The Novel Angiotensin I Converting Enzyme Inhibitory Peptide from Rainbow Trout Muscle Hydrolysate

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Abstract
The purpose of this study was the purification and characterization of an angiotensin I converting enzyme (ACE) inhibitory peptide purified from enzymatic hydrolysates of rainbow trout Oncorhynchus mykiss muscle. After removal of lipid, the approximate composition analysis of the rainbow trout revealed 24.4%, 1.7%, and 68.3% for protein, lipid, and moisture, respectively. Among six hydrolysates, the peptic hydrolysate exhibited the highest ACE inhibitory activity. We attempted to purify ACE inhibitory peptides from peptic hydrolysate using high performance liquid chromatography on an ODS column. The IC_{50} value of purified ACE inhibitory peptide was 63.9 μM. The amino acid sequence of the peptide was identified as Lys-Val-Asn-Gly-Pro-Ala-Met-Ser-Pro-Asn-Ala-Asn, with a molecular weight of 1,220 Da, and the Lineweaver-Burk plots suggested that they act as a competitive inhibitor against ACE. Our study suggested that novel ACE inhibitory peptides purified from rainbow trout muscle protein may be beneficial as anti-hypertension compounds in functional foods.

Key words: Angiotensin I converting enzyme, Rainbow trout muscle, Pepsin, Hydrolysates

Introduction
Hypertension is a worldwide problem of epidemic proportions that affects 15-20% of all adults. Its treatment is one of the major risk factors for the development of cardiovascular disease, stroke, and the end stage of renal disease (Zhang et al., 2006). Among the processes associated with hypertension, angiotensin I converting enzyme (ACE) plays an important role in the regulation of blood pressure. In the rennin-angiotensin system, ACE (peptidyl dipeptidase, EC 3.4.15.1) acts on decapptide angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to hydrolyze His-Leu from its C-terminal and produces the potent vasopressor octapeptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). While in the kinin-kallikrein system, ACE inactivates the vasodilator bradykinin (Bougatef et al., 2010).

Many synthetic ACE inhibitors including Captopril, Enalapril, and Lisinopril among others are available for clinical use, however some undesirable side effects may occur such as cough, loss of taste, renal impairment and angioneurotic oedema (Brown and Vaughan, 1998). In the past there has been a trend toward the development of natural ACE inhibitors isolated from various organism proteins (Fujita et al., 2000; Pihlanto-Leppälä et al., 2000). As a result of this research, various ACE inhibitory peptides through enzymatic hydrolysis have been isolated from marine organisms, including the skate skin (Lee et al., 2011), seaweed pipefish (Wijesekara et al., 2011), brownstripe red snapper (Khantaphant et al., 2011), tuna back bone (Lee et al., 2010), and sea cucumber (Zhao et al., 2007). Enzymatic hydrolysate showed several advantages when added to foods, such as improving water-binding ability, heat stability of myofibrillar protein, emulsifying stability, solubility of protein, and the nutritional quality of foods. Moreover, enzymatic hydrolysis has become a valuable tool...
for modifying the functionality of proteins (Korhonen et al., 1998). During hydrolysis, hydrophobicity of the amino-acid side chains is normally due to relatively small peptides, with molecular weights between 1,000 and 6,000 Da. Therefore, enzymatic hydrolysis was established as a source of bioactive peptides, which are short peptides released from food proteins by hydrolysis and have certain biological activities that may be beneficial for the organism (Je et al., 2005 a). Bioactive peptides usually contain 3-20 amino acid residues per molecule and are inactive within the sequence of the parent protein molecule. Moreover, bioactive peptides can be liberated by gastrointestinal digestion through proteolytic enzymes or during the fermentation process (Korhonen and Pihlanto, 2006).

The rainbow trout *Oncorhynchus mykiss* was most representative species on freshwater fish cultivation, and is a traditional food that is a good source of calcium, essential amino acids, n-3 polyunsaturated fatty acids, and vitamins (Jang et al., 1998). In a previous study, physiological and molecular approaches were used to investigate the existence of an intrarenal rennin angiotensin system in rainbow trout (Brown et al., 1998). In a previous study, physiological and molecular approaches were used to investigate the existence of an intrarenal rennin angiotensin system in rainbow trout (Brown et al., 1998). Several studies have examined the bioactivities of rainbow trout, such as its antioxidant (Li et al., 2010) as well as its anti-inflammatory (Schwaiger et al., 2004) and antimicrobial (Fernandes et al., 2002) activities. However, studies on the bioactivity peptide of rainbow trout muscle hydrolysates have not reported. The purpose of this study was to isolate ACE inhibitory peptides from rainbow trout muscle hydrolysates and establish the purified peptide with regards to the ACE inhibitory activity. Moreover, we also revealed the inhibition pattern of the isolated peptide on ACE.

