Evaluation of the ACE-Inhibitory Activity of Egg-White Proteins Degraded with Pepsin

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Key words: ovalbumin, hydrolysate, ACE-inhibitory peptide, egg-white proteins

 Increasing the potency of antihypertensive food-derived peptides is a critical and important step in the development of natural drugs for cardiovascular diseases prevention. We have proposed the egg-white protein precipitate (EWPP) obtained as a byproduct of cystatin and lysozyme isolation as a potential source of ACE-inhibitory peptides derived by pepsin digestion. The results indicated that hydrolysis of EWPP with pepsin produced the ACE inhibitory activity. During 3-h hydrolysis (DH: 38.3%), the IC₅₀ value of EWPP hydrolysate was significantly increased and finally reached IC₅₀ = 643.1 µg/mL. This hydrolysate was further fractionated by RP-HPLC. The peptide fraction exhibiting the highest ACE inhibitory activity was rechromatographed. Three peptide subfractions exhibiting ACE-inhibitory activities of 69.0, 25.0, and 37.6 µg/mL were further characterised. In each of them, mixtures of peptides with different molecular masses were observed.

INTRODUCTION

Biologically-active food-derived peptides are specific substances which exhibit a physiological effect in humans [Hartmann & Meisel, 2007; Erdmann et al., 2008]. They are inactive within the sequence of their parent protein and can be released by enzymatic hydrolysis in vitro during gastrointestinal digestion or ex vivo during food processing [Park et al., 2001; Erdmann et al., 2008]. A multitude of animal and plant proteins are sources of bioactive peptides, especially milk, egg, meat, soy, and rice proteins [Silva & Malcata, 2005; Sakana-ka & Tachibana, 2006; Saiga et al., 2003; Gibbs et al., 2004]. Biologically-active peptides exhibit diverse activities, including antimicrobial, immunomodulatory, antioxidative, mineral-binding, opiate-like, hypocholesterolemic, and antihypertensive actions. Many of them are multifunctional and can exert more than one biological effect [Davalô et al., 2004; Silva & Malcata, 2005; Hartmann & Meisel, 2007].

The World Health Organization estimates that by 2020, heart disease and stroke will have surpassed infectious diseases as the leading cause of death and disability worldwide [Erdmann et al., 2008]. Hypertension is one of the major risk factors for cardiovascular disease [Miguel et al., 2004]. Consequently, there has been an increased focus on finding effective antihypertensive agents. These include peptides displaying inhibitory activity against dipeptidyl-carboxypeptidase (angiotensin-converting enzyme or ACE, EC 3.4.15.1), which plays an important role in controlling the development of hypertension by regulating the rennin-angiotensin system. ACE is a drug target in the treatment of hypertension. It hydrolyzes angiotensin I into angiotensin II, which has a vasoconstrictive effect. Furthermore, ACE inactivates the vasodilator bradykinin, which may be involved in the control of blood pressure [Miguel et al., 2004].

Hen egg has traditionally been considered a source of highly valuable proteins in human nutrition. Nowadays it is also known as an important source of many bioactive substances which may find wide application in medicine and food production [Trziszka et al., 2006]. Many antihypertensive peptides derived from egg proteins by enzymatic hydrolysis have been described [Miguel et al., 2004; Sakana & Tachibana, 2006]. One of them is ovokinin, an octapeptide (Phe-Arg-Ala-Asp-His-Pro-Phe-Leu) isolated from the pepsin hydrolysate of ovalbumin. It has been shown that orally-administered ovokinin significantly lowered systolic blood pressure in spontaneously hypertensive rats (SHRs) [Fujita et al., 1995]. Furthermore, antihypertensive properties have also been observed for the ovokinin-derived peptide called ovokinin (2–7), a hexapeptide obtained by chymotrypsin digestion of ovalbu- min. Moreover, its synthetic analogs, such as Arg-Pro-Phe-His-Pro-Phe and Arg-Pro-Leu-Lys-Pro-Trp, also exhibited strong hypotensive activity in SHRs after oral administration [Matoba et al., 2001; Yamada et al., 2002]. Peptides identified in egg-white hydrolysate with pepsin, such as IVE, RADHP-FIL, and YAEERPIL, inhibit ACE in vitro and exhibit antihypertensive activity in SHRs at minimum effective doses of 2–4 mg/kg [Miguel et al., 2007]. The stimulated gastrointestinal digestion of RADHPFL and YAEERPIL yielding RADHP and YPI fragments exhibited a blood pressure-lowering effect [Miguel et al., 2007]. Antihypertensive activity was also dem-

