

Increase of γ -Glutamyl- β -Alanyl-Histidine Isopeptide in the Macromolecular Fraction of Model Chicken Extract during Heating and Distribution in Commercial Chicken Extracts

M. Kuroda*¹ and T. Harada†

*Seasoning Research & Development Department, Ajinomoto Co., Inc. 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan;
and †Food Research & Development Laboratories, Ajinomoto Co., Inc. 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan

ABSTRACT Changes in the amino acid composition and contents of γ -glutamyl- β -alanyl-histidine isopeptide in the macromolecular fraction were measured during heating of chicken extract. Increases of histidine, 1-methylhistidine, and β -alanine were observed, suggesting that carnosine and anserine were incorporated into the macromolecular fraction. The increase of γ -glutamyl- β -alanyl-histidine isopeptide in the macromolecular fraction of

chicken extract was also observed during the heating process. Furthermore, measurement of γ -glutamyl- β -alanyl-histidine isopeptide in commercial chicken extract showed that all kinds of chicken extract contained the above isopeptide (from 0.09 to 0.31 $\mu\text{mol/g}$ DM in the macromolecular fraction). These results suggest that the formation of γ -glutamyl- β -alanyl-histidine and related isopeptides occur during heating of chicken extract.

(Key words: γ -glutamyl- β -alanyl-histidine, chicken extract, isopeptide, carnosine, anserine)

2002 Poultry Science 81:590–594

INTRODUCTION

Chicken extract is used commercially as a seasoning and is usually produced by concentration after extraction from chicken meat with hot water. Meat extract has high viscosity due to soluble collagen (gelatin). Heating decreases viscosity (Yonemitsu et al., 1997) due to the changes in the structure of proteinaceous materials in the meat extract.

There have been many studies on the changes in amino acid residues during heating of food proteins. It has been reported that lysine, arginine, and methionine residues decrease when several kinds of protein are heated with reducing sugars (Hurrel et al., 1976; Hurrel and Carpenter, 1981). The formation of protein crosslinks such as pentosidine (Sell and Monnier, 1989; Dyer et al., 1991), pyrarrine ether crosslink (Nagaraj et al., 1996), and 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole (Pongor et al., 1984; Chang et al., 1985) from amino acids and reducing sugars by Maillard reaction during aging have been reported. It has also been reported that polymerization of protein occurs during heating with or without reducing sugars (Weder and Scharf, 1981a,b; Okitani et al., 1984). Forma-

tion of ϵ -(γ -glutamyl)-lysine during heating without reducing sugar has also been observed (Otterburn et al., 1977; Otterburn, 1983).

Solubilization of collagen occurs during preparation of beef soup stock solution, and the ratio of collagen to total protein in soup stock solution increases to approximately 30% (Tajima et al., 1991). That study suggested that the major proteinaceous material in beef soup stock solution and beef extract is soluble collagen (gelatin). Furthermore, it has also been reported that several sarcoplasmic proteins remain in the beef meat extract prepared by heating beef meat blocks or beef meat homogenate with hot water (Caldironi and Bazan, 1980; Tajima et al., 1989; Spanier et al., 1990). Based on these previous observations, beef and other meat extracts contain soluble collagen and sarcoplasmic proteins. These previous studies suggest that changes in amino acid composition in protein might occur during production of meat extract, resulting in decreased viscosity. However, there have been few studies on the changes of the protein itself during heating of meat extract.

Previously it was reported that histidine and β -alanine in the macromolecular fraction increased during heating of beef soup stock solution (Kuroda and Harada, 2000). Furthermore, it has been also reported that γ -glutamyl- β -alanyl-histidine is formed from glutamine and carnosine (β -alanyl-histidine) during heating in aqueous solution and that γ -glutamyl- β -alanyl-histidine increases in the macromolecular fraction during heating of beef soup

©2002 Poultry Science Association, Inc.

Received for publication May 4, 2001.

Accepted for publication November 30, 2001.

¹To whom correspondence should be addressed: motonaka_kuroda@ajinomoto.com.

stock (Kuroda et al., 2000). These results suggest that carnosine is incorporated into the macromolecular fraction and forms γ -glutamyl- β -alanyl-histidine isopeptide during heating.

