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

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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 IC_{50} value of EWPP hydrolysate was significantly increased and finally reached IC_{50}=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 vivo* 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; Sakanaka & Tachibana, 2006; Saiga *et al*., 2003; Gibbs *et al*., 2004]. Biologically-active peptides exhibit diverse activities, including antimicrobial, immunomodulatory, antioxidant, mineral-binding, opiate-like, hypocholesterolemic, and antihypertensive actions. Many of them are multifunctional and can exert more than one biological effect [Davalõ *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; Sakanaka & 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 ovalbumin. 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 IVF, RADHPFL, and YAEERYPIL, 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 YAEERYPIL 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 IC_{50} 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 Sokołowska *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 Ubichem (Hampshire, UK), acetonitrile 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 & Kołaczkowska [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 (5500 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-citriate, 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 $\times g$, 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 (IC_{50}).

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-C_{18} 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 trifluororacetic 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*

the electrospray interface (activation time: 1 min, flow: 220 μL/min). Spectra were recorded over the *m/z* range of 100 to 2200. Analysis was performed in the laboratory of BioCentrum sp. z o. o. in Crakow.

Peptide fractions' share in the mixture is expressed as relative concentration (%) of particular peptide in relation to the most intensive peak. If only one peak was detected, the relative concentration was established in relation to the residual spectrum.

Amino-acid content determination

Samples (10 μg) were dried and hydrolysed in 6 mol/L HCl containing 1% phenol. After three cycles of N_2 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 $p \leq 0.05$.

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).

TABLE 1. Angiotensin I-converting activity, degree of hydrolysis, and free amino group concentrations in the egg-white protein precipitate (EWPP) hydrolysate with pepsin after different times.

Time (h)	IC_{50} (μg/mL)	Degree of hydrolysis (%)	Free amino group concentration (μmol/L Gly/g protein)
0	Not detected[d]	9.3[a]	813.4[a]
0.5	Not detected[d]	17.1[b]	2131.1[b]
1	1371.1[c]	21.2[c]	3664.2[c]
2	736.2[b]	31.1[d]	5470.5[d]
3	643.1[a]	38.3[e]	5642.3[e]

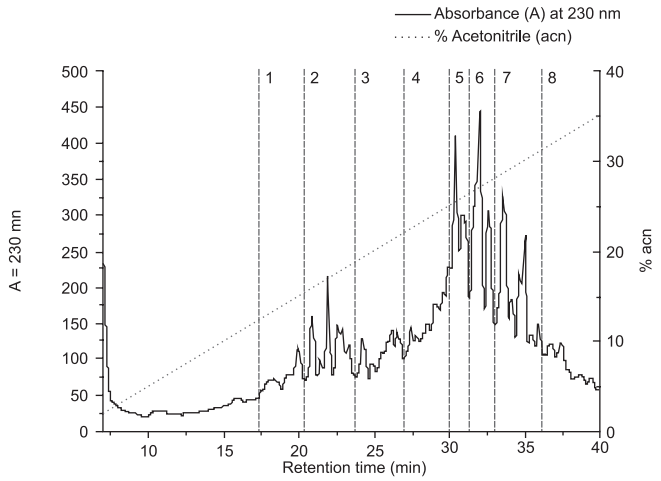
IC_{50} – the amount (concentration) of substance required to inhibit 50% of the ACE activity. [a,b] – the same letters indicate no statistically significant difference at p=0.05.

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 IC_{50} 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 IC_{50} 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 ($IC_{50} > 1800$ 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 ($IC_{50} > 750$ μg/mL). In contrast, Matoba *et al.* [2001] reported that ovalbumin exhibited antihypertensive activity on SHRs 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 IC_{50}=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- C_{18} 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 IC_{50} 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 (IC_{50}=55.8 μg/mL), which was 11.5 times higher than the activity of the 3-h hydrolysate. A lower activity

(A)



(B)

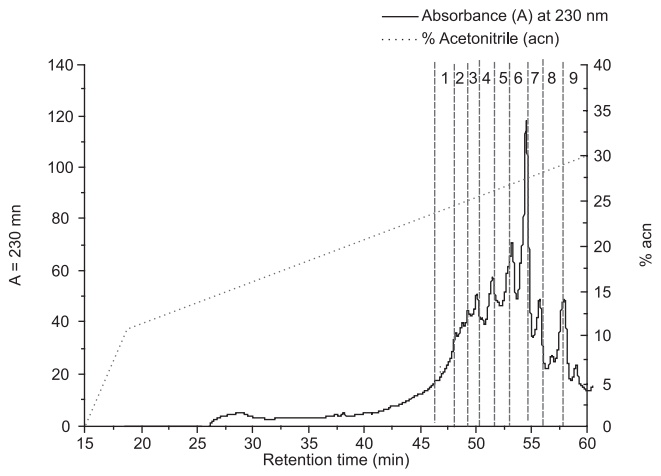
FRACTION NUMBER	ACTIVITY IC_{50} (μg/mL)
1	not detected[g]
2	267.0[f]
3	123.8[d]
4	55.8[a]
5	63.3[b]
6	162.5[e]
7	112.5[c]
8	not detected[g]

