Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005, Pages 176-183 藥物食品分析 第十三卷 第二期

Low-molecular-weight Peptides as Related to Antioxidant Properties of Chicken Essence

HUI-CHUN WU[1], BONNIE SUN PAN[1], CHE-LANG CHANG[2] AND CHYUAN-YUAN SHIAU[1]*

[1.] Department of Food Science, National Taiwan Ocean University, 2 Pei-Ning Rd., Keelung City 202, Taiwan, R.O.C.

[2.] Research and Development Center for Life Science, Great Wall Enterprise Co., Ltd., 222, Sec. 3, Ta-Tung Rd., Hsichih City, Taipei County 221, Taiwan, R.O.C.

(Received: October 22, 2004; Accepted: March 8, 2005)

ABSTRACT

Different antioxidant measurements, including the inhibition of linoleic acid autoxidation, scavenging effect on α,α-diphenyl-β-picrylhydrazyl free radical, reducing power, and chelating abilities of metal ions Cu^{2+} and Fe^{2+}, showed that the extract of chicken essence possessed antioxidant activities. The antioxidant activity increased with increasing concentration. Chicken essence contained a considerable amount of free amino acids, of which taurine was the predominant compound. Low-molecular-weight peptides were also present in a large quantity. An extraordinary feature was that the product contained high levels of potent antioxidants, anserine and carnosine. Three antioxidant peptide fractions of chicken essence were separated by size exclusion chromatography. The peptide with molecular weight of approximately 1400 Da possessed the strongest antioxidant activity, followed by peptides with 900 and 500 Da. Two antioxidant peptides were further isolated and identified, and their amino acid sequences were His-Val-Thr-Glu-Glu and Pro-Val-Pro-Ala-Glu-Gly-Val, respectively.

Key words: chicken essence, antioxidant activity, peptide, anserine, carnosine

INTRODUCTION

Chicken soup and chicken essence have been consumed traditionally as diet supplements for sickness, enhancement of mental efficiency, and recovery from mental fatigue[1,2]. Studies have shown that chicken essence increased the metabolic rate in healthy volunteers and rats[2,3] and increased the restoration of serum Fe after blood donation in females[4]. The consumption of chicken essence caused an increase in brain 5-hydroxyptrytamine (5-HT) in the cerebrospinal fluid of the rat and led to the activation of 5-HT-dependent physiological process such as sleep improvement, mood elevation and regulation of circadian rhythm[5]. Recent studies reported that chicken essence markedly suppressed the elevation of blood pressure, cardiovascular hypertrophy and renal damage in hypertension rats[6-8].

Oxidation is essential to living organisms to fuel biological processes. The uncontrolled production of free radical derived from oxidation may be associated with the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis[9]. Almost all organisms are well protected against free radical damage by enzymes such as catalase and superoxide dismutase, or antioxidant compounds such as ascorbic acid, tocopherols and glutathione[10]. When the antioxidant defenses are inadequate, deterioration of physiological functions may occur and result in diseases. Therefore, the antioxidants in human diet are of great interest as possible protective agents to help human body reduce oxidative damage. The chicken meat contained a considerable amount of histidine-containing dipeptides, carnosine (β-alanylhistidine) and anserine (β-alanyl-1-methylhistidine)[11,12]. Many studies have demonstrated these two dipeptides possessed antioxidatant and free radical-scavenging functions[12-14] which may contribute to vasodilatory actions[15] and the antihypertensive effect on animals[8].

Although efforts have been devoted to study the physiological functions of chicken essence[1-8], little information is available on the antioxidant properties. The present study was therefore undertaken to investigate the antioxidant activity of chicken essence. The methods including inhibition of linoleic acid autoxidation, scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical, reducing power, and chelating ability of metal ions were used to evaluate the activities. The levels and compositions of free amino acids and low-molecular-weight peptides were determined to find out their association with antioxidant activities. The potent antioxidant peptides from the chicken essence were also isolated and identified in the present study.

MATERIALS AND METHODS

I. Materials

DaChan Chicken Essence was supplied by the Great Wall Enterprise Co. Ltd. (Taipei, Taiwan). The product is a

* Author for correspondence. Tel: +886-2-24622192; Fax: +886-2-24634203; E-mail: cyshiau@mail.ntou.edu.tw

natural extract without the addition of ingredients such as Chinese medicines or plant extracts. Three groups were collected and at least three cans from each group were combined and homogenized for chemical analyses. The Sephadex G-25 column used for analyzing the molecular weight distribution was purchased from Pharmacia (Uppsala, Sweden). The TSK ODS 120 T column (5 μm, 4.6 × 250 mm) used for peptide separation was purchased from Toso (Tokyo, Japan).

II. *Extraction of Chicken Essence*

The extract of chicken essence was prepared according to the method of Konosu *et al.*[16]. Five grams of sample was homogenized for 2 min in 20 mL of 7% cold trichloroacetic acid (TCA) using a Polytron homogenizer. The homogenate was centrifuged at 4000 ×g (4˚C) for 20 min. The precipitate was extracted twice with TCA by the same procedure. The supernatants were combined and made up to 100 mL with TCA. A volume of 40 mL TCA-extracted supernatant was mixed with an equal amount of ether to remove the TCA. This procedure was repeated successively for five times. The aqueous solution was evaporated to dryness *in vacuo* at a temperature below 40˚C. The dried matter was diluted with water and made up to 25 mL for analyses of antioxidant activities and free amino acids and peptides. All analyses were done in three replicates.

