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Antihypertensive and antioxidative activity of peptides derived from pea sprouts (Pisum sativum) protein hydrolysates

Hipotensyjna i antyoksydacyjna aktywność peptydów pochodzących
z hydrolizatu białek kiełków grochu *(Pisum sativum)*

INTRODUCTION

Nowadays the production of high quality, healthy food that requires the application of raw materials containing bioactive substances, has became subject of interest of researchers and consumers. The peptides, which can be present on the food or release after enzymatic hydrolysis, as a bioactive food compound, may have preventive influence on our cardiovascular system and many degenerative diseases, such as cancers, atherosclerosis and diabetes [10]. On the other hand, the seeds of leguminous plants are the particular source of protein in the diet, due to their amino acid composition being similar to animal protein, carbohydrates (e.g. fibre, resistant starch and oligosaccharides), vitamins and minerals [5].

Angiotensin converting enzyme I (ACE, peptidyl dipeptide hydrolase EC 3.4.15.1) plays important role in rennin-angiotensin-aldosteron system (RAAS), involve in cardiovascular system regulation. This enzyme is responsible for conversion of angiotensin I to angiotensin II, which reveals a strong influence contracting the muscle membrane of blood vessels, which results in a blood pressure increase. Hypertension is the most common risk factor in heart diseases development and, unfortunately, is called "silent killer", because it shows no symptoms for years [7]. In recent years, the interest in natural compounds of plant origin (such as revealing ACE activity inhibition properties) has been growing, in view of the fact that numerous synthetic inhibitors often bring about serious side effects resulting in disorders of the alimentary, respiratory or nervous system. It has been shown that reactive oxygen species (ROS) and free radicals play an important role in many degenerative diseases, such as cancers, atherosclerosis and diabetes [4]. The creation of free radicals is an unavoidable consequence of the respiration of aerobic organisms. These radicals are very unstable and can quickly react with other groups or substances in the body, resulting in damage to tissues or cells. ROS results from disturbances between the production of free oxygen radicals and ability of cells to eliminate them [14]. The body has its own systems of protection against ROS, which include antioxidative enzymes (enzymatic system)

and antioxidative low molecular weight compounds (non-enzymatic system). Such compounds include peptides, which reduce the rate of the lipid autoxidation and lower the content of peroxide forms of fatty acids in food. They also limit the amount of free radicals by fulfilling the function of a peroxide decomposition promoter and by displaying the ability to bind heavy metals [9].

In this study protein hydrolysates derived from pea sprouts was a source of antioxidative and ACE (angiotensin I-converting enzyme) inhibitory peptides.

MATERIALS AND METHODS

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

Preparation of pea sprouts protein isolate (PSPI) and hydrolysate (PSPH)

The pea sprouts protein isolate was prepared according to the process described by Megias with minor modifications [12].

Protein hydrolysis was produced from the 4% protein isolate solution by using pepsin (enzyme-substrate ratio 1:20) under standard conditions (temperature at 37°C; pH 2.0) during 2h. Hydrolysis was stopped by heating to 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 frozen and stored at -20°C before further analysis.

The protein hydrolysate was used for purification and determination of antioxidative and ACE inhibitory activity of peptides.

Purification and determination of peptides with the highest biological activity

The PSPH was separated by chromatography on DEAE cellulose (linear gradient of NaCl from 0 to 0.5 M). Fractions (2ml 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 inhibitor activity was purified using a Sephadex G-10 gel filtration resin (flow rate: 0.8 ml/min). In the fractions the antioxidative and ACE inhibitor activity peptides were determined.

The peptides concentration was measured by TNBS (trinitrobenzene sulfonic acid) method by Adler-Nissen [1].

Antioxidant activity assay

The antiradical activity was determined by radical cation *2,2'-azino-bis*(*3-ethylbenzthiazoline-6 sulphonic acid*) ($ABTS^{\bullet+}$) decolorization assay [13]. The antiradical activity was expressed as the scavenging percent.

