Journal of Food and Drug Analysis, Vol. 11, No. 2, 2003, Pages 148-153 藥物食品分析 第十一卷 第二期

Antioxidant Activities of Carnosine, Anserine, Some Free Amino Acids and Their Combination

HUI-CHUN WU[1], CHYUAN-YUAN SHIAU[1]*, HUA-MING CHEN[2] AND TZE-KUEI CHIOU[1]

[1.] *Department of Food Science, National Taiwan Ocean University, 2 Peining Rd., 202 Keelung, Taiwan, R.O.C.*

[2.] *Department of Cosmetic Applications and Management, Tung-Fong Institute of Technology, 110 Tung-Fong Rd., Hunei, 829 Kaohsiung, Taiwan, R.O.C.*

(Received: July 16, 2002; Accepted: November 20, 2002)

ABSTRACT

For the evaluation of antioxidant activities of dipeptides (carnosine, anserine), and free amino acids (histidine, 1-methylhistidine, taurine, glycine, alanine, β-alanine), four methods were used, including the analysis of the inhibition of linoleic acid autoxidation, scavenging effect on α,α-diphenyl-β-picrylhydrazyl free radical, reducing power, and chelating ability of Cu^{2+}. Results showed that carnosine and anserine were antioxidants preventing lipid peroxidation in linoleic acid systems. They also possessed effective abilities as free radical scavengers, reducing agents, and copper ion chelators. The activities increased with increasing concentration. However, the constituent amino acids of dipeptides, β-alanine, histidine and 1-methylhistidine, were not as effective in inhibiting oxidation regardless of individual or combined usage. Taurine, glycine, alanine and β-alanine showed much weaker antioxidant activities than carnosine, anserine, histidine and 1-methylhistidine. No synergistic effects on antioxidation were found among compounds used in combination. The results suggested that histidine-containing compounds were related to antioxidation abilities, and the peptide linkage between β-alanine, histidine and 1-methylhistidine was involved in the antioxidant activities of carnosine and anserine.

Key words: antioxidant activity, anserine, carnosine, free amino acids

INTRODUCTION

Chicken essence is widely consumed in Southeast Asia as a traditional health food commonly used as a nutritional supplement, for the enhancement of mental efficiency, and recovery from mental fatigue[(1)]. Our previous study[(2)] revealed that chicken essences contained large amounts of free amino acids (FAAs) and dipeptides, of which anserine (β-alanyl-1-methylhistidine), carnosine (β-alanylhistidine) and taurine (2-aminoehanesulfonic acid) were the dominant compounds. In addition, glycine and alanine were also abundant in chicken essences. The role of these FAAs and dipeptides in human physiological functions has become interesting for studies.

Extracts of meat tissues contain FAAs, peptides and other non-protein nitrogenous compounds which are not incorporated in proteins. FAAs such as glycine and alanine have been implicated to be responsible for the characteristic taste of food[(3,4)]. Some specific FAAs play important roles in physiological functions such as buffer ability and osmoregulation in the tissues of animals. Free histidine, for instance, functions as an intracellular buffer in fish when vigorous movements result in the accumulation of acidic end products during periods of anaerobic metabolism[(3)]. Taurine is very widespread in animal tissues. It is an essential growth factor following the recognition of its role in bile acid synthesis and in the prevention of certain pathological problems. Its accumulation is necessary for regulating the functions of the eyes, heart, muscles, brain and central nervous system[(5-7)]. Carnosine and anserine were the major dipeptides in the skeletal muscle tissue of most vertebrates[(8-11)]. Like histidine, carnosine and anserine have been recognized as a potent intracellular pH-buffer[(11,12)]. Their antioxidative abilities have recently attracted considerable attention in the search for effective prevention of oxidation of foods and for eye disorder remedies[(10,12,13)].

Many studies[(13-17)] have been done on the antioxidant activities of carnosine, but related information on FAAs and other dipeptides are limited. The purpose of this study is to further confirm the antioxidant properties of carnosine and anserine with different measurements analyzing the inhibition of linoleic acid autoxidation, scavenging effects on DPPH (α,α-diphenyl-β-picrylhydrazyl) free radical, reducing power, and chelating ability of Cu^{2+}. In addition, the antioxidant activities of FAAs (histidine, 1-methylhistidine, taurine, glycine, alanine, β-alanine) are investigated. The effects of antioxidant activities among FAAs and dipeptides in combination are also evaluated in this study.