III. *Size Exclusion Chromatography*

Peptide fractions of chicken essence were separated using column chromatography as described by Chen *et al.*[17]. The extract of chicken essence was passed through a 5-K MWCO (molecular weight cut-off membrane) and fractionated by gel filtration on Sephadex G-25 column (1.5 × 85 cm), and eluted with deionized water. Each 5 mL fraction was collected at a flow rate of 30 mL/hr, and monitored at 280 nm absorbance to separate peptide fractions.

IV. *Purification of Antioxidant Peptides*

The antioxidant fractions were separated by TSK ODS 120 T column (5 μm, 7.5 × 250 mm) and analyzed by a Hitachi L-7100 high performance liquid chromatography (HPLC). A linear gradient of acetonitrile (0-40% in 60 min) in 0.1% trifluoroacetic acid (TFA) was accomplished at room temperature and flow rate of 1.5 mL/min. The elution peaks were monitored at 220 nm, and their antioxidant activities were measured. The antioxidant peptides were further isolated by HPLC using a TSK ODS 120 T column (5 μm, 4.6 × 250 mm) with a linear gradient of acetonitrile in 0.1% TFA at a flow rate of 1 mL/min.

V. *Measurements of Antioxidant Activities*

(I) *Inhibition of autoxidation in a linoleic acid system*

The antioxidant activity of chicken essence extract was determined according to the ferric thiocyanate method[17,18]. A volume of 0.5 mL of extract, 1.0 mL of 0.1 M sodium phosphate buffer (pH 7.0), and 1.0 mL of 50 mM linoleic acid in ethanol (95%) were mixed in a 5 mL tube. The test tubes were placed in dark at 60˚C to accelerate oxidation. To 50 μL of the reaction mixture were added 2.35 mL of 75% ethanol, 50 μL of 30% ammonium thiocyanate, and 50 μL of 20 mM ferrous chloride solution in 3.5% HCl. After the mixture was stirred for 3 min, the peroxide value was determined spectrophotometrically at 500 nm. The number of days taken to attain an absorbance (A500) of 0.3 was defined as the induction period[18] which was indicative of the relative antioxidant activity of the sample. The analyses of all samples were run in three replicates.

(II) *Scavenging effect on DPPH free radical*

The scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured by Shimada *et al.*[19] with some modifications. Sample solution (1.5 mL) was added to 1.5 mL of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at room temperature. Absorbance of the resulting solution was measured at 517 nm. The lower absorbance (A_{517}) represents higher DPPH scavenging activity, which is expressed as [(blank A_{517} − sample A_{517}) / blank A_{517}] × 100%.

(III) *Reducing power*

The reducing power was measured according to the method of Oyaizu[20]. A volume of 2 mL sample was added to 2 mL of 0.2 M phosphate buffer (pH 6.6) and 2 mL of 1% potassium ferricyanide. After the mixture was incubated at 50˚C for 20 min, 2 mL of 10% TCA was added to the reaction mixture. Two milliliter from the incubated mixture was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride in test tubes. After 10 min of reaction, the resulting solution was measured at 700 nm. Increased absorbance (A_{700}) of the reaction mixture indicated increased reducing power.

(IV) *Chelating of metal ions Cu^{2+} and Fe^{2+}*

The Cu^{2+}-chelating ability was measured according to the method of Shimada *et al.*[19]. After 2 mL of sample was mixed with 2 mL of 10 mM hexamine buffer containing 10 mM KCl and 3 mM $CuSO_4$, 0.2 mL of 1 mM tetramethyl murexide was added to the solution. The mixture was shaken and left for 3 min at room temperature, and the absorbance of the resulting solution was then measured at 485 nm. The chelating of Fe^{2+} by the sample was estimated by the method of Dinis *et al.*[21]. The extract (1 mL) was added to a solution of 2 mM ferrous chloride (0.1 mL) and 3.7 mL of deionized water. After 3 min, the reaction was inhibited by the addition of 5 mM ferrozine

(0.2 mL). The mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the resulting solution was measured at 562 nm. The chelating ability is expressed as [(blank A_{562} − sample A_{562}) / blank A_{562}] × 100%.

VI. *Determination of Free Amino Acids, Anserine and Carnosine*

Free amino acids (FAAs) and dipeptides, carnosine and anserine, were analyzed by a Hitachi L-8500 high-speed amino acid analyzer with a Hitachi 2622 SC packed column (4.6 mm × 60 mm). The buffers used were the standard lithium citrate buffers. Postcolumn derivatization with ninhydrin yielded amino acid derivatives which were measured at 570 nm and 440 nm. Analytical conditions and procedure were performed according to the method of Shiau *et al.*[22]. The levels of FAAs, carnosine and anserine were estimated based on peak areas of known concentrations of the standards (Wako, Ltd., Osaka, Japan) using a Hitachi D-2850 Chromato data processor.