Metal ions chelating activity was measured according to the method described by Decker and Welch, with minor modification [6].

ACE-inhibitory activity assay

The ACE-inhibitory activity was determined following the method described by Hollenberg with minor modifications [8]. 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 pea sprouts protein isolate (PSPI) used for the generation of protein hydrolysates was obtained by alkaline extraction and precipitation of proteins at their pI. Before pepsin proteolysis, the pea protein solution showed a lower peptides content 4.20 mg/ml, while the IC_{50} value of ACE inhibitory activity was 10.72 mg/ml, antioxidant activity was 20.37% against $ABTS^{\bullet+}$ and 7.72% ability to chelating of Fe^{2+} (Tab.1). During digestion, the peptides content was increased slightly to 21.86 mg/ml, the IC_{50} value of ACE inhibitory activity was 1.59 mg/ml and antiradical activity and ability to chelating of Fe^{2+}: 99.57% and 84.35%, respectively (Tab.1). The pea sprouts protein hydrolysates were separated by DEAE cellulose chromatography (Fig.1). The eight fractions were collected and concentrated in a vacuum concentrator. The ACE inhibitory and antioxidant activity of each fraction was measured (Fig.2) Sample with highest hypotension activity was fractionated using Sephadex G10 resin (Fig.3). The experimental results are shown in Tab.3.

Table 1. Peptides concentration, IC_{50} value of ACE inhibitory activity (mg/ml), antioxidant activity (%) in pea sprouts protein isolate (PSPI) and hydrolysate (PSPH)

Pea sprouts proteins	Peptides concentration (mg/ml)	IC_{50} value of ACE inhibitory activity (mg/ml)	Antiradical activity (%), peptides concentration 0.2mg/ml	Iron(II)chelating ability (%), peptides concentration 0.2mg/ml
PSPI	4.20	10.72	20.37	7.72
PSPH	21.86	1.59	99.57	84.35

Table 2. Peptides concentration, IC_{50} value of ACE inhibitory activity (mg/ml) and antioxidant activity (%) in sample obtained after separation of sixth fraction on Sephadex G10

peptides concentration	0.80 mg/ml
IC_{50} value of ACE inhibitory activity	0.69 mg/ml
antiradical activity	60.95%
iron(II)-chelating ability	75.38%

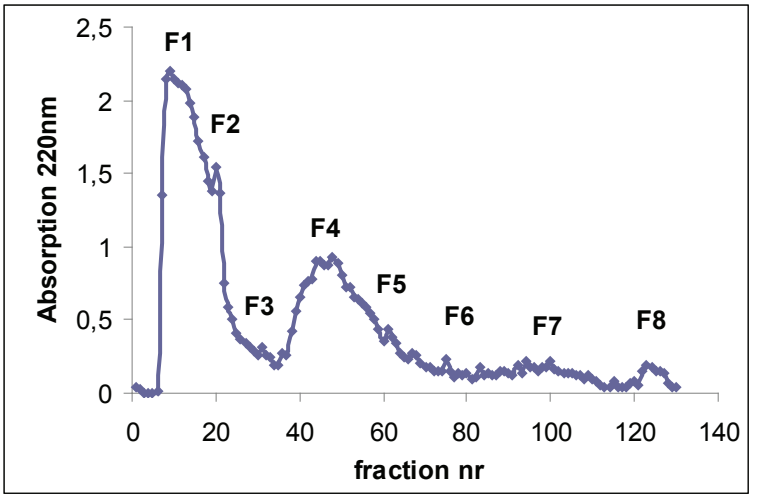

Fig.1. Ion exchange chromatography profile of the pepsin hydrolysate of pea sprouts protein on DEAE-cellulose column

