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Distribution of γ-Glutamyl-β-alanylhistidine Isopeptide in the Macromolecular Fractions of Commercial Meat Extracts and Correlation with the Color of the Macromolecular Fractions

MOTONAKA KURODA* AND TSUTOMU HARADA

Seasoning Research and Development Department, Ajinomoto Company, Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan

The measurement of γ-glutamyl-β-alanylhistidine isopeptide in the macromolecular fraction of various commercial meat extracts indicated that all of the commercial meat extracts tested contained the isopeptide, in concentrations ranging from 0.04 to 0.87 μmol/g of dry matter. This variation was suggested to be due to the differences between the processes of extraction and the differences in the initial amounts of carnosine. A positive correlation between the content of γ-glutamyl-β-alanylhistidine and the color of the macromolecular fraction was observed. These results suggested that γ-glutamyl-β-alanylhistidine is widely distributed in meat products and that the content can be used as an index of protein denaturation during the heating process.

KEYWORDS: γ-Glutamyl-β-alanylhistidine; isopeptide; beef extract; chicken extract; color

INTRODUCTION

The Maillard reaction plays an important role in the formation of color and aroma compounds during the heating of foods, and this reaction is known to occur during the production of various foods (*1*).

Meat extract is used commercially as a seasoning material and is usually produced by concentration after extraction of meat (beef, pork, or chicken) with hot water. As some commercial meat extracts have a dark brown color, browning reactions (Maillard reaction) are considered to occur during the production of meat extract, especially during the concentration process. It is known that meat extract has high viscosity, which was suggested to be due to the soluble collagen (gelatin) in the extract. The heating process decreases the viscosity of meat extracts (*2*), and it was suggested to be due to the changes in the structure of proteinaceous materials in the meat extract.

Solubilization of collagen occurs during the preparation of beef soup stock solution, and the ratio of collagen to total protein in the soup stock solution increases up to ∼30% (*3*). The present study suggested that the major proteinaceous material in beef soup stock solution and beef extract was soluble collagen (gelatin). Furthermore, it was also reported that several sarcoplasmic proteins remained in the beef meat extract prepared by heating beef meat cubes or beef meat homogenate with hot water (*4*−*6*). On the basis of these previous observations, beef extract and other meat extracts are considered to contain soluble collagen and sarcoplasmic proteins. These previous studies suggested that changes in the amino acid composition in protein might occur during production of the meat extract, resulting in a decrease in the viscosity. Meat extract could be used as a suitable model system for the investigation on the interaction between soluble components (especially soluble proteins and lower molecular weight components) in the meat products during the heating process. However, there have been few studies on the changes in protein itself during the heating of meat extract.

Recently, it was reported that levels of histidine and β-alanine in the macromolecular fraction (MMF) of beef soup stock solution increased during the heating process, and a tripeptide considered to be γ-glutamyl-β-alanylhistidine was isolated from the proteolytic digest of the MMF of heated beef soup stock (*7*). These findings suggested that a portion of carnosine (β-alanylhistidine) was incorporated into the MMF and formed γ-glutamyl-β-alanylhistidine. Furthermore, it was also reported that γ-glutamyl-β-alanylhistidine formed from glutamine and carnosine during heating in aqueous solution (*8*), suggesting the formation in the MMF during the heating of beef soup stock solution. In a previous study, the contents of γ-glutamyl-β-alanylhistidine in the MMF of heated beef soup stock were reported to increase during heating (*8*), showing the formation of γ-glutamyl-β-alanylhistidine from glutamine residue and carnosine.

Carnosine is a major imidazole compound in mammalian animal tissues (*9*−*11*). It was reported that a high content of carnosine exists in commercial meat extract, such as beef extract, mutton extract, and chicken extract (*12*), and in commercial meat products (*13*). From these observations and findings obtained previously, it might be possible that the γ-glutamyl-β-alanylhistidine isopeptide is widely distributed in processed meat products, especially in commercial meat extract.

In the present study, to investigate the generality of the formation of γ-glutamyl-β-alanylhistidine isopeptide in food,

* Author to whom correspondence should be addressed (telephone +81-44-210-5721; fax +81-44-244-0577; e-mail motonaka_kuroda@ajinomoto.com).

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the contents of the isopeptide in the MMF of commercial meat extracts were analyzed. Furthermore, to investigate the effect of the isopeptide formation on protein denaturation in the meat extracts, the correlation between the contents of γ-glutamyl-β-alanylhistidine isopeptide and the viscosity or color of the MMF was investigated.