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onstrated by peptides released from egg-white proteins by Alcalase [Liu et al., 2010; Zhipeng et al., 2011]. Among ovotransferrin-derived peptides, RVPSL peptide exhibited the highest level of ACE inhibitory activity of 20 µmol/L [Liu et al., 2010]. Zhipeng et al. [2011] also isolated three peptides from egg-white hydrolysate obtained with Alcalase. Only the peptide sequence QIGLF was a potential ACE inhibitor, with an IC50 value of 75 µmol/L. Moreover, QIGLF showed low gastrointestinal enzyme susceptibility and contained a relatively high amount of α-helix [Zhipeng et al., 2011].

The design of new health-promoting food products containing these bioactive peptides looks promising and would be attractive for consumers and producers [Miguel et al., 2007].

The aim of this study was to investigate the egg-white protein precipitate obtained as a byproduct of cystatin and lysozyme isolation as a potential source of ACE-inhibitory peptides derived by peptic digestion.

**MATERIALS AND METHODS**

**Materials**

Egg-white protein precipitate (EWPP) was obtained in our laboratory as a byproduct of lysozyme and cystatin isolation method according to Sokolowska et al. [2007]. EWPP was lyophilized and stored frozen until used. Porcine pepsin type A, trinitro-benzene sulfonic acid (TNBS), bovine serum albumin, angiotensin-converting enzyme (ACE) from rabbit lung, and hippuryl-histidyl-leucine (Hip-His-Leu) were obtained from Sigma Chemicals Co. (Poznan, Poland), trichloroacetic acid from LabScan (Poch-Gliwice, Poland), and trifluoroacetic acid (TFA) from Fluka (Steinhem, Switzerland).

**Enzymatic activity**

Pepsin activity was determined in the reaction with 2% acid-denaturized hemoglobin as the substrate according to Chrzanowska & Kotalczkowska [1998]. In brief, 650 µL of 0.2 mol/L phosphate-citrate buffer (pH 3.0) was preincubated at 37°C for 5 min. Then 100 µL of an enzyme solution (2–20 µg) were added. The reaction was started by adding 250 µL of hemoglobin. After 10 min the reaction was stopped by the addition of 1500 µL of 10% trichloroacetic acid. Then, the tubes were centrifuged (3500 rpm, 15 min, 20°C). The absorbance of the supernatants was measured at 280 nm. One unit of enzymatic activity of pepsin (U) was defined as the amount of enzyme producing an increase in absorbance at 280 nm of 0.1 under reaction conditions.

**Protein assay**

Protein concentration was determined according to the method of Lowry et al. [1951]. A standard curve was prepared for bovine serum albumin (BSA).

**Enzymatic hydrolysis**

The egg-white protein precipitate was dissolved in the reaction buffer (0.2 mol/L phosphate-citrate, pH 3.0) to a final concentration of 3.0 mg/mL. Hydrolysis was started by applying the enzyme (1 U per 1 mg of hydrolysed protein) and the reaction was run at 37°C for 3 h. It was stopped by heating at 100°C for 15 min. Then, the hydrolysate was centrifuged and the supernatant was lyophilised.

**Degree of hydrolysis (DH%)**

DH was expressed as the percentage ratio of protein soluble in 5% trichloroacetic acid (TCA) to total protein [Silvestre, 1997]. Five percent TCA was added to the hydrolysates (1:1). After 1 h of incubation at 4°C, the samples were centrifuged (5500 rpm, 15 min, 20°C). The concentration of the trichloroacetic acid-soluble product in the supernatant was measured spectrophotometrically at 280 nm.