MATERIALS AND METHODS

I. *Reagents*

All chemicals and reagents were of the highest purity available. Carnosine, anserine, histidine, β-alanine, 1-methylhistidine, taurine, glycine, alanine, linoleic acid, α,α-

* Author for correspondence. Tel:886-2-24622192 ext. 5129; Fax:886-2-24634203; E-mail:cyshiau@mail.ntou.edu.tw

diphenyl-β-picrylhydrazyl (DPPH), tetramethyl murexide (TMM), butylhydroxyanisole (BHA), α-tocopherol, citric acid, and ethylene diamine tetraacetic acid (EDTA) were obtained from Sigma Chemical Co. (St. Louis, MO). Ammonium thiocyanate (NH_4SCN), ferrous chloride ($FeCl_2$), potassium ferricyanide ($K_3Fe(SCN)_6$), ferric chloride ($FeCl_3$), trichloroacetic acid (TCA), hexamine, and copper sulfate ($CuSO_4$) were purchased from Riedel-de Haen Chemical Co., Ltd., Germany.

II. *Methods*

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

The antioxidant activity was determined according to the ferric thiocyanate method[18]. Various concentrations of carnosine, anserine, free amino acids and their combinations from 0.5 to 40 mM were prepared for the experiment. Deionized water, pretreated with reverse osmosis membrane, was used as the control sample. 0.5 mL of sample, 1.0 mL 0.1 M sodium phosphate buffer (pH 7.0), and 1.0 mL of 50 mM linoleic acid in ethanol (95%) were mixed in 5 mL tubes. The test tubes were placed in the dark at 60˚C to accelerate oxidation. To 50 μL of the reaction mixtures 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 stirring the mixtures for 3 minutes, the peroxide values were determined by recording absorbance readings at 500 nm. The number of days taken to attain an absorbance of 0.3 was defined as the induction period. The induction period indicates the relative antioxidative activity of the samples. The analyses of all samples were conducted in three replicates.

(II) *Scavenging effect on DPPH radical*

The scavenging effect of carnosine, anserine, and free amino acids on α,α-diphenyl-β-picrylhydrazyl (DPPH) radical was measured using a method published by Shimada *et al.*[19] with some modification. Chemical compounds and their combinations in concentrations from 0.5 to 40 mM were prepared for the experiment. Deionized water, pretreated with reverse osmosis membrane, was used as the blank sample. 1.5 mL of each sample was added to 1.5 mL of 0.1 mM DPPH in 95% ethanol. The mixtures were shaken and left alone for 30 minutes at room temperature. The absorbances of the resulting solutions were measured spectrophotometrically at 517 nm. The lower the absorbance, the higher the DPPH scavenging activity. The scavenging effect is expressed as [(blank absorbance–sample absorbance) / blank absorbance] ×100%.

(III) *Reducing power*

The reducing power of carnosine, anserine, and free amino acids was measured according to the method of Oyaizu[20]. Chemical compounds and their combinations in concentrations from 0.5 to 40 mM were prepared for the experiment. Deionized water, pretreated with reverse osmosis membrane, was used as the blank sample. 2 mL of each sample was added to 2 mL of 0.2 M phosphate buffer (pH 6.6) and 2 mL of 1% potassium ferricyanide. The mixtures were incubated at 50˚C for 20 minutes, and then 2 mL of 10% tricholoroacetic acid was added to each reaction mixture. 2 mL from each incubated mixture was mixed with 2 mL of distilled water and 0.4 mL of 0.1% ferric chloride in the tubes. After 10 minutes the resulting solutions were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

(IV) *Cu^{2+}-chelating ability*

Cu^{2+}-chelating ability of carnosine, anserine, and free amino acids was measured according to the method published by Shimada *et al.*[19]. Chemical compounds and their combinations in concentrations from 0.5 to 40 mM were prepared for the experiment. Deionized water, pretreated with reverse osmosis membrane, was used as the blank sample. 2 mL of each sample was mixed with 2 mL of 10 mM hexamine buffer containing 10 mM KCl and 3 mM $CuSO_4$. Then 0.2 mL of 1 mM tetramethyl murexide (TMM) was added. The mixtures were shaken and left alone for 3 minutes at room temperature. The absorbance of the resulting solutions was then measured at 485 nm. The chelating ability is expressed as [(blank absorbance–sample absorbance) / blank absorbance] ×100%