MATERIALS AND METHODS

Commercial Meat Extract Samples. Beef extracts were obtained from 11 different companies. Six were the meat extract type, three were the blended type (mixture of meat extract, bone extract, and other materials such as hydrolyzed vegetable protein), and two were the bone extract type. Chicken extracts were obtained from six different companies. Three were the meat extract type, and three were the blended extract type.

Preparation of Beef Soup Stock. Preparation and heating of beef soup stock were performed as reported previously (*7*). Briefly, 6 kg of beef shank (pieces of 5−10 cm) was boiled at 90−95 °C for 7 h with the addition of 8 L of water. After removal of beef meat, precipitate, and fat, the obtained beef soup stock was freeze-dried and stored at −25 °C until use.

Preparation and Heating of Model Chicken Extract. Commercial minced chicken meat (mechanically deboned chicken meat; 1 kg) was placed into an aluminum pot with 200 mL of water and boiled at 95−97 °C for 1 h. After removal of chicken meat by centrifugation (2500*g*, 10 min), the fat was removed by decantation. The obtained chicken extract was heated at 95 °C for up to 40 h.

Preparation of the Macromolecular Fraction. Commercial meat extract, heated beef soup stock, and heated model chicken extract were dissolved in distilled water and dialyzed against distilled water as reported previously (*7*). Dialysis was performed until the conductivity of the inner solution indicated <200 μS/cm. The obtained MMF were freeze-dried and stored at −25 °C until the analyses.

Chemicals. γ-Glutamyl-β-alanylhistidine was prepared by heating glutamine and carnosine and then isolated as reported previously (*8*). Trifluoroacetic acid (TFA; protein sequencing grade), acetonitrile (HPLC grade), phenylisothiocyanate (PITC; protein sequencing grade), and triethylamine (analytical grade) were purchased from Wako Pure Chemicals, Co., Ltd. (Osaka, Japan). All other reagents were of analytical grade.

Proteolytic Enzymes. *Streptomyces griseus* protease (Pronase E, EC 3.4.24.31, type XXV) and bovine pancreas carboxypeptidase A (EC 3.4.17.1) were purchased from Boehringer Mannheim GmbH (Germany). Porcine kidney leucine aminopeptidase (EC 3.4.11.1, type III) and prolidase (EC 3.4.21.26) were from Sigma Chemical Co. (St. Louis, MO). Wheat carboxypeptidase W (EC 3.4.16.1) was from Seikagaku Kogyo (Tokyo, Japan).

HPLC Analysis of γ-Glutamyl-β-alanylhistidine. *(1) Proteolytic Digestion.* Proteolytic digestion of the macromolecular sample was carried out as reported previously (*8*). Briefly, the macromolecular sample (5 mg/0.5 mL in 0.1 M borate buffer, pH 8.0) was digested by Pronase E (1.0 unit/mg of protein for 24 h, 37 °C). After inactivation by heating at 100 °C for 10 min, digestion was continued for 24 h by adding leucine aminopeptidase (1.0 unit/mg of protein), prolidase (0.5 unit/mg of protein), and carboxypeptidase A (3.0 units/mg of protein) followed by digestion with carboxypeptidase W (4.0 units/mg of protein for 24 h, 37 °C, pH 4.0). In these conditions, the recovery of total free amino acids after proteolytic digestion (ratio against the amounts of amino acids recovered by 6 M HCl hydrolysis) was ∼70% and almost the same in all samples (data not shown). This result suggested that most of the γ-glutamyl-β-alanylhistidine could be liberated by the proteolytic digestion.

(2) Solid-Phase Extraction (SPE) of γ-Glutamyl-β-alanylhistidine. The proteolytic digests obtained as described above were fractionated by SPE using Toyopack IC-SP cartridges (500 mg of resin; Toso Co. Ltd., Tokyo, Japan). The proteolytic digest (from 5 mg of protein) was diluted in 0.1 M HCl and applied to a Toyopack IC-SP cartridge equilibrated with 0.1 M HCl. After washing with 0.1 M HCl (6 mL, three times), the cartridge was air-dried, and the fraction containing γ-glutamyl-β-alanylhistidine was eluted with 5 mL of 80% methanol containing 1% ammonia. The fraction thus obtained was dried using a vacuum concentrator.