**Materials and Methods**

**Materials**

The bones and viscera were removed from the rainbow trout and separated muscle was stored at -80°C until used. Rainbow trout muscle lipid was removed using an organic solvent (n-hexane:ethanol = 1:2). ACE (from rabbit lung) and hippuryl-L-histidyl-L-leucine (HHL) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Various commercial enzymes, such as α-chymotrypsin, papain, pepsin and trypsin were purchased from Sigma Chemical Co. Alcalase and Neutrase were purchased from Novo Co. (Novo Nordisk, Bagsvaerd, Denmark). All other reagents used in this study were reagent grade chemicals.

**Analysis of approximate compositions**

Crude protein content was determined by the Kjeldahl method using an Auto Kjeldahl system (B-324/435/412; Buchi, Flawil, Switzerland). Crude lipid content was determined by the ether extraction method. Moisture content was determined by oven drying at 105°C for 24 h. Ash content was determined by a muffler furnace at 550°C for 4 h (Association of Official Analytical Chemist, 2000). Amino acids were analyzed using an automatic analyzer (835-50; Hitachi, Tokyo, Japan) with a C18 column (5 μm, 4.6 × 250 mm; Wathers, Massachusetts, MA, USA). The reaction was carried out at 38°C, with the detection wavelength at 254 nm and a flow rate of 1.0 mL/min. All chemical analyses (from each tank) were carried out in triplicate.

**Preparation of rainbow trout muscle hydrolysate**

For the production of ACE inhibitory activity peptide from rainbow trout muscle protein, enzymatic hydrolysis was performed using various commercial enzymes (Alcalase, α-chymotrypsin, Neutrase, papain, pepsin, and trypsin) at an enzyme/substrate ratio of 1/100 (w/w) for 6 h, under optimum pH and temperature conditions (Table 1). After the reaction, reactant was conducted by glass filter. Degree of hydrolysis (DH) was determined by measuring the soluble nitrogen content in 10% trichloroacetic acid as followed by Kim et al. (1990), and lyophilized hydrolysates were stored at -80°C until use.

**Determination of ACE inhibitory activity**

The ACE inhibition activity was measured using HHL as the substrate, according to Cushman and Cheung (1971) with slight modification. A 50 µL rainbow trout muscle hydrolysate solution with 50 µL of ACE solution (25 mU/mL) was pre-incubated at 37°C for 10 min, and the mixture was subsequently incubated with 100 µL of substrate (50 mM HHL in 50 mM sodium borate buffer) for 60 min at the same temperature. The reaction was terminated with the addition of 250 µL of 1 N HCl. The resulting hippuric acid was extracted with 500 µL of ethylacetate. After centrifugation (5,000 rpm, 10 min), 200 µL of the top layer (ethyl acetate layer) was transferred into a glass test tube and dried at -80°C for 1 h. The hippuric acid

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Buffer</th>
<th>pH</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcalase</td>
<td>50 mM sodium phosphate</td>
<td>7.0</td>
<td>50</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>50 mM sodium phosphate</td>
<td>7.0</td>
<td>37</td>
</tr>
<tr>
<td>Neutrase</td>
<td>50 mM sodium phosphate</td>
<td>7.0</td>
<td>50</td>
</tr>
<tr>
<td>Papain</td>
<td>50 mM sodium phosphate</td>
<td>7.0</td>
<td>37</td>
</tr>
<tr>
<td>Pepsin</td>
<td>20 mM glycine-HCl</td>
<td>2.0</td>
<td>37</td>
</tr>
<tr>
<td>Trypsin</td>
<td>50 mM sodium phosphate</td>
<td>7.0</td>
<td>37</td>
</tr>
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</table>
Results and Discussion

Proximate composition of rainbow trout muscle

The proximate analysis of the rainbow trout muscle is shown in Table 2. The protein content was 20.31%, while the lipid and moisture contents were 6.22% and 71.16%, respectively. Gokoglu et al. (2004) reported that the approximate compositions of rainbow trout raw were 73.40% moisture, 19.85% protein, 3.41% lipid, and 1.46% ash. In comparison with our study, the protein content was similar. After removal of lipid, the protein content increased by 24.44% and through enzymatic hydrolysis was considered valuable enough to use by industry standards. The most abundant amino acids in rainbow trout muscle were glycine, lysine, aspartic acid and leucine which accounted for 10.63%, 12.14%, 11.66%, and 11.10%, respectively (Table 3). Hong et al. (2008) reported