RESULTS AND DISCUSSION

I. *Inhibition of Linoleic Acid Autoxidation*

The inhibition of linoleic acid autoxidation by free amino acids and dipeptides was expressed as the relative antioxidant activity based on the days of the induction period according to the ferric thiocyanate method[18]. Table 1 shows the antioxidant activities of carnosine (β-alanylhistidine), anserine (β-alanyl-1-methylhistidine), histidine, β-alanine, 1-methylhistidine, taurine, glycine and alanine. Carnosine exhibited the strongest ability among all the compounds, and its activity increased with increasing concentration from 0.5 to 40 mM. Anserine, the other dipeptide, showed a lower activity than that of carnosine. The antioxidation activity of anserine reached the highest at a concentration of 10 mM, and no further increases were found at the higher concentrations. In addition to carnosine and anserine, other histidine-related compounds including 1-methylhistidine and histidine also possessed antioxidant abilities; however, the former had a much higher activity than the latter. Although glycine and alanine both showed antioxidation capabilities at concentrations of 20-40 mM, their activities were much lower as compared to those of carnosine and 1-methylhistidine. Taurine and β-alanine, on

Table 1. Antioxidant activity of dipeptides and free amino acids based on the induction period (day) * of linoleic acid autoxidation

	Concentration (mM)					
	0.5	2.5	5.0	10	20	40
Carnosine	5.70 ± 1.03**	8.83 ± 0.60	8.20 ± 0.92	9.17 ± 0.98	9.39 ± 0.85	10.66 ± 3.96
Anserine	2.03 ± 0.74	2.77 ± 1.19	3.08 ± 0.98	3.30 ± 0.93	2.97 ± 1.02	2.24 ± 0.57
Histidine	1.57 ± 0.36	1.58 ± 0.59	1.90 ± 0.47	2.14 ± 0.71	2.05 ± 1.26	1.59 ± 0.73
Taurine	1.20 ± 0.14	1.12 ± 0.25	1.16 ± 0.10	1.20 ± 0.21	1.12 ± 0.10	1.06 ± 0.16
Glycine	1.27 ± 0.07	1.95 ± 0.13	2.19 ± 0.80	2.50 ± 0.12	3.01 ± 0.14	2.91 ± 0.52
Alanine	1.33 ± 0.19	1.67 ± 0.34	2.09 ± 0.27	2.24 ± 0.55	2.67 ± 0.52	2.71 ± 0.55
β-Alanine	1.05 ± 0.04	1.11 ± 0.07	1.07 ± 0.05	1.17 ± 0.12	1.18 ± 0.08	1.21 ± 0.03
1-Methylhistidine	1.34 ± 0.12	2.79 ± 0.57	3.70 ± 0.60	5.98 ± 0.65	7.66 ± 0.29	8.39 ± 0.24
Carnosine + Anserine	3.61 ± 1.38	6.07 ± 3.19	6.39 ± 2.64	7.06 ± 2.90	6.10 ± 2.71	5.36 ± 1.04
Carnosine + Histidine	4.07 ± 1.09	7.69 ± 1.84	9.11 ± 1.73	8.79 ± 1.34	8.18 ± 1.90	6.21 ± 0.89
Anserine + Histidine	1.99 ± 0.36	2.15 ± 0.14	1.82 ± 0.08	1.71 ± 0.17	1.46 ± 0.00	1.47 ± 0.11
Carnosine + Anserine + Histidine	2.53 ± 0.04	4.03 ± 0.18	4.71 ± 0.31	4.45 ± 0.27	3.59 ± 0.16	2.94 ± 0.21
β-Alanine + Histidine	1.55 ± 0.03	1.84 ± 0.20	2.02 ± 0.09	2.23 ± 0.03	2.31 ± 0.01	2.26 ± 0.02
β-Alanine + 1-Methylhistidine	1.21 ± 0.08	1.44 ± 0.06	1.66 ± 0.16	2.11 ± 0.07	2.66 ± 0.01	1.86 ± 1.62

* : The results are shown as the relative antioxidative activity based on the days of induction period.
The induction period of control group is 1.19 day. The induction period of 0.05 and 0.5 mM BHA are 4.32 and 9.31 days, respectively.
The induction period of 0.05 and 0.5 mM α-tocopherol are 1.15 and 2.19 days, respectively.
** : Expressed as mean ± standard deviation (n = 3).