(3) Derivatization with Phenylisothiocyanate and High-Performance Liquid Chromatography (HPLC) Separation. Derivatization with PITC was performed according to the method reported previously (*7*). The derivatized sample (20 μL) was applied to an Inertsil ODS-3 column (250 mm × 4.6 mm i.d., particle size = 5 μm, GL Science). The separation was performed using a binary linear solvent gradient. Solution A consisted of 0.15 M ammonium acetate containing 7% (v/v) acetonitrile, and solution B consisted of acetonitrile and water (6:4). The gradient profiles were as follows: 0−15 min, 0% B; 15−25 min, 0−50% B; 25−35 min, 50−100% B. The flow rate was 1 mL/min, detection was performed at UV 269 nm, and column temperature was maintained at 40 °C.

Analyses of Amino Acid Composition of the Macromolecular Fraction. The aqueous solution (100 μL) of the MMF containing ∼2 μg of protein was put into glass tubes (3 mm i.d.), vacuum-dried, and hydrolyzed with 6 M HCl containing 1% phenol at 120 °C for 24 h under a nitrogen atmosphere. The hydrolysates in glass tubes were rinsed with 0.02 M HCl and filtered using Chromatodisk 4A membrane filters (GL Science Co., Ltd., Tokyo, Japan) and analyzed. Amino acid compositions were determined using a model L-8500 amino acid analyzer (Hitachi Co., Ltd., Tokyo) with lithium citrate buffer (PF series for nonhydrolyzed amino acid and peptide analysis; Mitsubishi Chemical Inc., Tokyo, Japan) by postcolumn derivatization with ninhydrin reagent (detected at 570 and 440 nm). The analyses were performed according to the external standard method.

Measurement of Color and Viscosity. The extract or the MMF was diluted with distilled water, passed through a Chromatodisk 25A filter (GL Science, Tokyo, Japan), and put into a cuvette (l = 10 mm), and the absorbance at 500 nm was measured using a U-3210 spectrophotometer (Hitachi Co. Inc., Tokyo, Japan). The viscosity of the MMF solution (5% solution in distilled water) was measured using a B-type viscometer (Vismetron VDA, Shibuya Systems Co. Ltd., Tokyo, Japan) at 35 °C at 60 rpm.

Statistical Analysis. Statistical comparison among means was performed by one-way ANOVA (analysis of variation). When ANOVA was significant, Tukey's multiple-range test was employed for the post hoc comparison. All statistical analyses were performed using SPSS 10.0J software (SPSS Japan Inc., Tokyo, Japan). Trends were considered to be significant when means of compared sets differed at $P < 0.05$.

RESULTS AND DISCUSSION

Measurement of γ-Glutamyl-β-alanylhistidine Isopeptide in Macromolecular Fractions of Commercial Beef Extract and Heated Beef Soup Stock. The contents of γ-glutamyl-β-alanylhistidine in the commercial beef extract and heated beef soup stock are shown in **Table 1**. The findings show that all kinds of commercial beef extract contained γ-glutamyl-β-alanylhistidine. The contents of γ-glutamyl-β-alanylhistidine isopeptide in the MMF of beef extract varied from 0.044 to 0.871 μmol/g of dry matter (DM). The results showed that the contents in the meat extract type were more than that in the blended type and the bone extract. This was possibly because muscular tissue contains high contents of carnosine. Carnosine has been reported to function as a buffering agent in muscle tissue (*10*, *11*) and was reported to be distributed in muscle tissue of many kinds of animals (*9*). In the meat extract type, the contents of γ-glutamyl-β-alanylhistidine isopeptide varied from 0.242 to 0.871 μmol/g of DM. These variations appeared to be due to differences in the contents of carnosine (*7*) and the condition of the heating process in the beef extract production. Furthermore, the contents of γ-glutamyl-β-alanylhistidine in the meat extract type and heated beef soup stock were compared with the contents of β-alanine in the MMF obtained by amino acid analyses (**Table 1**). The results indicate that the amount of γ-glutamyl-β-alanylhistidine was from 3 to 5% of the amount of β-alanine in the beef extract (meat extract type) and heated