**Determination of free amino group content**

The concentration of free amino groups was determined according to Kuchroo et al. [1983]. Samples of hydrolysates were diluted with 0.1 mol/L borate buffer to a final amount of 2 mL and mixed with 50 µL of TNBS reagent (0.03 mol/L). The samples were incubated in the dark for two hours at room temperature. The reaction was stopped by adding 2 mL of 0.1 mol/L sodium phosphate containing 1.5 mmol/L sodium sulfate and the absorbance was measured at 420 nm. A blank was prepared with water instead of hydrolysate. The results were expressed as µmol Gly/g by reference to a standard curve prepared with defined concentrations of glycine.

**ACE-inhibitory activity**

ACE-inhibitory activity was measured in vitro according to the method of Cushman & Cheung [1971] modified by Miguel et al. [2004]. Samples (40 µL) were preincubated with 100 µL of 0.1 mol/L borate buffer (pH 8.3) containing 5 mmol/L substrate (Hip-His-Leu) at 37°C for 5 min. The reaction was started by adding 20 µL of ACE solution (2 mU). After 30 min, the reaction was stopped by addition of 150 µL of 1 mol/L HCl. The hippuric acid formed was extracted with 1 mL of ethyl acetate and centrifuged (1500 ×g, 10 min). The organic phase (750 µL) was evaporated. The dry residue was dissolved in 800 µL of bi-distilled water and the absorbance was measured at 228 nm. Inhibitory activity was expressed as the amount (concentration) of substance required to inhibit 50% of ACE activity (IC50).

**Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)**

The samples of hydrolysate and the peptide fractions were dissolved in the mobile phase A (1:1) and applied to a Zorbax XDB-C18 column (4.6 × 250 mm, Agilent). The operation conditions were as follows: flow rate: 1 mL/min, gradient: 2% B/min, mobile phase A: 1 mL of trifluoroacetic acid (TFA) per liter in bi-distilled water, phase B: 1 mL of trifluoroacetic acid (TFA) per liter in acetonitrile, T 30°C, and retention time: 5 min. The absorbed peptides were eluted by gradient phase B. The absorption of the eluents was monitored at 230 nm.

**Molecular mass identification by mass spectrometry (MS) via the coupled with electrospray ionization (ESI)**

The peptide fractions were analyzed using an Esquire HCT quadrupole ion trap instrument (Bruker, Germany). The samples were injected into the mass spectrometer via
Amino-acid content determination

Samples (10 µg) were dried and hydrolysed in 6 mol/L HCl containing 1% phenol. After three cycles of N₂ purging and evacuation, the samples were placed in a hydrolysis oven and left at 115°C for 24 h. Amino-acid derivatization was accomplished by adding a freshly prepared solution of 10% PITC ((ethanol: water: TEA (triethylamine): PITC (phenylisothiocyanate) 7:1:1:1)) and leaving it for 20 min at room temperature. Then, the phenylthiocarbamyl derivatives were separated on a PicoTag 3.9 x 150 mm column (Waters, Milford, MA, USA) in an HPLC system. The column was calibrated with three different concentrations of an amino-acid standard composed of 23 pure amino acids (Pierce, USA).

Statistical analysis

All assays were conducted in triplicate. The results were analysed using the Statistica 7.0 program Analysis of Variance (ANOVA). Significance was set at the level of p≤0.05.