Table 2. Scavenging effect (%)* of dipeptides and free amino acids on α,α-diphenyl-β-picrylhrazyl (DPPH) radical

	Concentration (mM)					
	0.5	2.5	5.0	10	20	40
Carnosine	1.3 ± 1.0**	4.4 ± 2.9	9.2 ± 3.0	19.7 ± 4.8	30.9 ± 5.7	44.0 ± 4.7
Anserine	1.3 ± 1.8	7.8 ± 0.4	11.3 ± 2.0	19.1 ± 2.6	33.7 ± 2.4	50.6 ± 3.1
Histidine	0.2 ± 0.4	2.3 ± 1.2	2.7 ± 1.3	4.9 ± 1.8	7.4 ± 2.9	13.0 ± 1.1
Taurine	—***	—	—	—	—	—
Glycine	—	—	—	—	—	—
Alanine	—	—	—	—	—	—
β-Alanine	—	—	—	—	—	—
1-Methylhistidine	0.6 ± 0.5	1.9 ± 0.5	2.9 ± 0.5	5.0 ± 0.7	8.5 ± 0.3	14.4 ± 0.3
Carnosine + Anserine	1.6 ± 0.2	3.7 ± 0.3	6.9 ± 0.2	11.9 ± 0.3	18.9 ± 1.2	36.3 ± 0.5
Carnosine + Histidine	1.7 ± 0.3	3.5 ± 0.3	6.1 ± 0.8	12.2 ± 0.7	20.3 ± 2.0	34.0 ± 0.4
Anserine + Histidine	2.4 ± 0.6	4.1 ± 0.1	5.8 ± 0.2	8.3 ± 0.4	14.9 ± 0.7	25.1 ± 0.5
Carnosine + Anserine + Histidine	1.6 ± 0.2	4.3 ± 0.1	7.1 ± 0.5	10.9 ± 0.4	17.8 ± 1.2	32.7 ± 0.2
β-Alanine + Histidine	1.0 ± 0.3	1.4 ± 0.3	1.6 ± 0.3	2.1 ± 0.4	3.1 ± 0.2	4.4 ± 0.5
β-Alanine + 1-Methylhistidine	1.0 ± 0.3	1.2 ± 0.2	1.9 ± 0.1	2.6 ± 0.1	4.0 ± 0.6	5.7 ± 1.0

* : The results are shown as [(Blank absorbance–Sample absorbance)/Blank absorbance] × 100%. The scavenging effect of 0.05, 0.1, 0.5 mM BHA are 78.1%, 81.0%, 81.4%, respectively.
** : Expressed as mean ± standard deviation (n = 3).
*** : Not detectable.

the other hand, exhibited no inhibition abilities. No synergistic effects on the inhibition of linoleic acid autoxidation were found among carnosine, anserine and histidine. The combinations of two free amino acids, β-alanine and histidine, or β-alanine and 1-methylhistidine also showed no synergistic effects, and their activities were even weaker than carnosine and anserine alone.

Boldyrev *et al.*[14] demonstrated that carnosine and anserine could decrease membrane lipid/oxidation rates by measuring the level of thiobarbituric acid reactive substance (TBARS). Our study, using the method of inhibition in linoleic acid peroxidation, also revealed that carnosine possessed strong antioxidant activity. Inhibition of lipid oxidation by carnosine was concentration dependent with significant antioxidant activities occuring at concentrations comparable to those in skeletal tissues[8-11]. The induction period of linoleic acid peroxidation of 2.5 mM carnosine was slightly shorter than that obtained from 0.5 mM BHA, but much longer than that of 0.5 mM α-tocopherol. The constituent amino acids of carnosine, β-alanine and histidine, were not as effective at inhibiting oxidation regardless of individual or combined usage. Anserine, which differed from carnosine only in the methylation of imidazole group, showed a less-pronounced antioxidant activity than carnosine. The results indicated that the inhibition activity on the autoxidation of linoleic acid by histidine-containing dipeptides depended on amino acid composition.