Table 1. Contents of γ-Glutamyl-β-alanylhistidine Isopeptide in the Macromolecular Fraction (MMF) of Commercial Beef Extract and Heated Beef Soup Stock[a]

sample	type	yield of MMF[b] (%)	contents of γ-Glu-β-Ala-His[c] (μmol/g of DM)	contents of β-Ala[c] (μmol/g of DM)	relative viscosity at MMF 5% solution (mPa·s)
brand A	meat extract	23.2 ± 0.8	0.616 ± 0.082	18.97 ± 0.39	4.5 ± 1.0
brand B	meat extract	20.0 ± 0.6	0.345 ± 0.071	16.00 ± 2.00	4.4 ± 0.7
brand C	meat extract	25.5 ± 0.8	0.410 ± 0.058	17.45 ± 3.87	4.1 ± 0.5
brand D	meat extract	19.0 ± 0.6	0.871 ± 0.097	38.95 ± 0.53	3.8 ± 0.6
brand E	meat extract	21.9 ± 0.5	0.252 ± 0.049	9.59 ± 0.46	5.2 ± 0.5
brand F	meat extract	22.3 ± 0.6	0.242 ± 0.048	9.64 ± 0.22	4.1 ± 0.4
brand G	blended type	34.4 ± 1.2	0.110 ± 0.021	4.43 ± 0.59	12.8 ± 1.6
brand H	blended type	41.3 ± 1.1	0.396 ± 0.072	7.93 ± 0.67	28.7 ± 3.1
brand I	blended type	34.3 ± 1.4	0.264 ± 0.042	3.99 ± 0.78	17.8 ± 1.2
brand J	bone extract	61.9 ± 2.3	0.107 ± 0.021	1.25 ± 0.61	37.0 ± 3.1
brand K	bone extract	47.4 ± 1.9	0.044 ± 0.005	1.87 ± 0.34	25.0 ± 2.0
beef soup stock heated for 0 h		17.0 ± 1.2	0.288 ± 0.054[a d]	1.59 ± 0.82[a]	225.0 ± 11[a]
beef soup stock heated for 1 h		19.4 ± 1.1	0.456 ± 0.080[b]	8.51 ± 2.06[b]	22.0 ± 3.2[b]
beef soup stock heated for 3 h		19.2 ± 1.5	0.644 ± 0.126[c]	15.52 ± 4.73[c]	5.5 ± 2.1[c]
beef soup stock heated for 6 h		20.1 ± 0.9	0.664 ± 0.113[c]	19.30 ± 4.77[d]	4.2 ± 1.1[c]

[a] Means ± standard deviation of triplicate determinations. [b] Numbers show the yield of MMF from the extract (dry matter). [c] Numbers indicate the molar numbers of γ-glutamyl-β-alanyl-histidine or β-alanine in 1 g (dry matter) of the MMF. [d] Values in a column with different superscript letters are significantly different at $P < 0.05$.

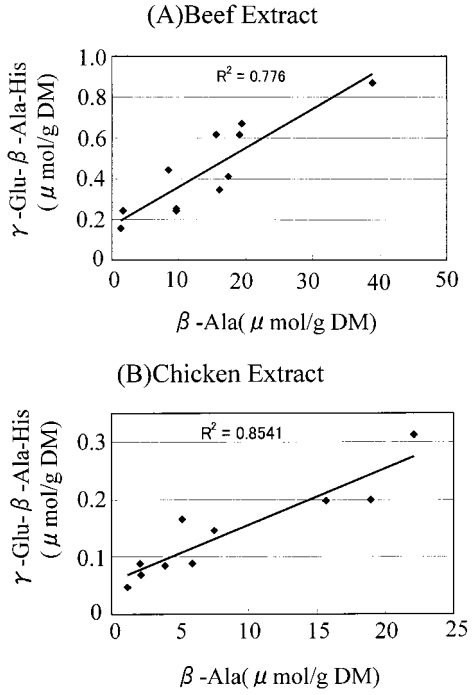

Figure 1. Correlation between the contents of β-alanine and γ-glutamyl-β-alanylhistidine in (A) beef extract samples and (B) chicken extract samples.

beef soup stock, and a positive correlation ($R = 0.881$) between the contents of γ-glutamyl-β-alanylhistidine and β-alanine was observed (**Figure 1A**), suggesting that γ-glutamyl-β-alanylhistidine is formed via a similar mechanism in the heating of beef soup stock and beef extract production. On the other hand, there was a lower correlation for the blended type and bone extract type (ratio of γ-glutamyl-β-alanylhistidine to β-alanine from 2.4 to 8.6%). It might be due to the difference of the beef extract contents and the effects of other kinds of meat extracts. Furthermore, these findings suggest the existence of other β-alanine-containing compounds in the MMF. Recently, it was reported that an incubation (at 37 °C for 2 h) of several proteins with 4-hydroxynonenal (lipd oxidation product) results in the modification of the histidine residue and formd cross-links (*14*, *15*). Thus, it may be suggested that some carnosine has been incorporated into the MMF by a reaction between the histidine moiety and the lipid oxidation products.