RESULTS AND DISCUSSION

Hen egg-white is an excellent source of many bioactive substances which may find application in the medical and food industries [Trziszka et al., 2006]. In this study, we investigated the possibility of applying enzyme hydrolysis to obtain a protein byproduct of the isolation of cystatin and lysozyme from hen egg white allowing the generation of ACE-inhibitory peptides. Egg-white protein precipitate (EWPP) containing mainly ovalbumin and ovotransferrin was hydrolysed with porcine pepsin [Graszkiewicz et al., 2007]. The progress of hydrolysis was monitored by determining the degree of hydrolysis (DH, %) and the concentration of the free amino groups (Table 1).

| Time (h) | IC₅₀ (µg/mL) | Degree of hydrolysis (%) | Free amino group concentration (µmol/L Gly/g protein) |
|---------|-------------|--------------------------|----------------------------------------------------|
| 0       | Not detected | 9.3a                    | 813.4  |
| 0.5     | Not detected | 17.3b                   | 2131.1b |
| 1       | 1371.1a      | 21.2c                   | 3664.2c |
| 2       | 736.2a       | 31.4d                   | 5470.5d |
| 3       | 643.1a       | 38.3e                   | 5642.3e |

IC₅₀ – the amount (concentration) of substance required to inhibit 50% of the ACE activity. a-b – the same letters indicate no statistically significant difference at p=0.05.

DH is an important parameter in the enzymatic modification of proteins and might be a factor controlling the composition and properties of the modified proteins [Ge & Zhang, 1993]. In our case, DH increased from 9.3 to 38.3% within 3 h of EWPP hydrolysis with pepsin. The final concentration of the free amino groups reached 5642.3 µmol Gly/g protein. Our results confirm the observation of other authors that pepsin is an efficient enzyme in egg-white protein degradation. Miguel et al. (2004) used pepsin (enzyme/substrate ratio: 1/100 wt/wt) to degrade whole egg-white. In their study, DH was 53.9% after 3 h of enzymatic reaction and increased to 74.3% after 24 h. Many studies [Fujita et al., 2000; Miguel et al., 2004] showed that hydrolysis of egg-white proteins with different proteolytic enzymes produced hydrolysates exhibiting a high ACE-inhibitory activity. The most potent hydrolysates were obtained after hydrolysis of egg-white with pepsin; however, the treatment with trypsin and chymotrypsin did not produce active hydrolysates. Fujita et al. (2000) also demonstrated that hydrolysates obtained from ovalbumin using pepsin and thermolysin exhibited an ACE-inhibitory activity. The IC₅₀ values for these hydrolysates were 45 and 83 mg/mL, respectively. In their further studies, they isolated six antihypertensive peptides from pepsin hydrolysates which had IC₅₀ values between 0.4 and 15 µmol/L [Fujita et al., 2000].

The antihypertensive properties of EWPP and its hydrolysates were monitored. The unhydrolyzed egg-white protein precipitate and the 0.5-h pepsin hydrolysate of EWPP did not show ACE-inhibitory activities (IC₅₀>1800 µg/mL). This is in line with the results obtained by Miguel et al. (2004), who reported that egg-white did not show ACE-inhibitory activities (IC₅₀>750 µg/mL). In contrast, Matoba et al. (2001) reported that ovalbumin exhibited antihypertensive activity on SHRats at a dose as high as 2 g/kg. An increase in ACE-inhibitory activity was observed as a result of hydrolysis. The highest inhibition of ACE was observed after 3 h of hydrolysis. The level of inhibitory activity of this hydrolysate (DH=38.3%) was IC₅₀=643.1 µg/mL, which is one third that noted for a 0.5-h pepsin hydrolysate of egg-white (DH=41.3%) obtained by Miguel et al. [2004]. However, Mullally et al. [1997] did not observe a relationship between the DH and the ACE inhibition index. For example, pepsin, chymotrypsin, and elastase hydrolysates of α-La had DHs of 5.10, 5.16, and 5.27%, respectively, whereas the corresponding ACE inhibitions were 84.4, 58.7, and 56.8%. The different levels of antihypertensive activity in our study might have resulted from the use of EWPP and not whole egg-white.