Table 2. Comparison of proximate composition of muscle and defatted muscle from rainbow trout

<table>
<thead>
<tr>
<th>Components</th>
<th>Muscle</th>
<th>Defatted muscle</th>
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<tr>
<td>Moisture</td>
<td>71.16</td>
<td>68.23</td>
</tr>
<tr>
<td>Protein</td>
<td>20.31</td>
<td>24.44</td>
</tr>
<tr>
<td>Lipid</td>
<td>6.22</td>
<td>1.70</td>
</tr>
<tr>
<td>Ash</td>
<td>1.90</td>
<td>1.92</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>0.41</td>
<td>3.71</td>
</tr>
</tbody>
</table>

Table 3. Amino acid contents of rainbow trout muscle

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>11.66</td>
</tr>
<tr>
<td>Thr</td>
<td>5.87</td>
</tr>
<tr>
<td>Ser</td>
<td>4.24</td>
</tr>
<tr>
<td>Glu</td>
<td>0.20</td>
</tr>
<tr>
<td>Gly</td>
<td>10.63</td>
</tr>
<tr>
<td>Ala</td>
<td>13.08</td>
</tr>
<tr>
<td>Val</td>
<td>6.66</td>
</tr>
<tr>
<td>Cys</td>
<td>1.46</td>
</tr>
<tr>
<td>Met</td>
<td>3.77</td>
</tr>
<tr>
<td>Ile</td>
<td>4.84</td>
</tr>
<tr>
<td>Leu</td>
<td>11.10</td>
</tr>
<tr>
<td>Try</td>
<td>3.32</td>
</tr>
<tr>
<td>Phe</td>
<td>4.33</td>
</tr>
<tr>
<td>Trp</td>
<td>0.17</td>
</tr>
<tr>
<td>Lys</td>
<td>12.14</td>
</tr>
<tr>
<td>Arg</td>
<td>5.64</td>
</tr>
<tr>
<td>Pro</td>
<td>0.89</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
trypsin and α-chymotrypsin were 0.61, 1.09, and 1.51 mg/mL, respectively. Among the various enzymatic hydrolysates, the peptic hydrolysate had exhibited the highest ACE inhibitory activity. The IC$_{50}$ value was lower than reported for shrimp (3.37 mg/mL) but higher than reported for Alaska pollack protein hydrolysates (0.21 mg/mL), sardine (0.01 mg/mL) and sea bream (0.57 mg/mL) (Wijesekara and Kim, 2010). Previous report (Simpson, 2000) have shown that pepsin is one of the major fish digestive proteolytic enzymes, commonly used for industrial application. Generally, pepsin is secreted from gastric mucosa in the stomach and had preferential specificity for the aromatic amino acids, phenylalanine, tyrosine, and tryptophan. Pepsin is an endopeptidase with broad specificity produced in the mucosal lining of the stomach and degrades proteins. Pepsin is one of three principal protein-degrading, or proteolytic, enzymes in the digestive system, the other two being chymotrypsin and trypsin (Cooper et al., 1990). Recent advances in biotechnology have proved the ability of enzymes to produce novel food products, modified food composition, or improved waste processing (Lee et al., 2011). Our next step in analysis required the use of HPLC to purify the ACE inhibitory peptide from peptic hydrolysate of rainbow trout muscle.

**Purification of ACE inhibitory peptide**

To identify the ACE inhibitory peptides derived from rainbow trout muscle hydrolysate that had the highest ACE inhibitory activity, the peptides were separated by RP-HPLC using an ODS preparative column into five fractions (F1-F5) (Fig. 3). Sub-fraction F3 possessed the highest ACE inhibitory activity. Subsequently, fraction F3 was further separated by RP-HPLC using the C$_{18}$ analytical column. Finally, we purified two fractions (A and B) from rainbow trout muscle hydrolysate (Fig. 4). Fraction A showed the most potent ACE inhibitory activity with an IC$_{50}$ value of 0.19 mg/mL.