Measurement of γ-Glutamyl-β-alanylhistidine Isopeptide in Macromolecular Fractions of Commercial Chicken Extract and Heated Model Chicken Extract. The contents of γ-glutamyl-β-alanylhistidine in the commercial chicken extract and heated model chicken extract are shown in **Table 2**, and the changes in amino acid composition of the MMF of the model chicken extract are shown in **Table 3**. The results in **Table 2** show that all kinds of commercial chicken extract contained γ-glutamyl-β-alanylhistidine, and the contents varied from 0.047 to 0.313 μmol/g. In the case of chicken extract, the amount of γ-glutamyl-β-alanylhistidine was ∼1% of the amount of β-alanine, and the ratio is less than that of beef extract. In the case of chicken extract, a positive correlation ($R = 0.924$) between the contents of γ-glutamyl-β-alanylhistidine and β-alanine was observed (**Figure 1B**). From these findings, it was also suggested that the other compounds containing β-alanine formed during the heating process of chicken extract. Furthermore, from the finding that the increase in the content of 1-methylhistidine in the MMF was observed (**Table 3**), it is suggested that the composition of anserine (β-alanyl-1-methylhistidine) has also been incorporated into the MMF during the heating process.

Correlation between Color and γ-Glutamyl-β-alanylhistidine Contents in Commercial Meat Extracts. The correlation between the color (absorbance at 500 nm) of the MMF and the contents of γ-glutamyl-β-alanylhistidine is shown in **Figure 2**. Positive correlations between the color of the MMF and the content of the isopeptide were observed in both the beef extract samples (**Figure 2A**; $R = 0.895$) and the chicken extract samples (**Figure 2B**; $R = 0.969$). The correlation between the color (absorbance at 500 nm) of the extract and the contents of γ-glutamyl-β-alanylhistidine is shown in **Figure 3**. Compared with the findings in **Figure 2**, lower correlations between γ-glutamyl-β-alanylhistidine contents and the color in both the beef extract samples (**Figure 3A**; $R = 0.650$) and chicken extract samples (**Figure 3B**; $R = 0.830$) were observed. These differences appeared to be due to the low correlation between the color of the MMF and the color of extracts (**Figure 4**; $R = 0.863$ in beef extract samples, $R = 0.910$ in chicken extract samples). The finding that the contents of γ-glutamyl-β-alanylhistidine specifically correlate with the color of the MMF

Table 2. Contents of γ-Glutamyl-β-alanylhistidine Isopeptide in the Macromolecular Fraction (MMF) of Commercial Chicken Extract and Heated Model Chicken Extract[a]

sample	type	yield of MMF[b] (%)	contents of γ-Glu-β-Ala-His[c] (μmol/g of DM)	contents of β-Ala[c] (μmol/g of DM)	relative viscosity at MMF 5% solution (mPa·s)
brand A	meat extract	32.8 ± 1.2	0.313 ± 0.086	22.1 ± 2.1	3.9 ± 0.5
brand B	meat extract	24.3 ± 1.1	0.197 ± 0.042	15.7 ± 0.9	3.8 ± 0.3
brand C	meat extract	50.5 ± 2.5	0.089 ± 0.013	5.9 ± 0.3	12.5 ± 1.1
brand D	blended type	51.9 ± 2.1	0.088 ± 0.016	2.1 ± 0.3	15.2± 1.1
brand E	blenden type	80.2 ± 3.1	0.047 ± 0.006	1.2 ± 0.11	8.7 ± 1.2
brand F	blended type	48.3 ± 2.1	0.167 ± 0.009	5.2 ± 0.4	25.5 ± 1.9
model chicken extract heated for 0 h		25.2 ± 1.1	0.069 ± 0.004[ad]	2.2 ± 0.7[a]	644.0 ± 11[a]
model chicken extract heated for 10 h		24.8 ± 0.9	0.086 ± 0.004[a]	3.9 ± 0.9[ab]	25.0 ± 3.2[b]
model chicken extract heated for 20 h		22.9 ± 0.8	0.146 ± 0.012[b]	7.5 ± 1.1[b]	4.8 ± 0.9[c]
model chicken extract heated for 40 h		25.4 ± 1.2	0.200 ± 0.008[c]	18.9 ± 4.0[c]	4.2 ± 0.7[c]