II. *Scavenging of DPPH Radical*

The scavenging activities of dipeptides and free amino

acids on DPPH radical are shown in Table 2. The histidine-related compounds including histidine, 1-methylhistidine, carnosine and anserine, possessed abilities to scavenge DPPH radicals, and activities increased with increasing concentration. However, non-histidine-related compounds such as taurine, glycine, alanine and β-alanine showed no scavenging activities at any concentration. No synergistic effect was found between carnosine and anserine. The constituent amino acids of carnosine and anserine including β-alanine, histidine and 1-methylhistidine also showed no synergistic effects on scavenging DPPH radicals, and their activities were even weaker than carnosine and anserine by themselves. The results showed that histidine and its related compounds were free radical inhibitors, as well as primary antioxidants that reacted with free radicals.

Rubtsov *et al.*[15] reported that carnosine possessed hydroxyl radical-scavenging capabilities and was effective in inhibiting oxidation. Aruoma *et al.*[16] indicated that carnosine could prevent biomacromolecules from •OH damage. Our study also revealed that carnosine and anserine possessed DPPH radical quenching abilities. The scavenging of DPPH by carnosine and anserine suggests that neither the imidazole group methylation nor the free carboxyl group amidation interferes greatly with quenching ability. The constituent amino acids, β-alanine, histidine and 1-methylhistidine, were not as effective at quenching DPPH radicals when used individually or in combination. This suggests that the peptide linkage between β-alanine, histidine and 1-methylhistidine was involved in the antioxidant activity of the histidine-contained dipeptides. The fact that only histidine-related compounds possessed the abilities suggested that the histidine group was responsible for the DPPH radical scavenging activity.

III. *Reducing Power*

Table 3 shows the reducing power (absorbance at 700 nm) of dipeptides, free amino acids and their synergistic effects. Anserine, with concentrations from 2.5 to 40 mM, exhibited the greatest reducing power among all compounds. The reducing power of anserine at 40 mM (0.90) was higher than 0.5 mM of BHA (0.78). Carnosine was the second compound with a strong reducing power, but that was only about half of anserine. The powers of these two dipeptides strengthened with increasing concentration. In contrast to dipeptides, free amino acids showed little activity at any concentration. The dipeptides had reducing power when used individually or in combination; however, no synergistic effect was found between compounds. The results revealed that anserine and carnosine were electron donors and could react with free radicals to covert them to more stable products and terminate radical chain reactions. The constituent amino acids of anserine and carnosine possessed little reducing power, suggesting that the peptide linkage between β-alanine, histidine and 1-methylhistidine was involved in the reducing capacity of the histidine-contained dipeptides.

IV. *Chelating Ability of Cu^{2+}*

The chelating abilities of Cu^{2+} by dipeptides and free amino acids are shown in Table 4. All compounds tested in this study increased the chelating abilities of Cu^{2+} with increasing concentration from 0.5 to 40 mM. At the same concentration, 1-methylhistidine and histidine showed stronger abilities than the other compounds. The chelating abilities of 1-methylhistidine and histidine at 40 mM were similar to that obtained from 4 mM EDTA (68%); however, they were much stronger than that of 4 mM citric acid (27%). Anserine exhibited the weakest ability among the histidine-related compounds. Non-histidine-related compounds, such as glycine, alanine and β-alanine, had abilities for chelating Cu^{2+}, but their abilities were only about half of histidine and 1-methylhistidine. Taurine, on