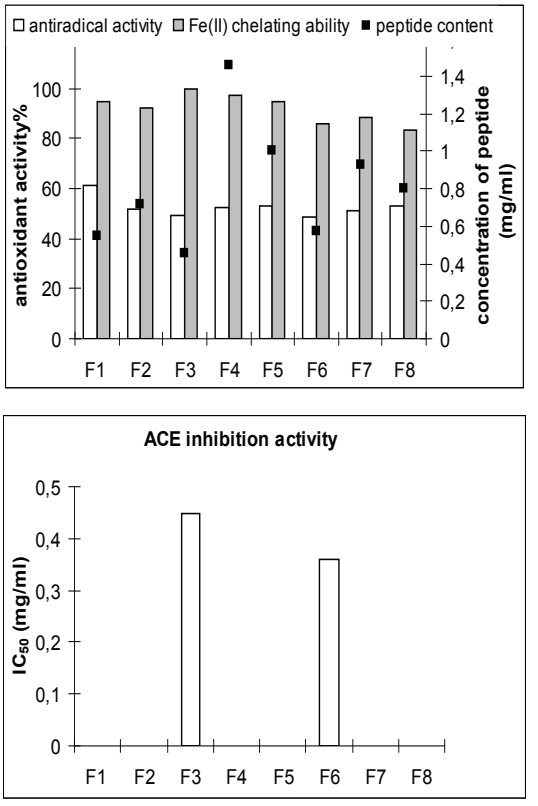

Fig. 2. Peptides concentration, antioxidant activity (%), IC_{50} value of ACE inhibitory activity (mg/ml) in fractions obtained after separated PSPH on DEAE cellulose

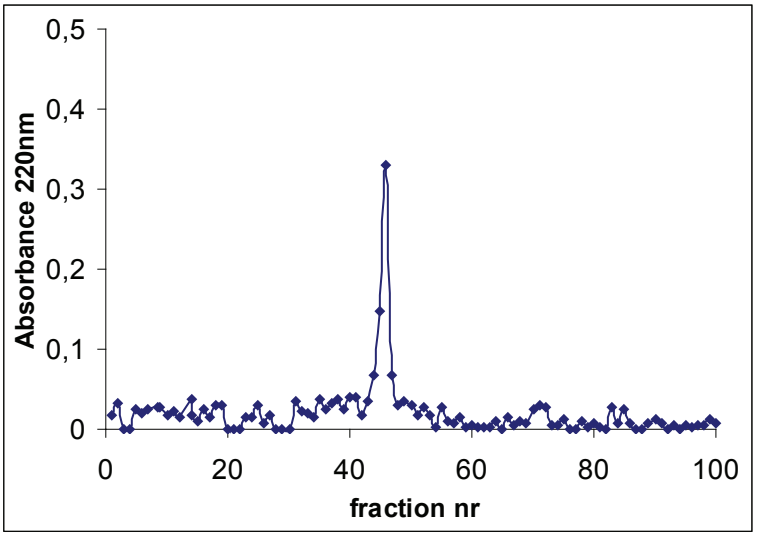

Fig. 3. Gel filtration profile of sixth fraction on Sephadex G10

DISCUSSION

During digestion of pea sprouts proteins, the peptides content slightly increased. Legume proteins are only partly hydrolysed because of the inability of most proteases to cleave phosphoproteins, glycoproteins, other post-translationally modified species, or domains that contain a higher number of disulfide bridges [3]. The biologically peptides activity depend on the degree of protein hydrolysis. The pea sprouts protein inhibited ACE activity and the IC_{50} value was obtained 10.72 mg/ml and after pepsin digestion hydrolysates showed the value of inhibition as IC_{50}=1.59 mg/ml. According to Akıllıoglu and Karakaya study, the ACE inhibitory activity of the peptide samples was increased during pepsin digestion of proteins. The highest ACE peptides inhibitory activity was observed for green lentil hydrolysates where after 50 min of digestion the IC_{50} value was determined as 0.008 mg/ml [2]. On the other hand, Marczak et al. reported that pepsin digestion has influence on hypertensive pepsin hydrolysates obtained from rapeseed proteins and this enzyme cased the highest ACE inhibitory activity and the value of IC_{50} after pepsin, pancreatin and pepsin-pancreatin was demonstrated as 0.16, 1.3 and 0.7 mg/ml, respectively [11].