In the present study, to investigate the general incorporation of carnosine and related peptides into the macromolecular fraction, the change in the amino acid composition was measured during heating of chicken extract. To confirm the formation of γ -glutamyl- β -alanyl-histidine isopeptide in the macromolecular fraction in food, the contents of the above peptide were quantified from the macromolecular fraction of heated model chicken extract and commercial chicken extract.

MATERIALS AND METHODS

Commercial Chicken Extract Samples

Commercial chicken extracts were obtained from three different companies. Two were from the United States^{2,3} and one was from France.⁴

Preparation of Model Chicken Extract

Commercial minced chicken meat (mechanically deboned chicken meat;⁵ 1 kg) was placed into an aluminum pot with 200 mL of water and was boiled at 95 to 97 C for 1 h. After removal of chicken meat by centrifugation (2,500 \times g, 10 min), the fat was removed by decantation. The resulting chicken extract was heated at 95 C for 40 h.

Preparation of the Macromolecular Fraction

Heated model chicken extract and commercial chicken extract were dialyzed against distilled water using cellulose membranes⁶ (separation molecular weight = 12,000 to 14,000). Dialysis was performed until the conductivity of the inner solution was less than 200 μ siemens/cm. The ratio of remaining free amino acids and peptides in the inner solution was less than 0.1% in this condition. The obtained macromolecular fractions were freeze-dried and stored at -25 C until analyzed.

Chemicals

γ -Glutamyl- β -alanyl-histidine was prepared by heating glutamine and carnosine and was isolated as reported

previously (Kuroda et al., 2000). Amino acid standard mixtures (type H, A/N, and B), trifluoroacetic acid (protein sequencing grade), acetonitrile (HPLC grade), phenylisothiocyanate, and triethylamine were purchased.⁷ All other reagents were analytical grade.

Analyses of Amino Acid Composition of the Macromolecular Fraction

The aqueous solution (100 μ L) of the macromolecular fraction containing approximately 2 μ g 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 were filtered using Chromatodisk 4A membrane filters⁸ and analyzed. Amino acid compositions were determined with a Model L-8500 amino acid analyzer⁹ with lithium citrate buffer.¹⁰

Analyses of Other Components

The components in the heated model chicken extract samples were quantified by the methods as follows. Free amino acids and several peptides such as carnosine and anserine were analyzed with a Model L-8500 amino acid analyzer with lithium citrate buffer (PF-series for nonhydrolyzed amino acid and peptide analysis). Sugars were analyzed by HPLC equipped with an anion exchange column with detection at 425 nm after coloring with orcinol-sulfuric acid reagent (Georg and Christian, 1986). Contents of nucleotides were analyzed by HPLC equipped with a Hitachi #3013 column⁹ with detection at 254 nm. Organic acids were analyzed with a carbonic acid analyzer,¹¹ equipped with an anion exchange column with detection at 530 nm after coloring with a solution comprising 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-hydrochloride, 2-nitrophenyl hydrazine, and NaOH.

HPLC Analysis of γ -Glutamyl- β -Alanyl-Histidine

Proteolytic Digestion. Proteolytic digestion of the macromolecular sample was carried out as reported previously (Kuroda and Harada, 2000). 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 U/mg protein for 24 h, 37 C), after inactivation heating at 100 C for 10 minutes, digestion was continued for 24 h by adding leucine aminopeptidase (1.0 U/mg protein), prolidase (0.5 U/mg protein), and carboxypeptidase A (3.0 U/mg protein) followed by the digestion with carboxypeptidase W (4.0 U/mg protein for 24 h, 37 C, pH 4.0).

Solid-Phase Extraction of γ -Glutamyl- β -Alanyl-Histidine. The proteolytic digests obtained as described above were fractionated by solid-phase extraction using Toyopack IC-SP cartridges¹² (500 mg resin). The proteolytic digest (from 5 mg protein) was diluted in 0.1 M HCl

²IDF Co., Inc., Springfield, MO 65809.

³AMPC Inc., Ames, IA 50010.

⁴SPI-DIANA Inc., 56230 Berric, France.