Table 3. Reducing power* of dipeptides and free amino acids

	Concentration (mM)					
	0.5	2.5	5.0	10	20	40
Carnosine	0.06 ± 0.00**	0.09 ± 0.00	0.11 ± 0.01	0.15 ± 0.01	0.25 ± 0.02	0.45 ± 0.05
Anserine	0.08 ± 0.01	0.16 ± 0.08	0.27 ± 0.18	0.39 ± 0.27	0.60 ± 0.38	0.90 ± 0.49
Histidine	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
Taurine	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Glycine	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
Alanine	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01
β-Alanine	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00
1-Methylhistidine	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.06 ± 0.00
Carnosine + Anserine	0.07 ± 0.00	0.10 ± 0.01	0.14 ± 0.01	0.21 ± 0.01	0.35 ± 0.01	0.49 ± 0.02
Carnosine + Histidine	0.06 ± 0.00	0.08 ± 0.00	0.10 ± 0.00	0.13 ± 0.01	0.18 ± 0.01	0.31 ± 0.01
Anserine + Histidine	0.07 ± 0.00	0.09 ± 0.01	0.11 ± 0.01	0.16 ± 0.01	0.25 ± 0.01	0.42 ± 0.02
Carnosine + Anserine + Histidine	0.07 ± 0.00	0.09 ± 0.00	0.12 ± 0.01	0.17 ± 0.01	0.28 ± 0.01	0.46 ± 0.01
β-Alanine + Histidine	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
β-Alanine + 1-Methylhistidine	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00

* : Absorbance at 700 nm. The reducing power of control group is 0.05. The reducing power of 0.05, 0.1, 0.5 mM BHA are 0.09, 0.10 and 0.78, respectively.

** : Expressed as mean ± standard deviation (n = 3).

Table 4. Chelating effect (%)* of dipeptides and free amino acids on Cu^{2+}

	Concentration (mM)					
	0.5	2.5	5.0	10	20	40
Carnosine	17.4 ± 6.2**	27.1 ± 3.2	32.7 ± 2.9	52.7 ± 1.5	61.2 ± 1.3	62.5 ± 2.3
Anserine	18.8 ± 2.7	25.5 ± 3.7	26.9 ± 3.7	28.2 ± 3.6	29.3 ± 3.3	31.3 ± 3.6
Histidine	15.5 ± 1.4	27.0 ± 3.3	37.1 ± 3.4	59.4 ± 1.8	66.1 ± 0.6	66.1 ± 0.2
Taurine	4.5 ± 1.6	3.5 ± 0.5	3.4 ± 1.3	3.3 ± 2.4	4.9 ± 0.9	6.9 ± 0.5
Glycine	11.3 ± 3.5	25.8 ± 2.4	27.3 ± 3.1	29.5 ± 3.2	30.7 ± 3.6	33.2 ± 3.1
Alanine	12.9 ± 1.5	25.5 ± 3.3	28.9 ± 2.8	30.0 ± 3.0	30.8 ± 3.7	32.4 ± 2.7
β-Alanine	4.2 ± 0.6	20.0 ± 3.8	35.7 ± 4.5	41.7 ± 3.7	42.3 ± 3.6	44.5 ± 4.4
1-Methylhistidine	14.1 ± 2.4	43.0 ± 2.6	48.9 ± 2.2	60.4 ± 1.6	66.3 ± 1.0	67.6 ± 0.8
Carnosine + Anserine	7.9 ± 0.2	38.8 ± 0.4	40.8 ± 0.6	43.6 ± 0.7	48.2 ± 0.4	57.6 ± 0.2
Carnosine + Histidine	6.1 ± 0.6	32.9 ± 1.3	41.8 ± 0.5	47.7 ± 0.2	58.5 ± 0.1	71.5 ± 0.1
Anserine + Histidine	9.4 ± 0.7	39.3 ± 0.3	40.9 ± 0.3	44.9 ± 0.7	51.4 ± 0.3	66.6 ± 0.2
Carnosine + Anserine + Histidine	7.8 ± 1.1	38.4 ± 0.8	40.8 ± 0.8	45.4 ± 0.6	52.1 ± 0.2	67.0 ± 0.5
β-Alanine + Histidine	5.0 ± 1.5	26.3 ± 4.3	39.2 ± 4.0	41.1 ± 4.7	46.2 ± 5.6	64.2 ± 1.8
β-Alanine + 1-Methylhistidine	5.9 ± 1.4	23.4 ± 4.3	37.8 ± 4.2	39.9 ± 4.9	42.8 ± 6.5	57.8 ± 1.3

* : The results are shown as [(Blank absorbance–Sample absorbance) / Blank absorbance] × 100%. The scavenging effect of 4 mM EDTA and citric acid are 68.0% and 26.5%, respectively.

