil P.: Questions keep sprouting about sprouts. FDA Consumer Rep, 33, 18 1999
7. Li G-H et al.: Novel angiotensin I-converting enzyme inhibitory peptides isolated from alcalase hydrolysate of mung bean protein. J. Pept. Sci, 12, 509, 2006
8. Megias C. et al.: Production of copper-chelating peptides after hydrolysis of sunflower proteins with pepsin and pancreatin. LWT - Food Science and Technology, 41, 1973 2008
9. Pihlanto A. et al.: ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chemistry, 109, 104, 2008
10. Raghavan S., Kristinsson H.: ACE-inhibitory activity of tilapia protein hydrolysates. Food Chemistry, 117, 582, 2009
11. Rho S. et al.: Purification and identification of an angiotensin I-converting enzyme inhibitory peptide from fermented soybean extract. Process Biochemistry, 44, 490 2009
12. Sica D.: Angiotensin-converting enzyme inhibitors. in Hypertension Primer, 3rd ed.; Izzo, J. L., Black, H. R., Goodfriend,T. L., Sowers, J. R. et al. Eds. Lippincott Williams & Wilkins: Baltimore, MD, 426, 2003
13. Sieber R. et al.: ACE-inhibitory activity and ACE-inhibiting peptides in different cheese varieties, Dairy Sci. Technol. , 90, 47, 2010
14. Srinivas S., Prakash V.: Bioactive peptides from bovine milk *a*-casein: isolation, characterization and multifunctional properties. Int. J. Pept. Res. Ther., 16, 7, 2010
15. Vermeirssen V. et al.: Fractionation of angiotensin I converting enzyme inhibitory activity from pea and whey protein in vitro gastrointestinal digests. Journal of the Science of Food and Agriculture, 85, 399, 2005

16. Vidal-Valverde C. et al.: New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. European Food Research and Technology, 215, 472, 2002
17. Yokomizo A. et al.: Antioxidative activity of peptides prepared from okara protein. Food Sci.Technol. Res., 8, 357, 2002

SUMMARY

Pea sprouts are a promising source for the production of bioactive compounds that can be beneficial to human health. In this study the peptides were obtained from trypsin hydrolysate from pea sprouts proteins with antiradical and antihypertensive activity. $ABTS^{+}$ was used to measure the antiradical capacities, and ACE (angiotensin I-converting enzyme) inhibitory activity of peptides was determined. The protein hydrolysate was fractionated by ion exchange chromatography on DEAE cellulose. The highest activity was noted in the fist fraction (100 % antiradical activity for 0.2 mg/ml peptides concentration and IC_{50} value of 0.24 mg/ml for ACE inhibitory activity). Further, this fraction was purified by Sephadex G-15 where the highest antiradical activity was measured in the fourth fraction (28.68 %) and ACE inhibitory activity of peptides was IC_{50} 0.007 mg/ml in the twelfth fraction. Results of this study indicate that enzymatic hydrolysates of pea sprouts protein possess potent antioxidative and antihypertensive activity.

Keywords: pea sprouts, peptide, ACE inhibitory, ABTS

STRESZCZENIE

Kiełki grochu stanowią potencjalne źródło bioaktywnych składników, które mogą pozytywnie wpływać na zdrowie człowieka. W pracy badano aktywność przeciwrodnikową i przeciwnadciśnieniową peptydów otrzymanych w wyniku trypsynowej hydrolizy białek kiełków grochu. Określono aktywność przeciwrodnikową wobec $ABTS^{+}$ oraz przeciwnadciśnieniową peptydów hamujących ACE (enzym konwertujący angiotensynę I). Hydrolizat białkowy poddano rozdziałowi przy użyciu chromatografii jonowymiennej na kolumnie DEAE-celuloza. Najwyższą aktywność przeciwrodnikową (100 % przy stężeniu peptydów 0.2 mg/ml) i inhibitującą ACE (IC_{50} 0,24 mg/ml) uzyskano w pierwszej peptydowej frakcji. Następnie frakcja ta została poddana rozdziałowi na Sephadexie G-15, gdzie największą aktywność przeciwrodnikową (28,68 %) zanotowano we frakcji czwartej, natomiast inhibitującą ACE (IC_{50} 0,007 mg/ml) w dwunastej. Na podstawie przeprowadzonych badań można stwierdzić, że w wyniku enzymatycznej hydrolizy białek kiełków grochu uwalniane są peptydy wykazujące aktywność przeciwrodnikową i przeciwnadciśnieniową.

Słowa kluczowe: kiełki grochu, peptydy, inhibitory ACE, ABTS