⁵Nippon Meat Packers, Inc., Tokyo 108-0074, Japan.

⁶UC-30-32-100, Sanko Junyaku Co., Ltd., Tokyo 101-0032, Japan.

⁷Wako Pure Chemical Industries, Ltd., Osaka 541-0045, Japan.

⁸GL Science Inc., Tokyo 163-1130, Japan.

⁹Nissei Sangyo Co., Ltd., Tokyo 105-0003, Japan.

¹⁰PF-series for nonhydrolyzed amino acid and peptide analysis; Mitsubishi Chemical Inc., Tokyo 100-0005, Japan.

¹¹S-3000 system, Tokyo Rika Inc., Tokyo 103-0023, Japan.

¹²Tosoh Co. Ltd., Tokyo 107-0052, Japan.

and applied to a Toyopack IC-SP cartridge equilibrated with 0.1 M HCl. After being washed with 0.1 M HCl (6 mL \times three washes), the cartridge was air-dried, and the fraction containing γ -glutamyl- β -alanyl-histidine was eluted with 5 mL of 80% methanol containing 1% ammonia. The fraction thus obtained was dried in a vacuum concentrator.

Derivatization with Phenylisothiocyanate and HPLC Separation. Derivatization with phenylisothiocyanate was performed by a method reported previously (Kuroda et al, 2000). The derivatized sample (20 μ L) was applied onto an Inertsil ODS-3 column⁸ (250 mm \times 4.6 mm i.d., particle size 5 μ m). The separation was performed with a binary linear solvent gradient. Solution A comprised 0.15 M ammonium acetate containing 7% (vol/vol) acetonitrile, and solution B comprised acetonitrile and water (6:4). The gradient profiles were as follows: 0 to 15 min, 0% B; 15 to 25 min, 0 to 50% B; 25 to 35 min, 50 to 100% B. Flow rate was 1 mL/min; detection was at 269 nm UV, and column temperature was maintained at 40 C.

Statistical Analysis

Statistical comparison among means was performed by one-way ANOVA. When ANOVA was significant, the Tukey's multiple-range test was used for the posthoc comparison. All statistical analyses were performed with SPSS 10.0J¹³ software. Trends were considered significant when means of compared sets differed at $P < 0.05$.

RESULTS AND DISCUSSION

Changes of General Components in Model Chicken Extract During Heating

The changes in the components in beef soup stock solution (DM = 30%) are shown in Table 1. A slight decrease of glutamine was observed (changes were not different), and other amino acids and peptides were almost unchanged in these conditions. The decreases of reducing sugars such as glucose and ribose were observed. These results suggest that Maillard reaction between reducing sugars and free amino acids and peptide occur in heating of model chicken extract.

Changes in Amino Acid Composition of the Macromolecular Fraction of Model Chicken Extract During Heating

Macromolecular fractions were obtained from heated chicken extract by dialysis. As shown in Table 2, increases in contents of histidine, 1-methylhistidine and β -alanine were observed. The increases in molar amounts of other amino acids were also almost unchanged during heating

under these conditions. The results suggested that carnosine and anserine (β -alanyl-1-methylhistidine) in chicken extract were incorporated into the macromolecular fraction during heating.

Measurement of γ -Glutamyl- β -Alanyl-Histidine Isopeptide in the Macromolecular Fractions of Heated Model Chicken Extract