[a] Means ± standard deviation of triplicate determinations. [b] Numbers show the yield of MMF from the extract (dry matter). [c] Numbers indicate the molar numbers of γ-glutamyl-β-alanylhistidine or β-alanine in 1 g (dry matter) of the MMF. [d] Values in a column with different superscript letters are significantly different at $P < 0.05$.

Table 3. Changes in Amino Acid Composition of the Macromolecular Fraction of Chicken Extract during Heating (Micromoles per Gram of DM)[a−c]

amino acid	0 h	10 h	20 h	40 h
Asx	530.4 ± 13.5	544.4 ± 12.9	541.6 ± 18.9	540.2 ± 15.6
Thr	206.8 ± 8.4	220.2 ± 9.1	222.4 ± 8.9	210.8 ± 10.1
Ser	302.8 ± 8.9	312.8 ± 9.2	311.0 ± 11.5	293.0 ± 10.8
Glx	1336.8 ± 23.6	1316.0 ± 28.1	1342.2 ± 25.1	1340.8 ± 32.1
Gly	1421.6 ± 33.2	1456.6 ± 38.1	1417.0 ± 40.3	1384.8 ± 35.4
Ala	880.8 ± 25.8	887.6 ± 30.6	911.0 ± 28.9	970.8 ± 31.2
Val	371.8 ± 10.2	365.4 ± 12.1	378.6 ± 13.1	398.4 ± 9.8
Met	116.4 ± 6.7	122.8 ± 5.8	112.4 ± 4.9	128.4 ± 5.2
Ile	190.0 ± 8.9	201.0 ± 6.8	182.8 ± 7.8	198.6 ± 8.2
Leu	416.2 ± 12.4	448.6 ± 20.4	432.4 ± 19.8	423.0 ± 17.9
Tyr	89.2 ± 4.1	87.4 ± 5.1	94.0 ± 4.9	89.6 ± 4.6
Phe	64.2 ± 2.3	68.0 ± 3.9	62.8 ± 3.1	57.2 ± 3.3
Lys	644.0 ± 14.9	635.0 ± 21.0	644.4 ± 18.6	660.4 ± 21.0
His	34.3 ± 2.8[a]	38.4 ± 4.9[b]	46.8 ± 3.9[c]	48.0 ± 2.4[c]
1-Me-His	5.1 ± 0.8[a]	6.2 ± 1.4[b]	13.0 ± 1.3[c]	32.1 ± 2.8[d]
Arg	366.2 ± 10.8	381.2 ± 15.2	380.6 ± 16.8	370.6 ± 14.3
Hypro	749.0 ± 26.2	764.6 ± 22.9	792.0 ± 23.9	690.0 ± 21.7
Pro	648.2 ± 25.9	682.0 ± 28.2	668.8 ± 30.5	653.8 ± 26.7
β-Ala	2.2 ± 0.7[a]	3.9 ± 0.9[ab]	7.5 ± 1.1[b]	18.9 ± 4.0[c]

[a] Means ± standard deviations of three replicates. [b] Numbers show the molar amounts recovered from 1 g (dry matter) of the MMF. [c] Values in a column with different superscript letters are significantly different at $P < 0.05$.

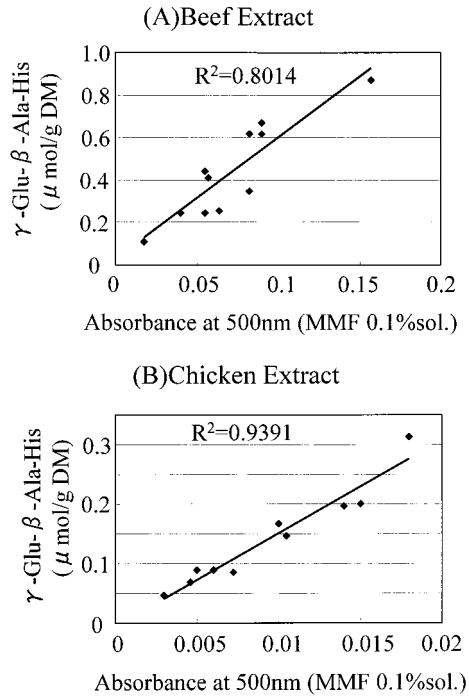

Figure 2. Correlation between the color (absorbance at 500 nm) of the macromolecular fraction (MMF; 0.1% solution) and the contents of γ-glutamyl-β-alanylhistidine in (A) beef extract samples and (B) chicken extract samples.