VII. *Determination of Amino Acids of Low-molecular-weight Peptides*

Amino acids were measured before and after hydrolysis of the TCA extracts using 6 N HCl at 110°C for 24 hr in an evacuated sealed tube. The difference between the amino acids obtained without the pre-hydrolysis and the values obtained after the HCl hydrolysis was the constituted amino acids of low-molecular-weight peptides[23].

VIII. *Analyses of Amino Acid Sequence*

The amino acid sequences of the functional peptides with antioxidant activity were identified using *N*-terminal sequencer (Model ABI/LC 494 protein sequencer) by Taiwan Protein Inc. (Taipei, Taiwan).

RESULTS

I. *Antioxidant Activities*

The inhibition on autoxidation of linoleic acid by the chicken essence extract was expressed as the relative antioxidant activity based on the induction period using the ferric thiocyanate method[17,18]. Longer induction period on linoleic acid peroxidation indicates stronger antioxidant activity. The induction periods increased from 3 to 12 days as the chicken essence concentrations increased from 5 to 160 mg/mL (Figure 1). The result indicated chicken essence exhibited noticeable antioxidant activity which was concentration dependent.

DPPH is a stable free radical that shows maximum A_{517} in ethanol. When DPPH encounters a proton-donating substance such as an antioxidant, the radical would be scavenged and the absorbance reduced[19]. Based on this principle, the antioxidant activity of a substance can be expressed as its ability to scavenge the DPPH radical. In this study, scavenging ability of the chicken essence extract on DPPH radical increased with increasing concentration, and reached a maximum of 59% as the concentration increased to 120 mg/mL (Figure 2). The chicken essence extract might contain electron donors and could react with free radicals to covert them to more stable products and terminate radical chain reaction.

The reducing power (measured as A_{700}) and levels of chicken essence extract are directly proportional (Figure 2). The result indicated that reductone-related products appeared to be present in chicken essence. Chicken essence

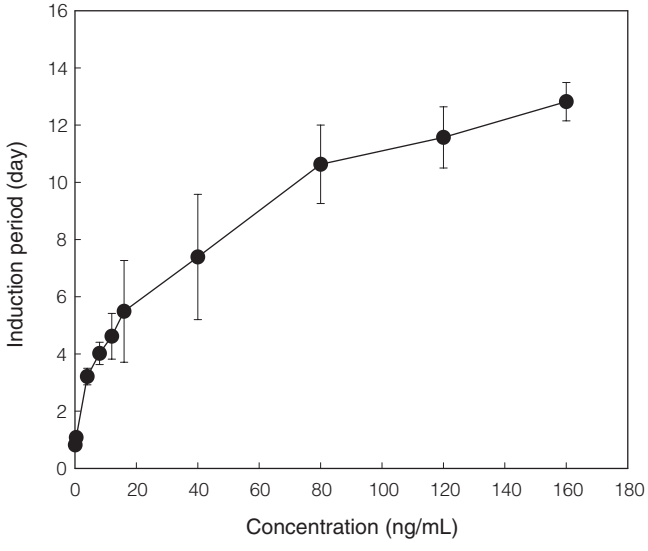

Figure 1. The inhibition on linoleic acid autoxidation by TCA extract of chicken essence at various concentrations. The number of the relative antioxidant activity is based on the induction period, which was 0.78 day for the control group. Each value is expressed as mean ± SD (n = 3).

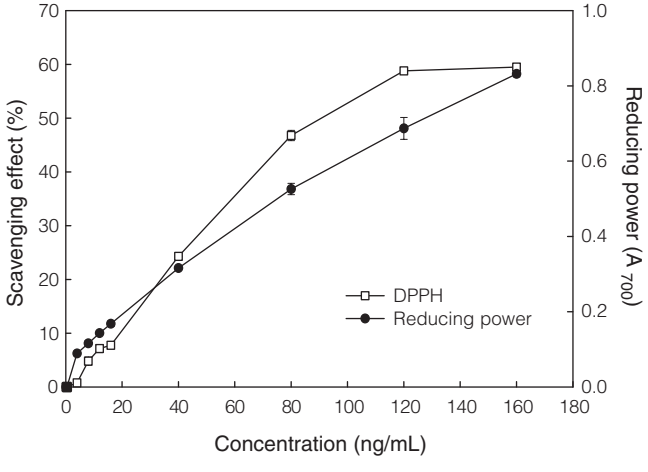

Figure 2. The α,α-Diphenyl-β-Picrylhydrazyl (DPPH) free radical scavenging effects and reducing power (A_{700}) of chicken essence TCA extract at various concentrations. Each value is expressed as mean ± SD (n = 3).

Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005

also showed increased chelating activities on Cu^{2+} and Fe^{2+} at higher concentration (Figure 3). The results obtained from this study showed that chicken essence demonstrated capacities for binding Cu^{2+} and Fe^{2+}.

II. *Free Amino Acids, Anserine, Carnosine and Low-molecular-weight Peptides*

DaChan chicken essence was consisted of moisture 89.9%, protein 10.3%, and ash 1.0% (data not shown). Fat was negligible. On a dry weight basis, protein was the predominant proximate composition in the product, followed by ash.