The contents of γ -glutamyl- β -alanyl-histidine in the macromolecular fractions from heated chicken extract were measured by HPLC. The results of the analyses are shown in Table 3. The result showed that the contents of γ -glutamyl- β -alanyl-histidine in the macromolecular fraction increased during heating. This result suggests that γ -glutamyl- β -alanyl-histidine isopeptide was formed during the heating of chicken extract. Previously, it was reported that histidine and β -alanine in the macromolecular fraction increased during the heating of beef soup stock solution (Kuroda and Harada, 2000), and that γ -glutamyl- β -alanyl-histidine in the macromolecular fraction during heating of beef soup stock (Kuroda et al., 2000). From the result obtained in this study, the incorporation of carnosine into the macromolecular fraction and formation of γ -glutamyl- β -alanyl-histidine isopeptide also occur during heating of chicken extract. Furthermore, the data obtained was compared to the data of the increase in β -alanine and contents in macromolecular fractions obtained by amino acid analyses followed by 6 M HCl hydrolysis (Table 2). The results indicate that the amount of increased γ -glutamyl- β -alanyl-histidine was about 1% of the amount of increased β -alanine. From these results, it was suggested that the other compounds containing β -alanine formed during heating of chicken extract. Recently, it has been reported that incubation (at 37 C for 2 h) of several proteins with 4-hydroxynonenal (lipid-oxidation product) results in the modification of histidine residue and form the crosslinks (Uchida and Stadtman, 1992, 1993). From these previous observations, it seemed possible that some of carnosine has been incorporated into the macromolecular fraction by a reaction between the histidine moiety and lipid-oxidation products. Furthermore, the increase in contents of 1-methylhistidine in the macromolecular fraction suggests that anserine was also incorporated into the macromolecular fraction during heating.

Measurement of γ -Glutamyl- β -Alanyl-Histidine Isopeptide in the Macromolecular Fractions of Commercial Chicken Extract

The contents of γ -glutamyl- β -alanyl-histidine isopeptide in the commercial chicken extract were shown in Table 4. The result shows that all kinds of commercial chicken extract contained γ -glutamyl- β -alanyl-histidine. The contents in the macromolecular fraction varied from 0.09 to 0.31 μ mol / g DM. This variation seemed due to differences in the content of carnosine and the condition

¹³SPSS Japan Inc., Tokyo 150-0012, Japan.

TABLE 1. Changes of general components in model chicken extract during heating at 95 C (μ mol/g DM)¹

Component	0 h	10 h	20 h	40 h
Free amino acids				
Tau	168.8 \pm 8.2	180.0 \pm 5.9	182.1 \pm 10.3	177.9 \pm 9.8
Asp	22.8 \pm 1.6	25.5 \pm 1.4	25.9 \pm 1.1	25.5 \pm 1.4
Asn	3.8 \pm 0.4	4.3 \pm 0.7	3.8 \pm 0.3	3.6 \pm 0.5
Thr	22.8 \pm 3.1	25.5 \pm 1.9	25.9 \pm 1.8	25.5 \pm 1.3
Ser	37.3 \pm 2.4	40.2 \pm 3.5	40.8 \pm 4.1	40.8 \pm 3.2
Glu	66.0 \pm 4.7	70.6 \pm 5.7	70.0 \pm 3.7	66.8 \pm 4.2
Gln	53.7 \pm 4.4	52.5 \pm 4.5	53.2 \pm 4.3	51.9 \pm 4.3
Gly	46.0 \pm 3.9	51.6 \pm 3.8	52.3 \pm 4.3	52.4 \pm 3.6
Ala	58.2 \pm 11.9	61.0 \pm 8.8	61.7 \pm 13.0	61.8 \pm 6.2
Val	17.0 \pm 1.3	18.8 \pm 1.6	18.9 \pm 1.4	19.0 \pm 1.7
Met	7.7 \pm 0.7	8.5 \pm 0.4	8.3 \pm 0.8	8.3 \pm 0.4
Ile	9.2 \pm 1.1	10.2 \pm 1.2	10.2 \pm 0.7	9.8 \pm 0.9
Leu	19.1 \pm 1.4	21.4 \pm 1.9	21.6 \pm 1.8	21.2 \pm 2.1
Tyr	7.8 \pm 0.7	8.7 \pm 0.6	8.8 \pm 0.5	8.6 \pm 0.8
Phe	7.0 \pm 0.4	7.8 \pm 0.5	8.1 \pm 0.4	8.3 \pm 0.4
Lys	19.9 \pm 1.2	22.2 \pm 2.4	22.5 \pm 3.0	22.1 \pm 1.8
His	7.0 \pm 1.4	7.3 \pm 1.7	7.2 \pm 1.7	7.0 \pm 0.8
Arg	16.5 \pm 1.4	18.2 \pm 1.8	17.9 \pm 1.3	16.4 \pm 0.9
Peptides				
Carnosine	110.0 \pm 5.5	117.6 \pm 6.3	116.5 \pm 6.2	111.8 \pm 5.1
Anserine	355.3 \pm 12.7	382.7 \pm 21.1	384.3 \pm 23.4	372.8 \pm 21.3
Nucleotide				
Inosinic acid	67.7 \pm 4.1 ^a	59.0 \pm 3.1 ^b	50.7 \pm 3.7 ^c	33.6 \pm 2.9 ^d
Sugars				
Glucose	24.8 \pm 1.3 ^a	20.4 \pm 1.5 ^b	12.4 \pm 0.8 ^c	6.5 \pm 0.3 ^d
Ribose	2.6 \pm 0.1 ^a	1.7 \pm 0.1 ^b	0.9 \pm 0.06 ^c	0.3 \pm 0.03 ^d
Organic acids				
Lactic acid	520.4 \pm 32.1	519.0 \pm 26.2	517.6 \pm 27.1	520.4 \pm 30.6
Pyroglutamic acid	117.3 \pm 7.8 ^a	131.6 \pm 11.9 ^b	138.7 \pm 6.9 ^b	142.8 \pm 11.4 ^b
Succinic acid	6.9 \pm 0.5	7.8 \pm 0.4	7.8 \pm 0.5	7.8 \pm 0.5
Acetic acid	11.0 \pm 0.8 ^a	13.2 \pm 1.2 ^a	17.5 \pm 2.2 ^b	26.3 \pm 3.0 ^c