suggests the possibility that γ-glutamyl-β-alanylhistidine contents can be used as an index of the denaturation of the MMF.

Correlation between Viscosity of the Macromolecular Fraction and γ-Glutamyl-β-alanylhistidine Contents in Commercial Meat Extracts. As shown in **Tables 1** and **2**, the viscosity of the MMF drastically decreased during the heating of the beef soup stock (from 225 to 22 mPa·s after 1 h of heating) and the model chicken extracts (from 664 to 25 mPa·s after 10 h of heating). A plot of the viscosity of the MMF against the γ-glutamyl-β-alanylhistidine content (**Figure 5**) shows a trend that samples with high γ-glutamyl-β-alanylhistidine contents had lower viscosity. It is known that the viscosity of the meat extract is mainly due to the hydrogen bonds between soluble collagen molecules. From the results above, it might be possible that the formation of γ-glutamyl-β-alanylhistidine inhibits the formation of hydrogen bonds between soluble collagen.

In the manufacture of meat extract, it is known that the heating process decreases the viscosity of the extract (*2*), which was suggested to be due to the change in the structure of proteinaceous materials in the extract. It was suggested that the incorporation of carnosine or anserine into the proteinaceous materials may be a reason for the decrease in the viscosity during the heating of meat extract. It was also reported that the tenderness of cooked meat is controlled by the heat-induced changes in collagenous connective tissues (*16*), and it might be possible that the incorporation of carnosine into the proteinaceous materials contributes to the change in the texture during the cooking of meat and meat products.

Furthermore, there have been many studies on the changes in amino acid residues during the heating of food proteins via Maillard reaction. It has been reported that the contents of lysine, arginine, and methionine residues decreased when several kinds of protein are heated with reducing sugars (*17*). Furthermore, recent studies have demonstrated that the amino−carbonyl reaction between proteins and amino acids occurs under physiological conditions (ex. at 37 °C). The formation of protein cross-links such as pentosidine (*18*, *19*) and pyrraline ether cross-

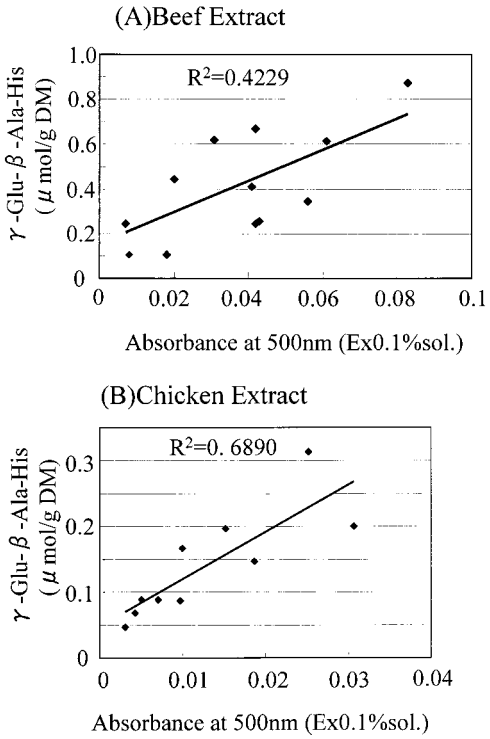

Figure 3. Correlation between the color (absorbance at 500 nm) of the extract (DM 0.1% solution) and the contents of γ-glutamyl-β-alanylhistidine in (A) beef extract samples and (B) chicken extract samples.