**Amino acid sequence of purified ACE inhibitory peptide**

Fraction A was found to have the highest ACE inhibitory activity more than fraction B. Amino acids sequence of fraction A was identified using MS/MS and shown to be Lys-Val-Asn-Gly-Pro-Ala-Met-Ser-Pro-Asn-Ala-Asn with an ACE inhibitory IC$_{50}$ value of 63.9 µM and 1,220 Da molecular weights (Fig. 5). In this study, the purified ACE inhibitory peptide was found to have a similar sequence compared to other reports, including the algae protein waste (Val-Glu-Cys-Tyr-Gly-Pro-Asn-Arg-Pro-Gln-Phe, IC$_{50}$ = 29.6 µM) (Sheih et al., 2009) and sauce of fermented blue mussel (Glu-Val-Met-Ala-Gly-Asn-Leu-Try-Pro-Gly, IC$_{50}$ = 2.9 µM) (Je et al., 2005b) and porcine hemoglobin (Val-Val-Tyr-Pro-Trp, IC$_{50}$ = 6.0 µM) (Yu et al., 2006). Our peptide had valine at the N-terminus which may be the reason our peptide yielded larger IC$_{50}$ values. Regarding the relationship between structure and activity of ACE that many ACE inhibitory peptides contained glycine, leucine, proline, tyrosine and phenylalanine indicating that rainbow trout muscle may have ACE inhibitory peptides and exhibit potential antihypertensive activity.

**ACE inhibitory activity of hydrolysates**

The rainbow trout muscle protein hydrolysates were prepared by hydrolysis using commercial proteases including Alcalase, α-chymotrypsin, Neutrase, papain, pepsin, and trypsin. The extent of protein degradation by enzymatic hydrolysates was estimated by evaluating the DH. Analysis revealed that DH of pepsin, α-chymotrypsin and trypsin were 49.12%, 30.52%, and 28.75%, respectively (Fig. 1). Peptides from six hydrolysates were evaluated for their ACE inhibitory activities by IC$_{50}$ value (mg/mL). As shown in Fig. 2, ACE inhibitory activity of extracts produced by various enzymes, pepsin,
Fig. 3. High performance liquid chromatography (HPLC) chromatogram of hydrolysates prepared with pepsin. Separation was performed with linear gradient of acetonitrile from 0% to 35% in 30 min at a flow rate of 1.5 mL/min. Elution was monitored at 280 nm (A). The fractions showing angiotensin I converting enzyme inhibitory activity was designated as F1-F5 on upper layer (B).

Fig. 4. High performance liquid chromatography (HPLC) chromatogram of potent angiotensin I converting enzyme (ACE) inhibitory fraction A, B was isolated. Separation was performed with linear gradient of acetonitrile from 0% to 30% in 40 min at a flow rate of 1.5 mL/min. Elution was monitored at 280 nm (A). ACE inhibitory activity of each fraction (B).

Fig. 5. Identification of molecular mass and amino acid sequence of the purified peptides from rainbow trout muscle peptic hydrolysate by high performance liquid chromatography (HPLC). Tandem mass spectrometry (MS/MS) experiments were performed on a quadrupole time-of-flight (Q-TOF) tandem mass spectrometer equipped with a nano-electrospray ionization source.
inhibitory peptides, Cheung et al. (1980) reported that those peptides with valine and isoleucine at the N-terminus showed highly potent inhibitory activity. It has been confirmed that functional peptides are dependent on amino acid sequence and structure (Elias et al., 2008). Thus, the sequencing and structure of peptides could be related to ACE inhibitory activity. For example, Val-Tyr-Ala-Pro (IC$_{50}$ = 6.1 µM) exhibiting a potent antihypertensive peptide was derived from cuttlefish muscle protein hydrolysate (Balti et al., 2010). Similarly, other structure-activity correlation studies have indicated that ACE binding is strongly affected by the C-terminal tripeptide sequence of the substrate and that the tripeptide could interact with subsites S1, S’1, and S’2 of ACE (Pihlanto-Leppälä, 2000). The amino sequencing, strongly affects potential ACE inhibition because of the inclusion of hydrophobic amino acid residues (aromatic or branched side chains) at the C-terminal (Cheung et al., 1980). Hydrophobic amino acid residues in the ACE inhibitor sequence are a critical factor in inhibitory activity (Li et al., 2004). Therefore, we concluded that the purified peptide exhibited low ACE inhibition activity due to non-distribution of hydrophobic amino acids at the C-terminal. ACE inhibitory peptide purified from rainbow trout muscle was composed of hydrophilic amino acids at the C-terminal with an IC$_{50}$ of 63.9 µM. This IC$_{50}$ value exhibited lower or similar activity compared to those of peptides derived from oyster protein (Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe, IC$_{50}$ = 66.0 µM) (Wang et al., 2008), however, it had a higher activity than those of peptides from the hydrolysate of skate skin (Pro-Gly-Pro-Leu-Gly-Leu–Thr-Gly-Pro, IC$_{50}$ = 95.0 µM) (Lee et al., 2011). The hydrophilic amino acid residues in the peptide sequence could also affect inhibitory activity by disrupting the access of the peptide to the active site of ACE. In this study, Lys-Val-Asn-Gly-Pro-Ala-Met-Ser-Pro-Asn-Ala-Asn from rainbow trout muscle contained hydrophilic amino acid such as asparagine at the C-terminal peptide sequence, which may contribute to the ACE inhibitory activity we observed.