^{a-d}Values in a row with different superscripts are significantly different at $P < 0.05$.

¹Means \pm standard deviation of triplicate determinations.

of processing. These results suggest that the incorporation of carnosine and the formation of γ -glutamyl- β -alanyl-histidine isopeptide occur during the process of the commercial chicken extract.

In meat extract manufacturing, the heating process decreases viscosity (Yonemitsu et al., 1997), which is supposed to be due to the change of the structure of proteinaceous materials in the extract. It was supposed that the

TABLE 2. Changes in the amino acid composition of the macromolecular fraction of chicken extract during heating (μ mol/g DM)¹

Amino acid ²	0 h	10 h	20 h	40 h
Asx	530.4 \pm 13.5	544.4 \pm 12.9	541.6 \pm 18.9	540.2 \pm 15.6
Thr	206.8 \pm 8.4	220.2 \pm 9.1	222.4 \pm 8.9	210.8 \pm 10.1
Ser	302.8 \pm 8.9	312.8 \pm 9.2	311.0 \pm 11.5	293.0 \pm 10.8
Glx	1,336.8 \pm 23.6	1,316.0 \pm 28.1	1,342.2 \pm 25.1	1,340.8 \pm 32.1
Gly	1,421.6 \pm 33.2	1,456.6 \pm 38.1	1,417.0 \pm 40.3	1,384.8 \pm 35.4
Ala	880.8 \pm 25.8	887.6 \pm 30.6	911.0 \pm 28.9	970.8 \pm 31.2
Val	371.8 \pm 10.2	365.4 \pm 12.1	378.6 \pm 13.1	398.4 \pm 9.8
Met	116.4 \pm 6.7	122.8 \pm 5.8	112.4 \pm 4.9	128.4 \pm 5.2
Ile	190.0 \pm 8.9	201.0 \pm 6.8	182.8 \pm 7.8	198.6 \pm 8.2
Leu	416.2 \pm 12.4	448.6 \pm 20.4	432.4 \pm 19.8	423.0 \pm 17.9
Tyr	89.2 \pm 4.1	87.4 \pm 5.1	94.0 \pm 4.9	89.6 \pm 4.6
Phe	64.2 \pm 2.3	68.0 \pm 3.9	62.8 \pm 3.1	57.2 \pm 3.3
Lys	644.0 \pm 14.9	635.0 \pm 21.0	644.4 \pm 18.6	660.4 \pm 21.0
His	34.3 \pm 2.8 ^a	38.4 \pm 4.9 ^b	46.8 \pm 3.9 ^c	48.0 \pm 2.4 ^c
1-Me-His	.1 \pm 0.8 ^a	6.2 \pm 1.4 ^b	13.0 \pm 1.3 ^c	32.1 \pm 2.8 ^d
Arg	366.2 \pm 10.8	381.2 \pm 15.2	380.6 \pm 16.8	370.6 \pm 14.3
Hypro	749.0 \pm 26.2	764.6 \pm 22.9	792.0 \pm 23.9	690.0 \pm 21.7
Pro	648.2 \pm 25.9	682.0 \pm 28.2	668.8 \pm 30.5	653.8 \pm 26.7
β -Ala	2.2 \pm 0.7 ^a	3.9 \pm 0.9 ^{ab}	7.5 \pm 1.1 ^b	18.9 \pm 4.0 ^c