Table 1 shows the profile of free amino acids (FAAs , expressed as mg/100g wet weight) in the TCA extract of chicken essence. The predominant FAA was taurine (2-aminoethanesulfonic acid), which accounted for 37% of the total FAAs. Other FAAs abundant in chicken essence included glutamic acid, glycine and alanine which accounted for 9, 6 and 9% of the total FAAs, respectively.

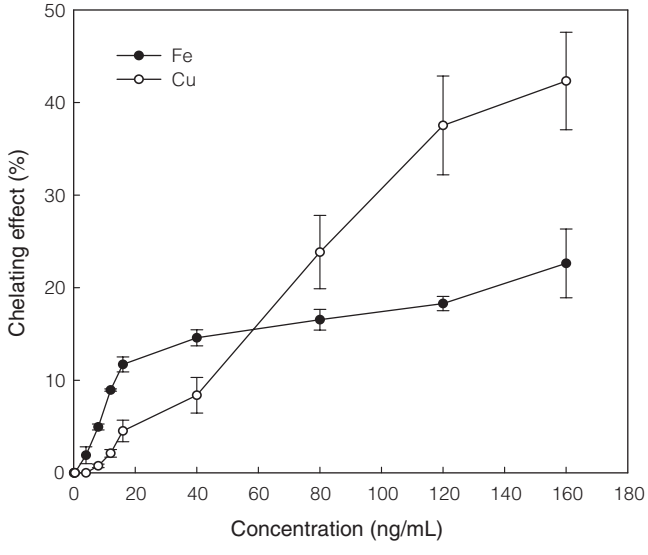

Figure 3. Chelating abilities on copper and ferrous ions of chicken essence TCA extract at various concentrations. Each value is expressed as mean ± SD (n = 3).

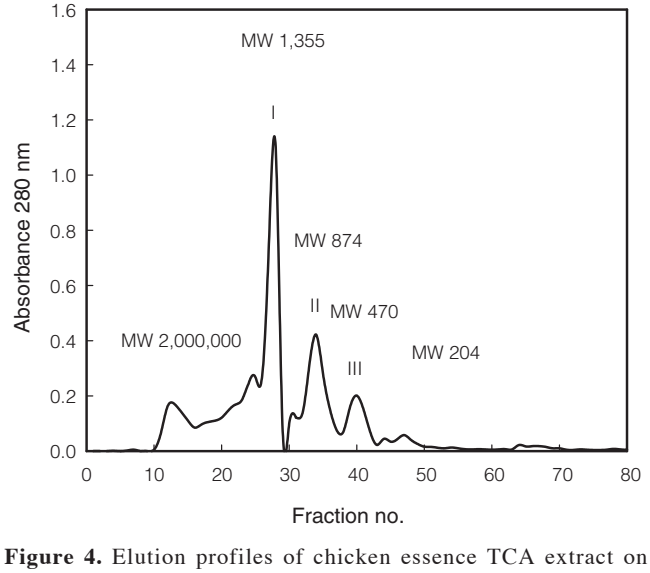

Figure 4. Elution profiles of chicken essence TCA extract on Sephadex G-25. Fractionation: 5 mL; flow rate: 30 mL/hr; eluent: water.

Table 1. The contents (mg/100g) of free amino acid, anserine, carnosine and constituted amino acids of peptides in chicken essence

Amino acid	Free form	Peptide form
Phosphoserine	3.3 ± 0.5[a]	—[b]
Taurine	66.3 ± 14.8	—
Aspartic acid	7.7 ± 1.6	204.4 ± 40.7
Threonine	5.4 ± 0.8	69.4 ± 13.8
Serine	8.3 ± 1.1	90.1 ± 16.2
Glutamic acid	16.0 ± 2.3	229.4 ± 76.9
Glycine	10.4 ± 1.7	675.5 ± 152.3
Alanine	15.1 ± 2.0	269.3 ± 51.8
Valine	7.2 ± 0.3	57.1 ± 19.3
Methionine	1.7 ± 0.3	34.7 ± 10.3
Isoleucine	2.0 ± 0.4	47.0 ± 19.2
Leucine	3.8 ± 0.6	107.5 ± 25.6
Tyrosine	3.5 ± 0.5	24.7 ± 4.0
Phenylalanine	2.6 ± 0.4	61.4 ± 11.8
β-Alanine	1.9 ± 0.4	93.6 ± 18.7
Ornithine	0.3 ± 0.2	0.8 ± 0.7
Lysine	7.0 ± 1.3	126.7 ± 28.6
1-Methylhistidine	—	152.3 ± 27.8
Histidine	2.1 ± 0.3	78.8 ± 16.4
3-Methylhistidine	—	2.7 ± 1.7
Arginine	5.3 ± 0.9	227.0 ± 48.6
Proline	7.5 ± 0.5	328.6 ± 77.5
Total	177.3 ± 31.0	2876.7 ± 501.3
Carnosine	94.1 ± 35.0	—
Anserine	267.8 ± 73.9	—

[a]Each value is expressed as mean ± SD (n = 3).
[b]Not detectable.