ACE-inhibitory activity in the hydrolysates is probably due to the presence of a range of different peptide sequences. The 3-h pepsin hydrolysate was separated by RP-HPLC. The pepsin hydrolysate was applied on a Zorbax XDB-C₁₈ column. The absorbed peptides were eluted with 0.1% TFA in an acetonitrile gradient and eight fractions were collected (Figure 1A). All the fractions were lyophilized and the ACE-inhibitory activity was determined in each of them (Figure 1B). The IC₅₀ inhibition values of the peptide fractions were different. The peptides in fraction no. 4 eluted with acetonitrile concentrations of 22–25% exhibited the highest ACE inhibition activity (IC₅₀=55.8 µg/mL), which was 11.5 times higher than the activity of the 3-h hydrolysate. A lower activity
was noted for fraction no. 5 (IC\textsubscript{50}=63.3 µg/mL). The most active fraction (no. 4) was re-chromatographed on the same column (Figure 2 A). The presence of many peptides with different hydrophobic properties was observed in the elution profile. This fraction was then divided into nine subfractions, three of which, labeled 4.2; 4.3, and 4.4, exhibited ACE-inhibitory activities of 69.0, 25.0, and 37.6 µg/mL, respectively (Figure 2B). A similar antihypertensive peptide isolation procedure was proposed by Matoba et al. [2001], who fractionated chymotrypsin hydrolysate of ovalbumin by RP-HPLC on an octadecyl silica (ODS) column. Further purification of the active peak on a phenyl silica column allowed the isolation of an active peptide in a pure form (ovokinin (2–7)). Miguel et al. [2004] used another peptide isolation procedure involving membrane filtration (3-kDa cut-off membrane) and RP-HPLC with an A Prep Nova Pak HR C\textsubscript{18} column and obtained three antihypertensive fractions, nos. F6; F7, and F8, exhibiting ACE-inhibitory activity (IC\textsubscript{50} <20, <10, and <30 µg/mL).

The molecular masses of the most active fractions were analysed by MS-ESI (Table 2). In each fraction of peptic-degraded EWPP, products with different molecular masses were obtained (Table 2). In the most active fraction (no. 4.3), ten peptides with different molecular masses were confirmed. The main component of this mixture was a peptide with a molecular mass of 34.98 kDa. Peptides with molecular masses of 37.33 kDa and 2.19 kDa also exhibited relatively high concentrations (88.07 and 77.82, respectively). A 52.90 kDa peptide originating from ovotransferrin confirmed that this protein was much more resistant than other egg-white proteins to proteolytic enzymes. This is in line with results obtained by Miguel et al. [2004], who reported that peptides obtained during crude egg-white hydrolysis with pepsin were derived mainly from ovalbumin. In fraction no. 4.4, many peptides exhibiting different molecular masses were also observed. Only fraction no. 4.2 was homogenous and the presence of only one protein fragment with a molecular mass of 26.58 kDa was confirmed. Our results differ from those of other authors studying ACE-inhibitory peptides. Miguel et al. [2004] demonstrated that ACE inhibition was mainly attributable to peptide components with molecular masses lower than 3 kDa. Other authors also confirmed that the ACE-inhibitory activi-

![Figure 1](image1.png)

**FIGURE. 1.** RP-HPLC peptide profile of the 3-h hydrolysate of egg-white precipitate hydrolysed with pepsin (3 h) (A) and ACE-inhibitory activity of the RP-HPLC fractions (B). IC\textsubscript{50} - the amount (concentration) of substance required to inhibit 50% of the ACE activity. a, b - the same letters indicate no statistical significant difference at p=0.05.

![Figure 2](image2.png)

**FIGURE. 2.** Re-chromatography (RP-HPLC) of fraction no. 4 isolated from the hydrolysate fraction obtained from egg-white precipitate hydrolysed with pepsin (3 h) (A) and ACE-inhibitory activity of the RP-HPLC subfractions (B). IC\textsubscript{50} - the amount (concentration) of substance required to inhibit 50% of the ACE activity. a, b - the same letters indicate no statistical significant difference at p=0.05.
ties of hydrolysates obtained by peptic hydrolysis of egg white and its low-molecular-mass fraction were small and fitted within the concentration range exerting an antihypertensive activity [Gobbetti et al., 2000; Mullally et al., 1997].