In this study radical activity was inhibited around 20 and 99% for pea sprouts protein isolates and hydrolysates, respectively and it is compared to results obtained by Philanto et. al. - antiradical activity changed from 5.6 to 89.4% [13]. According to Zhang et al. the Fe^{2+} chelating was estimated to be 63.08% of the purified peptide obtained from chickpea protein hydrolysates [16]. This result is compared to our study where the Fe^{2+} chelating capability in purified fraction was demonstrated at 75.38 %. The purified peptides fraction exhibited ACE inhibitory activity – for 0.8 mg/ml the value of ion chelating was observed at 75.38%. But not only legume can cause ion chelating, on the other hand the alfalfa protein hydrolysates showed 65.15 % chelating effect for 0.8 mg/ml of peptides [15].

The correlation between the highest ACE inhibitory activity and antioxidative activity wasn't observed. This suggests that the biological activity of peptides may depend on the specific proteases used and the nature of the peptides released.

CONCLUSION

The pepsin hydrolysis of pea sprout protein enables production of low molecular weight products with ACE-inhibitory and antioxidative activity. These peptides can be promising bioactive compounds for functional foods aimed at preventing and/or treating lifestyle diseases, particularly hypertension.

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REFERENCE

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. Akıllıog˘lu H, Gul Ć, Karakaya S: Effects of heat treatment and in vitro digestion on the angiotensin converting enzyme inhibitory activity of some legume species. Eur Food Res. Technol., 229, 915, 2009.
3. Bamdad F et al.: The impact of germination and *in vitro* digestion on the formation of angiotensin converting enzyme (ACE) inhibitory peptides from lentil proteins compared to whey proteins. Int. J. Biol. Sci., 5, 2, 2009.
4. Beckman K, Ames B: The free radical theory of aging matures. Physiol. Rev., 78, 547, 1998.
5. Boye J et al. Ribereau S, Mondor M., Farnworth, E.: Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. Food Res. Internat., 43, 2, 537, 2010.
6. Decker E, Welch B: Role of ferritin as a lipid oxidation catalyst in muscle food. J. Agric. Food Chem., 38, 674, 1990.
7. Gao D et al.: Angiotensin I-converting enzyme inhibitor derived from cottonseed protein hydrolysates. Afr. J. Biotechnol., 53, 8977, 2010.
8. Hollenberg N.: Treatment of hypertension: the place of angiotensin-converting enzyme inhibitors in the nineties. J. Cardiovasc. Pharmacol., 20, 29, 1992.
9. Kansci G et al.: The antioxidant activity of carnosine and its consequences on the volatile profiles of liposomes during iron/ ascorbate induced phospholipid oxidation. Food Chem., 60, 165, 1995.
10. Mallikarjun G et al.: Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11S globulin of soybean (Glycine max). J Agric Food Chem., 54, 4568, 2006.
11. Marczak E et al.: New antihypertensive peptides isolated from rapeseed. Peptides, 24, 791, 2003.
12. Megias C et al.: Purification of an ACE inhibitory peptide after hydrolysis of sunflower (*Helianthus annuus* L.) protein isolates. J. Agric. Food Chem., 52, 1928, 2004.

13. Pihlanto A, Akkanen S, Korhonen H.: ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chem., 109, 104, 2008.
14. Sokołowska A, Polanowski A: Rodzina białek bogatych w prolinę – struktura, niektóre funkcje i perspektywy wykorzystania. Praca zbiorowa pod red. Koroniak H., Barciszewski J., Na pograniczu chemii i biologii, Wyd. UAM Poznań,16, (2005).
15. Xie Z et al.: Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysates. Food Chem. 111, 370, 2008.
16. Zhang T et al.: Purification and characterisation of a new antioxidant peptide from chickpea (*Cicer arietium* L.) protein hydrolysates. Food Chem., 28, 2011.