Large amount of histidine-containing dipeptides, anserine and carnosine, were present in the chicken meat[11,12]. The chicken essence also had high concentrations of anserine and carnosine (Table 1). Anserine was found much higher than carnosine, and even higher than the total FAAs.

The constituted amino acids of low-molecular-weight peptides in chicken essence were much higher than the FAAs present in the product in Table 1. Glycine, proline, alanine, glutamic acid, arginine and aspartic acid were the major constituted amino acids of the small peptides in chicken essence. Taurine, the most predominant FAA, was not detected, and apparently existed in a free form in the extract. Carnosine and anserine were included in the low-molecular-weight peptides. After hydrolysis of the TCA extract using high concentration of HCl, the constituted amino acids of carnosine and anserine including β-alanine, histidine and 1-methylhistidine were detected in high levels as shown in Table 1.

III. *Separation and Identification of Antioxidant Peptides*

To analyze molecular weight distribution and relative

antioxidant activities of the desired peptides in chicken essence, the extract was separated by size exclusion chromatography on Sephadex G-25. Three major fractions were present as shown in Figure 4. Fraction I, II, and III were estimated to be about 1400, 900, and 500 Da compared to the molecular weight standards. Table 2 shows the antioxidant activities of three peptide fractions. Fraction I possessed a strong antioxidant activity, followed by fraction II and III. Results showed that the peptide from chicken essence with molecular weight of approximately 1400 Da

Table 2. The antioxidant activity of different fractions from chicken essence

Fraction	Inhibition on autoxidation of linoleic acid[a]	Scavenging effect on DPPH radical (%)	Reducing power (700 nm)	Chelating effect on Cu^{2+} (%)
I[b]	6.05	10.4	0.23	6.1
II	3.18	5.2	0.19	5.6
III	1.37	—[c]	0.08	4.2

[a]The results are shown as the relative antioxidant activity based on the induction period. The induction period of control group was 1.09 day.
[b]The concentrations of I, II, and III were 97, 123, and 38 mg/100 mL, respectively.
[c]Not detectable.