The amino-acid composition of these subfractions was analysed (Table 3). Fraction no. 4.2 had a high concentration of amino-acid residues; the concentrations of valine, serine, and alanine amounted to 19.02, 18.49, and 17.51 nmol/10 µg, respectively. The highest concentration of leucine (18.09 nmol/10 µg) was noted in fraction no. 4.3. Proline was found in all samples and its concentration ranged from 7.50 to 8.75 nmol/10 µg. According to Hartmann & Meisel, 2007; Smacchi & Gobbetti, 2000. The presence of low-molecular-weight ACE-inhibitory peptides in several ripened cheeses (e.g. medium-aged Gouda, Parmesan or Gorgonzola) has been observed. Bioactive peptides were liberated by proteolytic enzymes from Lactic Acid Bacteria (LAB) during cheese ripening [Smacchi & Gobbetti, 2000]. It suggests that egg-white hydrolysates exhibiting the ACE-inhibitory activity may find application in functional food production.

### CONCLUSIONS

1. Pepsin was an effective enzyme in egg-white protein precipitate (EWPP) degradation. During 3-h digestion, the enzyme caused DH 38.3%.

2. The hydrolysis of egg-white protein precipitate with pepsin produced the ACE inhibitory activity.

3. The RP-HPLC fractionation led to obtaining three peptide subfractions exhibiting a significant ACE-inhibiting activity of 25.0, 37.6 and 69.0 µg/mL.

### ACKNOWLEDGMENTS

This study was co-financed by the European Social Found and Human Capital National Cohesion Strategy for Lower Silesian “GRANT wsparcie prac badawczych poprzez stypendia naukowe dla doktorantów” and Scientific Project no. R1201401 (2007–2010).
REFERENCES

1. Chrzanowska J., Kolaczkowska M., Production of extracellular proteolytic enzymes by *Beauveria bassiana*. Acta Myc., 1998, 33, 277–285.

2. Cushman D.W., Cheung H.S., Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol., 1971, 20, 1637–1648.

3. Davalos A., Miguel M., Bartolome B., López–Fandíno R., Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. J. Food Prot., 2004, 67, 1939–1944.

4. Erdmann K., Cheung B.W.Y., Schröder H., The effect of pH-drop method. Acta Biotech., 1993, 13, 151–160.

5. Fujita H., Sasaki R., Yoshikawa M., Potentiation of the anti-hypertensive activity of orally administered Ovokin, a vasorelaxing peptide derived from ovalbumin, by emulsification in egg phosphatidyl-choline. Biosci. Biotechnol. Biochem., 1995, 59, 2344–2345.

6. Fujita H., Yokoyama K., Yoshikawa M., Classification and anti-hypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. J. Food Sci., 2000, 65, 564–569.

7. Ge S.J., Zhang L.X., Control of the degree of hydrolysis of a protein modification with immobilized protease by the pH-drop method. Acta Biotech., 1993, 13, 151–160.

8. Gibbs B.F., Zougman A., Masse R., Mulligan C., Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. Food Res. Int., 2004, 37, 123–131.

9. Gobbetti M., Ferranti P., Smacchi E., Goffredi F., Addeo F., Production of angiotensin-I-converting enzyme inhibitory peptides in fermented milks started by *Lactobacillus delbrueckii* subsp. *bulgaricus* SS1 and *Lactococcus lactis* subsp. *cremoris* FT4. Appl. Environ. Microbiol., 2000, 66, 3898–3904.

10. Graszkiewicz A., Żelazko M., Trziszka T., Polanowski A., Antioxidative capacity of hydrolysates of hen egg proteins. Pol. J. Food Nutr. Sci., 2007, 57, 195–199.

11. Hartmann R., Meisel H., Food-derived peptides with biological activity; from research to food applications. Curr. Opin. Biotechnol., 2007, 18, 163–169.

12. Kuchroo C.N., Rahilly J., Fox P.F., Assessment of proteolysis in cheese by reaction with trinitrobenzenesulphonic acid. Irish J. Food Sci. Technol., 1983, 7, 129–133.