** : Expressed as mean ± standard deviation (n = 3).

the other hand, showed a very weak ability even at the higher concentration of 40 mM. Carnosine, anserine, histidine, β-alanine and 1-methylhistidine showed no synergistic effects on chelating abilities of Cu^{2+}. Erickson and Hultin[21] indicated that, under inhibitory conditions, histidine's ability to inhibit lipid peroxidation might stem from its ability to coordinate with iron, thus preventing the reduction of Fe^{3+} to Fe^{2+}. Nuclear magnetic resonance (NMR) studies revealed that the chelation of metal ions with the imidazole ring group of histidine-related compounds has been indicated by the loss of the C-2 and C-4 peaks of the imidazole ring[21]. The results obtained from our study showed that histidine-related compounds demonstrated capacities for Cu^{2+} binding, suggesting that their activities as peroxidation protectors might be related to its metal binding capacity of the imidazole group.

CONCLUSIONS

The histidine-containing dipeptides, carnosine and anserine, are antioxidants preventing lipid peroxidation in linoleic acid systems. They also possessed effective abilities as free radical scavengers, reducing agents, and copper ion chelators. These activities increased with increasing concentration. However, the constituent amino acids, β-alanine, histidine and 1-methylhistidine, were not as effective at inhibiting oxidation regardless of individual or combined usage. Taurine, glycine, alanine and β-alanine showed much weaker antioxidant activities than carnosine, anserine, histidine and 1-methylhistidine. No synergistic effects were found on antioxidation among compounds used in combination. The results indicated that an imidazole group of histidine-containing compounds was related to the ability of antioxidation, and the peptide linkage between β-alanine, histidine and 1-methylhistidine was also involved in the activities of the dipeptides. The roles of dipeptides as endogenous antioxidants in meat essences cannot be underestimated. These compounds could be applied to inactivate lipid oxidation catalysts and to chelate metal ions in medicine and food preservation.

ACKNOWNLEDGEMENT

This work was supported by a grant (NSC-89-2313-B-019-065) from the National Science Council, Taiwan, R.O.C.

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. Wu, H. C. and Shiau, C. Y. 2002. Proximate composition, free amino acids and peptides contents in commercial chicken and other meat essences. J. Food Drug Anal. 10: 170-177.
3. Konosu, S. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. In "Chemistry and Biochemistry of Marine Food Products". pp. 367-404. Martin, R. E., Flick, G. J., Hebard, E. and Ward, D. R. eds. AVI Publishing Co., Westport, CT, U.S.A.
4. 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, UK.
5. Sturman, J. A. 1993. Taurine in development. Physiol. Rev. 73: 119-147.
6. Stapleton, P. P., Charles, R. P., Redmond, H. P. and

Bouchier-Hayes, D. J. 1997. Taurine and human nutrition. Clin. Nutr. 16: 103-108.
7. Redmond, H. P., Stapleton, P. P., Neary, P. and Bouchier-Hayes, D. 1998. Immunonutrition: The role of taurine. Nutr. 14: 599-604.
8. Crush, K. G. 1970. Carnosine and related substances in animal tissues. Comp. Biochem. Physiol. 34: 3-30.
9. Suyama, M., Suzuki, T., Maruyama, M. and Satio, K. 1970. Determination of carnosine, anserine, and balenine in the muscle of animal. Bull. Jap. Soc. Sci. Fish. 36: 1048-1053.
10. Chan, K. M. and Decker, E. A. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. Nutr. 34: 403-426.
11. Harris, R. C., Marlin, D. J., Dunnett, M., Snow, D. H. and Hultmam, E. 1990. Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. Comp. Biochem. Physiol. 97A: 249-251.
12. Abe, H. 1995. Histidine-related dipeptides: distribution, metabolism, and physiological function. In "Biochemistry and Molecular Biology of Fishes". Vol. 4. pp. 309-333. Hochachka, P. W. and Mommsen, T. P. eds. Elsevier, Amsterdam, Holland.
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. 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.
15. 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.
16. 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.
17. Decker, E., Crum, A. and Calvert, J. 1992. Differences in the antioxidant mechanism of carnosine in the presence of copper and iron. J. Agric. Food Chem. 40: 756-759.
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. Erickson, M. C. and Hultin, H. O. 1992. Influence of histidine on lipid peroxidation in sarcoplasmic reticulum. Arch. Biochem. Biophys. 292: 427-432.