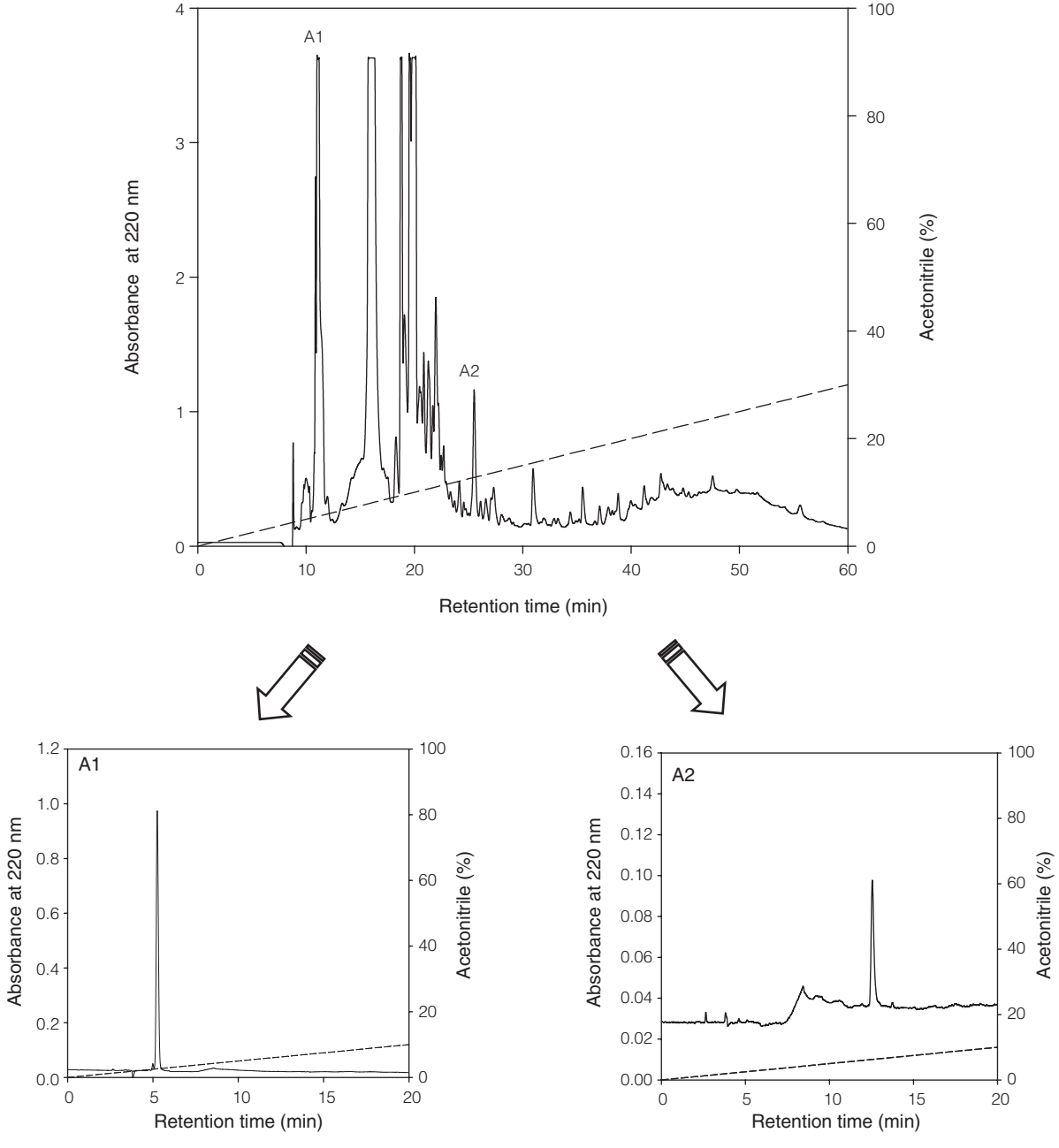

Figure 5. Isolation and purification of antioxidant peptides from fraction II by reverse-phase C18 column and HPLC analysis.

Journal of Food and Drug Analysis, Vol. 13, No. 2, 2005

Table 3. Amino acid sequences and antioxidant activities of isolated antioxidant peptides from chicken essence

Peptide	Amino acid sequences	Inhibition on autoxidation of linoleic acid (day)[a]	Days/μg
A1[b]	His-Val-Thr-Glu-Glu	2.49	0.003
A2[b]	Pro-Val-Pro-Ala-Glu-Gly-Val	6.50	0.722

[a]The results are shown as the relative antioxidant activity based on the induction period. The induction period of control group was 1.20 day.
[b]The solid content of A1 was 763 μg/mL and A2 was 9 μg/mL.

and 900 Da possessed stronger antioxidant activity.

Fraction I and II were further used for the separation and purification of the antioxidant peptides by reversed-phase HPLC using a 0.1% TFA-acetonitrile system. Two peaks in Fraction I and 3 peaks in Fraction II of HPLC chromatograms were collected for further analyses of amino acid sequences. However, only two peaks in Fraction II (Peak A1 and A2 in Figure 5) could be successfully identified for their amino acid sequences. The two peptides comprised amino acid residues of hydrophobic amino acids, histidine (His) and proline (Pro) at *N*-terminal position, and of valine (Val), threonine (thr), Pro, glutamic acid (Glu), alanine (Ala) and glycine (Gly) in the sequence. The amino acid sequence of A1 was His-Val-Thr-Glu-Glu, and that of A2 was Pro-Val-Pro-Ala-Glu-Gly-Val (Table 3). The induction periods of A1 and A2 were 2.5 and 6.5 days, respectively (Table 3).

DISCUSSION

The extract of chicken essence possessed antioxidant activities including the inhibition of linoleic acid autoxidation, scavenging effect on DPPH free radical, reducing power, and chelating ability of metal ions. Results of our another experiment, based on linoleic acid peroxidation, showed that the induction periods of 0.02 mg/mL butylated hydroxyanisol (BHA), 80 mg/mL chicken essence, 0.04 mg/mL α-tocopherol and 1.8 mg/mL ascorbic acid were 11, 10, 4 and 2 days, respectively (data not shown). Accordingly, the antioxidant activity of 80 mg/mL of chicken essence was stronger than 0.04 mg/mL of α-tocopherol and 1.8 mg/mL of ascorbic acid, but slightly weaker than 0.02 mg/mL of BHA. However, chicken essence at concentration of 120 mg/mL or higher would possess stronger antioxidant activity than BHA at 0.02 mg/mL.

The major FAAs in chicken essence were taurine, glutamic acid, alanine and glycine. The later three FAAs are recognized as the taste-active components of various foods[24]. These FAAs present in chicken essence may contribute to the taste of the product. Taurine is an essential growth factor followed recognition of its role in bile acid synthesis and in prevention of certain pathological problems. Its accumulation is necessary for functional regulation of the eyes, heart, muscle, brain and central nervous system[25-27]. Some studies also indicated that taurine functions as an antioxidant in certain tissues[27-29], but the mechanisms were unclear.

Chicken essence contained high levels of anserine and carnosine which have many physiological functions. Their antioxidant abilities have recently attracted considerable attention in search for effective eye disorder remedies[12,14]. Boldyrev *et al.*[30] demonstrated that anserine and carnosine could decrease membrane lipid/oxidation rates by measuring thiobarbituric acid reactive substance. Proposed mechanisms of antioxidative activities of carnosine and anserine are ascribed to their oxidation based on free radical quenching reaction, reducing power and metal chelating abilities. Rubtsov *et al.*[31] reported that carnosine possessed a hydroxyl radical-scavenging activity and became effective on inhibiting oxidation. Similarly, Aruoma *et al.*[14] also indicated that carnosine could prevent biomacromolecules from •OH damage. Erickson and Hultin[32] indicated that, under inhibitory conditions, histidine-related compounds capable to inhibit lipid peroxidation might stem from its ability to coordinate with iron, thus preventing reduction of Fe^{3+} to Fe^{2+}. Furthermore, nuclear magnetic resonance (NMR) studies showed the chelation of metal ion by the imidazole ring of histidine-related compounds, as indicated by the loss of the C-2 and C-4 peaks of imidazole ring[32]. The fact that the extract of chicken essence possessed DPPH free radical quenching activity, reducing power, and chelating ability of metal ions may be associated with its high levels of carnosine and anserine. In addition to antioxidant activity, the biological roles of these two dipeptides have been postulated to include the control of enzyme activity[33], neurotransmitter function[13,34], and intracellular buffer[35]. Hence, the roles of anserine and carnosine as nutritional ingredients and as endogenous antioxidants in meat essences cannot be underestimated.