13. Liu J.B., Yu Z.P., Zhao W.Z., Lin S.Y., Wang E.L., Zhang Y., Hao Y., Wang Z.Z., Chen F., Isolation and identification of angiotensin-converting enzyme inhibitory peptides from egg-white protein hydrolysates. Food Chem., 2010, 122, 1159–1163.

14. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 1951, 193, 265–275.

15. Matoba N., Doyama N., Yamada Y., Maruyama N., Utsumi S., Yoshikawa M., Design and production of genetically modified soybean protein with anti-hypertensive activity by incorporating potent analogue of Ovokin(2–7). FEBS Lett., 2001, 497, 50–54.

16. Miguel M., Alvarez Y., Lopez-Fandíno R., Alonso M., Salaices M., Vasodilator effects of peptides derived from egg white proteins. Reg. Peptides, 2007, 140, 131–135.

17. Miguel M., Recio I., Gomez-Ruiz J.A., Ramos M., Lopez-Fandíno R., Angiotensin-I-converting enzyme inhibitory activity of peptides derived from egg white proteins by enzymatic hydrolysis. J. Food Prot., 2004, 67, 1914–1920.

18. Mullally M.M., Meisel H., Fitz-Gerald R.J., Angiotensin-I-converting enzyme inhibitory activities of gastric and pancreatic protease digests of whey protein. Int. Dairy J., 1997, 7, 299–303.

19. Nakamura Y., Yamamoto N., Sakai K., Okubo A., Yamazaki S., Takano T., Purification and characterization of angiotensin-I-converting enzyme inhibitors from sour milk. J. Dairy Sci., 1995, 78, 777–783.

20. Park PJ., Jung WK., Nam KS., Shahidi F., Kim SK., Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. J. Am. Oil Chem. Soc., 2001, 78, 651–656.

21. Saiga A., Okamura T., Makihara T., Katsuda S.I., Miorimatsu F., Nishimura T., Action mechanism of an angiotensin-I-converting enzyme inhibitory peptide derived from chicken breast muscle. J. Agric. Food Chem., 2006, 54, 942–945.

22. Saiga A., Tanabe S., Nishimura T., Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J. Agric. Food Chem., 2003, 51, 3661–3667.

23. Sakanaka S., Tachibana Y., Active oxygen scavenging activity of egg-yolk protein hydrolysates and their effects on lipid oxidation in beef and tuna homogenates. Food Chem., 2006, 95, 243–249.

24. Silva S.V., Malcata FX., Caseins as source of bioactive peptides. Int. Dairy J., 2005, 15, 1–15.

25. Silvestre M.P.C., Review of methods for the analysis of protein hydrolysates. Food Chem., 1997, 60, 263–271.

26. Smacchi E., Gobbetti M., Bioactive peptides in dairy products: synthesis and interaction with proteolytic enzymes. Food Microbiol., 2000, 17, 129–141.

27. Sokolowska A., Kubiak A., Trziszka T., Polanowski A., Rapid one step separation of proteinaceous agents of antimicrobial activity from hen's egg white. Pol. J. Food Nutr. Sci., 2007, 57, 499–502.

28. Trziszka T., Polanowski A., Saleh Y., Kopec W., Egg white isolation and its medical application. http://www.animalscience.com/uploads/additionalfiles/wpsaverona.htm, 2006.

29. Yamada Y., Matoba N., Usui H., Onishi K., Yoshikawa M., Design of a highly potent anti-hypertensive peptide based on ovo-kinin(2–7). Biosci. Biotechnol. Biochem., 2002, 66, 1213–1217.

30. Zhipeng Y., Wenzhu Z., Jingbo L., Jing L., Feng C., QIGLF, a novel angiotensin-I-converting enzyme-inhibitory peptide from egg white protein. J. Sci. Food Agric., 2011, 6, 921–926.

Submitted: 8 December 2011. Revised: 13 March 2012. Accepted: 6 June 2012. Published on-line: 16th of November 2012.