ACE inhibition pattern of purified peptides

The ACE inhibition pattern of the fraction A of the purified peptides was analyzed by the Lineweaver-Burk plot and was found to be competitive (Fig. 6). Thus, ACE inhibitor from rainbow trout muscle hydrolysate binds competitively with the substrate at the active site of ACE. Moreover, the ACE inhibitory peptide from oyster was also found to be competitive (Je et al., 2005 a) along with other types such as rotifer (Lee et al., 2009). Captopril has been reported to show competitive inhibition competition with the substrate for binding to active ACE site (Tsai et al., 2006). The purified peptide in this study also exhibited an inhibition pattern of being able to bind to the active site. Based on the results of this study, it appears that novel ACE inhibitory peptide may be beneficial to the bioactivity of materials and functional foods related antihypertension. In this study, in order to improve the utilization of rainbow trout muscle, enzymatic hydrolysis was performed on ACE inhibitory peptides using various enzymes. The purified peptide from rainbow trout muscle was shown to exhibit potent ACE inhibitory activity with an IC$_{50}$ value of 63.9 µM, and a molecular weight of 1,220 Da. The results of this study suggest that the ACE inhibitory peptide from rainbow trout muscle protein has the potential to be beneficial as a food additive or a pharmaceutical agent.

Acknowledgments

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References


Balti R, Nedjar-Arroume N, Bougafet A, Guillochon D and Nasri M. 2010. Three novel angiotensin I-converting enzyme (ACE) inhibi-