In addition to carnosine and anserine, a considerable amount of low-molecular-weight peptides were present in chicken essence. Some natural food products contained oligopeptides that can reduce the rate of autoxidation in foods. Potatoes and mushrooms contained low-molecular-weight peptides (approximately 1,000 Da) which inhibit polyphenol oxidase activity[36]. Similarly, a peptide found in honey (approximately 600 Da) inhibited polyphenol oxidase in apple slices, grape juice, and model food system[37]. Several peptides derived from protein hydrolyzates also possessed antioxidant activities[17,38,39]. Wu *et al.*[39] indicated that the peptide of mackerel protein hydrolysate with molecular weight approximately 1,400 Da showed stronger *in vitro* antioxidant activity than other peptides.

Chen *et al.*[38] demonstrated that antioxidant activity of the peptides depended on amino acid composition and their

sequences. Histidine-containing peptides possessed a higher activity than others, and can be attributed to the chelating and lipid radical trapping abilities of the imidazole ring[38,40,41]. Several amino acids, such as histidine, threonine, lysine, and methionine, showed antioxidant activity in sunflower oil emulsions[42]. Antioxidative capacity of proline was equivalent to that of BHA in sardine oil[43], and a nitroxide derivative of proline was reported to have antioxidant activity[44]. Due to the desired amino acids including histidine, threonine and proline comprised in the sequence, the identified peptide A1 and A2 in chicken essence are expected to possess strong antioxidant activity. The separation and identification of more active peptides need further investigations.

CONCLUSIONS

Chicken essence had noticeable antioxidant property, and the activity was concentration dependent. Dipeptides, anserine and carnosine, and other low-molecular-weight peptides such as His-Val-Thr-Glu-Glu and Pro-Val-Pro-Ala-Glu-Gly-Val were associated with the antioxidant activity.

REFERENCES

1. Nagai, H., Harada, M., Nakagawa, M., Tanaka, T., Gunadi, B., Setiabudi, M. L. J., Uktolseja, J. L. A. and Miyata, Y. 1996. Effects of chicken extract on the recovery from fatigue caused by mental workload. Appl. Human Sci. 15: 281-286.
2. Geissler, G., Boroumand-Naini, M. and Tomassen, C. 1989. Large acute thermic response to chicken essence in humans. Nutr. Rep. Int. 39: 547-556.
3. Ikeda, T., Nishijima, Y., Kiso, Y., Shibata, H., Ono, H. and Moritani, T. 2001. Effects of chicken essence tablets on resting metabolic rats. Biosci. Biotechnol. Biochem. 65: 2083-2086.
4. Williams, A. T. and Scey, S. A. 1993. Use of traditional blood remedy. A study on regular donors. Int. J. Food Sci. Nutr. 44: 17-20.
5. Xu, C. L. and Sim, M. K. 1997. Effect of oral feeding of essence of chicken on the level of 5-hydroyindole acetic acid in the cerebrospinal fluid of the rat. Int. J. Food Sci. Nutr. 48: 113-117.
6. Matsumura, Y., Okui, T., Ono, H., Kiso, Y. and Tanaka, T. 2001. Antihypertensive effects of chicken extract against deoxycorticosterone acetate-salt-induced hypertension in rats. Biol. Pharm. Bull. 24: 1181-1184.
7. Sim, M. K. 2001. Cardiovascular action of chicken-meat extract in normo- and hypertensive rats. British J. Nutr. 86: 97-103.
8. Matsumura, Y., Kita, S., Ono, H., Kiso, Y. and Tanaka, T. 2002. Preventive effect of a chicken extract on the development of hypertension in stroke-prone spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. 66: 1108-1110.
9. Halliwell, B. and Gutteridage, J. M. C. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1-4.
10. Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. JAOCS 75: 199-212.
11. Crush, K. G. 1970. Carnosine and related substances in animal tissues. Comp. Biochem. Physiol. 34: 3-30.
12. Chan, K. M. and Decker, E. A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. Nutr. 34: 403-426.
13. Boldyrev, A. A., Stvolinsky, S. L., Tyulina, O. V., Koshelev, V. B., Hori, N. and Carpenter, D. O. 1997. Biochemical and physiological evidence that carnosine is an endogenous neuroprotector against free radicals. Cell. Molecul. Neurobiol. 17: 259-271.
14. Aruoma, O. I., Laughton, M. J. and Halliwell, B. 1989. Carnosine, homocarnosine and anserine: could they act as antioxidants *in vivo*? Biochem. J. 264: 863-869.
15. Ririe, D. G., Roberts, P. K., Shouse, M. N. and Zaloga, G. P. 2000. Vasodilatory actions of the dietary peptide carnosine. Nutrition 16: 168-172.
16. Konosu, S., Watanabe, K. and Shimizu, T. 1974. Distribution of nitrogenous constituents in the muscle extracts of eight species of fish. Bull. Jpn. Soc. Sci. Fish. 40: 909-915.
17. Chen, H. M., Muramoto, K. and Yamaguchi, F. 1995. Structural analysis of antioxidative peptides from soybean β-conglycinine. J. Agric, Food Chem. 43: 574-578.
18. Mitsuda, H., Yasumoto, K. and Iwami, K. 1966. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyoto Shokuryo 19: 210-214.
19. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the antioxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 40: 945-948.
20. Oyaizu, M. 1988. Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakkaishi 35: 771-775.
21. Dinis, T. C. P., Madeira, V. M. C. and Almeida, L. M. 1994. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315: 161-169.
22. Shiau, C. Y., Pong, Y. J., Chiou, T. K. and Chai, T. 1996. Free amino acids and nucleotide-related compounds in milkfish (*Chanos chanos*) muscle and viscera. J. Agric. Food Chem. 44: 2650-2653.
23. Chiou, T. K. and Konosu, S. 1988. Changes in extractive components of dried mullet roe. Nippon Suisan Gakkaishi 54: 307-313.
24. Fuke, S. 1994. Taste-active components of seafoods with special reference to umami substances. In