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_{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.

was noted for fraction no. 5 (IC_{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 *el 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_{18} column and obtained three antihypertensive fractions, nos. F6; F7, and F8, exhibiting ACE-inhibitory activity (IC_{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-

(A)



(B)

SUBFRACTION NUMBER	ACTIVITY IC_{50} (μg/mL)
4.1	79.7[d]
4.2	69.0[c]
4.3	25.0[a]
4.4	37.6[b]
4.5	820.0[e]
4.6	1831.0[f]
4.7	not detectedg
4.8	not detectedg
4.9	not detectedg

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_{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.

TABLE 2. Molecular masses of the peptides in the subfractions isolated from 3-h peptic hydrolysate of egg-white precipitate.

Fraction number	Experimental mass (kDa)	Relative concentration (%)[1]
4.2	26.58	36.18
4.3	37.33	88.07
	52.90	37.05
	36.20	38.71
	10.10	26.88
	31.30	26.86
	27.70	29.78
	34.98	100.00
	2.19	77.82
	27.31	38.00
	45.69	34.27
4.4	24.71	22.85
	31.99	56.29
	16.09	86.45
	23.07	40.17
	52.57	34.81
	35.01	28.08
	19.59	100
	52.17	22.68
	42.04	22.33
	21.36	22.55

[1] Relative concentration (%) in relation to the most intensive peak. If only one peak was detected, the relative concentration was established in relation to the residual spectrum.

TABLE 3. Amino-acid composition (nmol/10 μg) of subfractions isolated from 3-h peptic hydrolysate of egg-white precipitate

Amino acid	Subfraction		
	4.2	4.3	4.4
Asx*	18.41	18.51	17.56
Glx*	20.77	22.80	23.57
Ser	18.49	15.72	17.85
Gly	13.79	12.48	13.12
His	1.43	1.13	0.72
Arg	12.86	8.94	10.40
Thr	10.19	10.52	9.99
Ala	17.51	15.62	15.72
Pro	7.83	7.50	8.75
Tyr	5.46	5.79	6.46
Val	19.02	14.33	12.68
Met	10.63	5.60	5.48
Ile	9.81	10.53	11.07
Leu	16.24	18.09	18.07
Phe	13.63	10.66	11.74
Lys	8.73	9.21	8.11

Asx = Asn+Asp, Glx=Gln+Glu

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 [Gobbeti *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], ACE-inhibitory peptides are generally short-chain peptides, often carrying polar amino-acid residues such as proline. The hypotensive peptides Val-Pro-Pro and Ile-Pro-Pro, for example, can be released from β-casein and κ-casein by enzymes from *Lactobacillus helveticus* [Hartmann & Meisel, 2007; Smacchi & Gobbetti, 2000]. Other authors reported that ACE-inhibitory activity is stronger when there is a dipeptide, such as -His-Leu, -Phe-Arg, or -Ala-Pro [Saiga *et al.,* 2006]. Miguel *et al.* [2007], in contrast, indicated that longer-chain peptides present in egg-white hydrolysate with pepsin (RADHPFL and YAEERYPIL) can also exert ACE-inhibitory activity. Saiga *et al.* [2006] also found an ACE-inhibitory long-chain peptide (Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe) in an extract of chicken breast muscle digested by gastric enzymes (trypsin-chymotrypsin and small intestinal enzymes).

There are several reports on the antihypertensive effect of milk protein-derived hydrolysates in rat and human feeding studies [Nakamura *et al.,* 1995; Smacchi & Gobbetti, 2000]. This does not speak against the application of food hydrolysates in the prevention of hypertension, unlike the synthetic antihypertensive drug Captopril, which would have undesirable effects [Mullally *et al.,* 1997]. Nowadays, bioactive peptides (in unpurified form as hydrolysates) are constituents of many commercially-available functional foods, such as sour milk and fermented milk under the names Calpis AMEEL S (Japan) and Evolus (Finland), respectively [Hartmann & Meisel, 2007]. 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.

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