Fig. 6. Angiotensin I converting enzyme inhibition pattern of purified peptide, estimated using Lineweaver-Burk plots.
tory peptides from cuttlefish (Sepia officinalis) using digestive pro-
tes. Food Res Int 43, 1136-1143.
Bougatof A, Balti R, Nedjar-Aroumne N, Royallec R, Adjé EY, Sou-
issi N, Lassoudi E, Guillochen D and Nasri M. 2010. Evaluation of
angiotensin I-converting enzyme (ACE) inhibitory activities of
smooth hound (Mustelus mustelus) muscle hydrolysates
enerated by gastrointestinal proteases: identification of the most
Brown JA, Paley RK, Amer S and Aves SJ. 2000. Evidence for an intra-
renal renin-angiotensin system in the rainbow trout, Oncorhynchus
mykiss. Am J Physiol Regul Integr Comp Physiol 278, R1685-
R1691.
hibitors. Circulation 97, 1411-1420.
Bush K, Hery PR and Slusarchyk DS. 1984. Muracinsmuramyl pep-
tides produced by Norcardia orientalis as angiotensin converting
Cheung HS, Wang FL, Miguel AO, Emily FS and David WC. 1980.
Binding of peptide substrates and inhibitors of angiotensin-con-
Cooper JB, Khan G, Taylor G, Tickle IJ and Blundell TL. 1990. X-ray
yses of aspartic proteinases. II. Three-dimensional structure of
the hexagonal crystal form of porcine pepsin at 2.3 A\textdegree resolution.
J Mol Biol 214, 199-222.
Cushman DW and Cheung HS. 1971. Spectrophotometric assay and
roperties of the angiotensin-converting enzyme of rabbit lung. Bio-
chem Pharmacol 20, 1637-1648.
Elias RJ, Kellery SS and Decker EA. 2008. Antioxidant activity of pro-
Fernandes JMO, Kemp GD, Molle MG and Smith VJ. 2002. Anti-mi-
crobial properties of histone H2A from skin secretions of rainbow
out, Oncorhynchus mykiss. Biochem J 368, 611-620.
Fujita H, Yokoyama K and Yoshikawa M. 2000. Classification and an-
thypertensive activity of angiotensin I converting enzyme inhibi-
tory peptides derived from food proteins. J Food Sci 65, 564-569.
Gokoglu N, Yerlikaya P and Cengiz E. 2004. Effects of cooking meth-
ods on the proximate composition and mineral contents of rainbow
out (Oncorhynchus mykiss). Food Chem 84, 19-22.
Hong F, Ming L, Yi S, Zhanxia L, Yongguan W and Chi L. 2008. The
thypertensive effect of peptides: a novel alternative to drugs?
Peptides 29, 1062-1071.
Jang SI, Marsden MJ, Secombes CJ, Choi MS, Kim YG, Kim KJ and
Chung HT. 1998. Effect of glycyrrhizin on rainbow trout Oncorhynchus
Je JY, Park JY, Jung WK, Park PJ and Kim SK. 2005a. Isolation of
angiotensin I converting enzyme (ACE) inhibitor from fermented
I converting enzyme (ACE) inhibitory peptide derived from the
auce of fermented blue mussel, Mytilus edulis. Bioresour Technol
96, 1624-1629.
and in vivo evaluation of novel angiotensin-I-converting enzyme
Khantaphant S, Benjakul S and Ghomi MR. 2011. The effects of pre-
treatments on antioxidative activities of protein hydrolysates from
the muscle of brownstripe red snapper (Lutjanus vitta). LWT Food
Sci Technol 44, 1139-1148.
Kim SY, Park PSW and Rhee KC. 1990. Functional properties of pro-
teolytic enzyme modified soy protein isolate. J Agric Food Chem
38, 651-656.
Korhonen H and Pihlanto A. 2006. Bioactive peptides: production and
functionality. Int Dairy J 16, 945-960.
Korhonen M, Pihlanto-Leppälä A and Tupsela T. 1998. Impact of pro-
cessing on bioactive proteins and peptides. Trends Food Sci Technol
9, 307-319.
Lee JK, Hong S, Jeon JK, Kim SK and Byun HG. 2009. Purification and
characterization of angiotensin I converting enzyme inhibitory pep-
tides from the rotifer, Brachionus rotundiformis. Bioresearch Technol
100, 5255-5259.
Lee JK, Lee MS, Park HG, Kim SK and Byun HG. 2010. Angiotensin
I converting enzyme inhibitory peptide extracted from freshwater
Lee JK, Jeon JK and Byun HG. 2011. Effect of angiotensin I con-
verting enzyme inhibitory peptide purified from skate skin hydrolysate.
Food Chem 125, 495-499. Li GH, Le GW, Shi YH and Shrestha S.
2004. Angiotensin I-converting enzyme inhibitory peptides derived
m from food proteins and their physiological and pharmaco-
logical effects. Nutr Res 24, 469-486.
Li ZH, Velisek J, Zlabek V, Grabic R, Machova J, Kolarova J and Ran-
dak T. 2010. Hepatic antioxidant status and hematological parame-
ters in rainbow trout, Oncorhynchus mykiss, after chronic exposure
Pihlanto-Leppälä A. 2000. Bioactive peptides derived from bovine
whey proteins: opioid and ACE-inhibitory peptides. Trends Food
Pihlanto-Leppälä A, Koskinen P, Piilola K, Tupsela T and Korho-
nen H. 2000. Angiotensin I-converting enzyme inhibitory properties
of whey protein digestes: concentration and characterization of active
Schwaiger J, Ferling H, Mallow U, Wintemayr H and Negele RD.
2004. Toxic effects of the non-steroidal anti-inflammatory drug di-
clofenac. Part I. histopathological alterations and bioaccumulation
in rainbow trout. Aquat Toxicol 68, 141-150.
Sheih IC, Fang TJ and Wu TK. 2009. Isolation and characte-
risation of a novel angiotensin I-converting enzyme (ACE) inhibitory
Simpson BK. 2000. Digestive proteinases from marine animals. In: Sea
food Enzymes: Utilization and Influence on Postharvest Seafood
quality. Haard NF, Simpson BK, eds. Marcel Dekker, New York,
US, pp. 531-540.
Tsai JS, Lin TC, Chen JL and Pan BS. 2006. The inhibitory effects of
freshwater clam (Corbicula fluminea, Muller) muscle protein hy-
drolysates on angiotensin I converting enzyme. Process Biochem
41, 2276-2281.
fication and identification of a ACE inhibitory peptide from oyster

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