"Seafoods: Chemistry, Processing Technology and Quality". pp. 115-139. Shahidi, F. and Botta, J. R. eds. Blackie Academic & Professional. Glasgow, U. K.

25. Huxtable, R. J. 1992. Physiological actions of taurine. Physiol. Rev. 72: 101-163.
26. Sturman, J. A. 1993. Taurine in development. Physiol. Rev. 73: 119-147.
27. Timbrell, J. A., Seabra, V. and Waterfield, C. J. 1995. The *in vivo* and *in vitro* protective properties of taurine. Gen. Pharmac. 26: 453-462.
28. Pasantes-Morales, H. and Cruz, C. 1985. Taurine and hypotaurine inhibit light-induced lipid peroxidation and protect rod outer segment structure. Brain Res. 330: 154-157.
29. Schuller-Levis, G., Quinn, M. R., Wright, C. and Park, E. 1994. Taurine protects oxidant-induced lung injury: possible mechanism(s) of action. Adv. Exp. Med. Biol. 359: 31-39.
30. Boldyrev, A., Dupin, A., Pindel, E. and Severin, S. 1988. Antioxidative properties of histidine-containing dipeptides from skeletal muscles of vertebrates. Comp. Biochem. Physiol. 89B: 245-250.
31. Rubtsov, A. M., Schara, M., Senticurc, M. and Boldyrev, A. A. 1991. Hydroxyl radical-scavenging activity of carnosine: a spin trapping study. Acta. Pharm. Jugosl. 41: 401-407.
32. Erickson, M. C. and Hultin, H. O. 1992. Influence of histidine on lipid peroxidation in sarcoplasmic reticulum. Arch. Biochem. Biophys. 292: 427-432.
33. Ikeda, T., Kimura, K., Hama, T. and Tamaki, N. 1980. Activiation of rabbit muscle fructose 1,6-biphosphatase by histidine and carnosine. J. Biochem. 87: 179-185.
34. Boldyrev, A. A. 2001. Carnosine as a modulator of endogenous Zn^{2+} effects. Trends Pharmacol. Sci. 22: 112-113.
35. Abe, H. 1995. Histidine-related dipeptides: distribution, metabolism, and physiological function. In "Biochemistry and Molecular Biology of Fishes". pp. 309-333. Hochachka, P. W. and Mommsen, T. P. eds. Elsevier. Amsterdam, Netherlands.
36. Lyengar, R. and McEvily, A. J. 1992. Anti-browning agents: alternatives to the use of sulfites in foods. Trends Food Sci. Technol. 3: 60-64.
37. Oszmianski, J. and Lee, C. Y. 1990. Inhibition of polyphenol oxidase activity and browning by honey. J. Agric. Food Chem. 38: 1892-1895.
38. Chen, H. M., Muramoto, K., Yamaguchi, F., Fujimoto, K. and Nokihara, K. 1998. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J. Agric. Food Chem. 46: 49-53.
39. Wu, H. C., Chen, H. M. and Shiau, C. Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Res. Int. 36: 949-957.
40. Uchida, K. and Kawakishi, S. 1988. Selective of tryptophan and histidine residues in protein through the copper-catalyzed autoxidation of L-ascorbic acid. Agric. Biol. Chem. 52: 1529-1535.
41. Uchida, K. and Kawakishi, S. 1990. Formation of the 2-imidazolone structure within a peptide mediated by a copper(II)/ascorbate system. J. Agric. Food Chem. 38: 1896-1899.
42. Riison, T., Sims, R. J. and Fioriti, J. A. 1980. Effect of amino acids on autoxidation of sunflower oil in emulsions. JAOCS 57: 354-359.
43. Revankar, G. D. 1974. Proline as an antioxidant in fish oil. J. Food Sci. Technol. 11: 10-11.
44. Lin, J. S., Tom, T. C. and Olcott, H. S. 1974. Proline nitroxide. J. Agric. Food Chem. 22: 526-528.