SUMMARY

Proteins of leguminous plants being a source of bioactive peptides characterized by antioxidant and blood pressure lowering properties, can contribute to the prevention of the development of many civilization diseases by inhibiting free radical reactions and delaying the process of cell aging. In this study antioxidative and ACE (angiotensin I-converting enzyme) inhibitory activity of peptides obtained from pepsin hydrolysis of proteins of pea sprouts, were studied. During hydrolysis, the peptides content increased slightly to 21.86 mg/ml, the IC_{50} value of ACE inhibitory activity was 1.59 mg/ml and antiradical activity and ability to chelating of Fe^{2+} was 99.57% and 84.35%, respectively. The pea sprouts protein hydrolysate was separated by DEAE cellulose. The highest hypotension activity was obtained in sixth fraction (IC_{50}-0.36mg/ml), which was fractionated using Sephadex G10 resin. The received fraction demonstrated inhibitory activity at 60.95 % with regard to $ABTS^{\bullet+}$, and ability to chelating of Fe^{2+} at 75.38%, while IC_{50} value of ACE inhibitory peptide was 0.69 mg/ml. In conclusion, pea sprouts are a source of bioactive peptides, which have antihypertensive and antioxidant properties, released in the process of their digestion using pepsin. This suggests that these hydrolysates can be used for production of new preparations having a favorable effect on human health or can be applied as additives to functional food increasingly popular among customers.

Keywords: peptides, antihypertensive properties, antioxidative properties, legumes

STRESZCZENIE

Białka roślin strączkowych są prekursorami bioaktywnych peptydów o przeciwnadciśnieniowych i antyoksydacyjnych właściwościach, które poprzez hamowanie reakcji wolnorodnikowych opóźniają starzenie się komórek i mogą mieć znaczenie w zapobieganiu rozwoju wielu chorób cywilizacyjnych. W niniejszej pracy zbadano właściwości przeciwutleniające oraz hamujące aktywność ACE (enzymu konwertującego angiotensynę I) peptydów otrzymanych w wyniku pepsynowej hydrolizy białek kiełków grochu (*Pisum sativum* odm. Bajka). Podczas hydrolizy zawartość peptydów wzrosła do 21.86 mg/ml, a wartość IC_{50} peptydowych inhibitorów ACE wynosiła 1.59 mg/ml, natomiast aktywność antyrodnikowa i zdolność do chelatowania jonów Fe^{2+} odpowiednio: 99.57% i 84.35%. Hydrolizat białek kiełków grochu poddano rozdziałowi na złożu DEAE-celuloza. Najwyższą aktywność przeciwnadciśnieniową uzyskano we frakcji szóstej (IC_{50} -0.36mg/ml), którą następnie

poddano rozdziałowi na złożu Sephadex G10. Otrzymana frakcja wykazywała 60.95 % inhibicji wobec $ABTS^{\bullet+}$ i 75.38% zdolności do chelatowania jonów Fe^{2+}, natomiast wartość IC_{50} peptydowego inhibitora ACE wyniosła 0.69 mg/ml. Białka kiełków grochu są potencjalnym źródłem biologicznie aktywnych peptydów o właściwościach przeciwnadciśnieniowych i przeciwutleniających, które uwalniane zostają podczas pepsynowej proteolizy. Tego typu hydrolizaty mogą zostać wykorzystane do produkcji nowych preparatów korzystnie wpływających na organizm ludzki lub jako dodatki do żywności funkcjonalnej, coraz bardziej popularnej wśród konsumentów.

Słowa kluczowe: peptydy, właściwości przeciwnadciśnieniowe, właściwości antyoksydacyjne, rośliny strączkowe