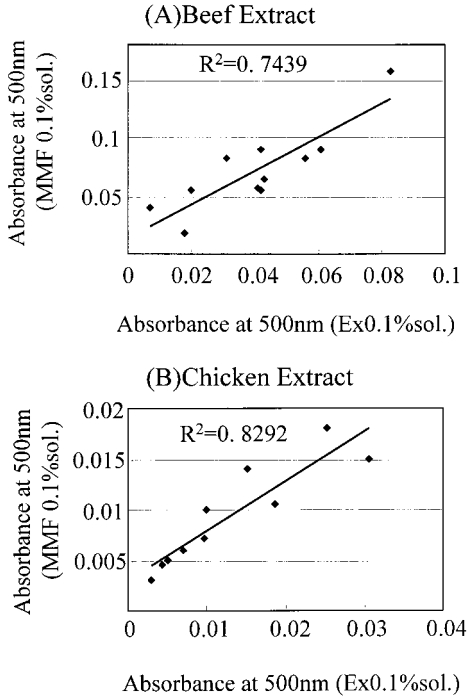

Figure 4. Correlation between the color (absorbance at 500 nm) of the extract (DM 0.1% solution) and the color of the macromolecular fraction (MMF; 0.1% solution) in (A) beef extract samples and (B) chicken extract samples.

links (*20*) from amino acids and reducing sugars by the Maillard reaction in aging has been reported. Furthermore, it was reported that one of these cross-links, pentosidine, can be used as an index of the protein glycation during aging (*21*). In the present study, correlations between the color and γ-glutamyl-β-alanylhistidine contents were investigated. From the finding that the contents of γ-glutamyl-β-alanylhistidine specifically correlate

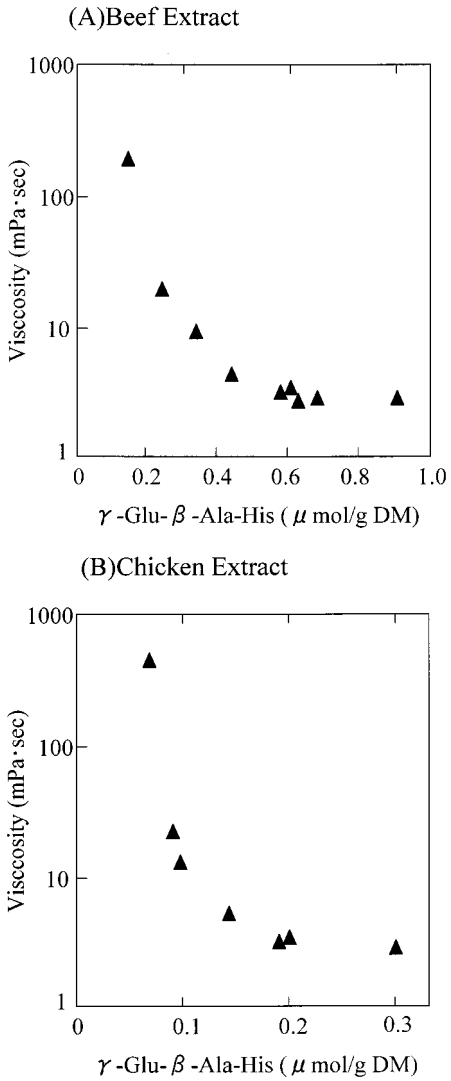

Figure 5. Correlation between the content of γ-glutamyl-β-alanylhistidine and viscosity in (A) beef extract samples and (B) chicken extract samples.

with the color of the MMF, it is suggested that the γ-glutamyl-β-alanylhistidine contents can be used as an index of the denaturation of protein in the MMF.

It is known that carsosine is widely distributed in animal cells (*9*, *10*); therefore, it is suggested that γ-glutamyl-β-alanylhistidine widely exists in animal-derived processed food. Furthermore, in some kinds of animals, it is known that anserine (in chicken and in many kinds of fish such as tuna, cod, and salmon) and balenine (β-alanyl-3-methylhistidine; in whales and snakes) are major imidazole compounds (*9*), making it possible that these related compounds are also incorporated into the protein and form isopeptides similar to γ-glutamyl-β-alanylhistidine. The measurement of γ-glutamyl-β-alanylhistidine in other food sources and measurements of related isopeptides in food protein are now in progress in our laboratories.

In conclusion, these findings suggest that γ-glutamyl-β-alanylhistidine isopeptide is widely distributed in commercial meat extract, and the contents of the above isopeptide can be used as an index of the denaturation of protein in the meat extract.

ABBREVIATIONS USED

HPLC, high-performence liquid chromatography; PITC, phenylisothiocyanate; MMF, macromolecular fraction; SPE, solid-phase extraction; DM, dry matter.

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