^{a-d}Values in a row with different superscripts are significantly different at $P < 0.05$.

¹Means \pm standard deviations of three replicates.

²The numbers show the molar amounts recovered from one gram (dry matter) of the macromolecular fractions.

TABLE 3. Contents of γ -glutamyl- β -alanyl-histidine isopeptide in the macromolecular fraction of heated model chicken extract ($\mu\text{mol/g DM}$)^{1,2}

Heating (h)	γ -Glu- β -Ala-His
0	0.069 \pm 0.011 ^a
10	0.086 \pm 0.014 ^a
20	0.146 \pm 0.012 ^b
40	0.200 \pm 0.021 ^c

^{a-c}Values in a column with different superscript letters are significantly different at $P < 0.05$.

¹Means \pm standard deviation of three replicates.

²Values indicate the molar numbers in 1 g of dry matter of the macromolecular fraction.

TABLE 4. Contents of γ -glutamyl- β -alanyl-histidine isopeptide in the macromolecular fraction of commercial chicken extract ($\mu\text{mol/g DM}$)^{1,2}

Samples	γ -Glu- β -Ala-His
Brand A	0.313 \pm 0.025
Brand B	0.197 \pm 0.020
Brand C	0.089 \pm 0.011

¹Means \pm standard deviation of three replicates.

²Values indicate the molar numbers in 1 g of dry matter of the macromolecular fraction.

incorporation of carnosine or anserine into the proteinaceous materials might be a reason for the decrease in viscosity during the heating of meat extract. It has also been reported that the tenderness of cooked meat is controlled by the heat-induced changes in collagenous connective tissues (Kruggel and Field, 1971), and it might be possible that the incorporation of carnosine into the proteinaceous materials contributes to the change of the texture during the cooking of meat and meat products. The analysis of γ -glutamyl- β -alanyl-histidine in various food sources is currently in progress in our laboratory. Furthermore, studies on the correlation between the increase of γ -glutamyl- β -alanyl-histidine and related isopeptides in the macromolecular fraction and the change in the viscosity of the extract are now being investigated.

REFERENCES

- Caldironi, H. A., and N. G. Bazan. 1980. Quantitative determination of low-salt-soluble proteins in bovine muscles cooked at different temperatures by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Food Sci.* 45:901-904.
- Chang, J. C. F., P. C. Ulrich, R. Bucala, and A. Cerami. 1985. Detection of an advanced glycation product bound to protein in situ. *J. Biol. Chem.* 260:7970-7974.
- Dyer, D. G., J. A. Blackledge, S. R. Thorpe, and J. W. Baynes. 1991. Formation of pentosidine during nonenzymatic browning of proteins by glucose. *J. Biol. Chem.* 266:11654-11660.
- Georg, A., and W. Christian. 1986. An automated method for the quasi-continuous analysis of degradation and transfer products during the enzymatic hydrolysis of oligosaccharides. *Anal. Biochem.* 153:144-150.
- Hurrell, R. F., and K. J. Carpenter. 1981. The estimation of available lysine in foodstuffs after Maillard reactions. *Prog. Food Nutr. Sci.* 5:159-176.
- Hurrell, R. F., K. J. Carpenter, W. J. Sinclair, M. S. Otterburn, and R. S. Asquith. 1976. Mechanism of heat damage in proteins. 7. The significance of lysine-containing isopeptides and of lanthionine in heat proteins. *Br. J. Nutr.* 35:383-395.
- Kruggel, W. G., and R. A. Field, 1971. Soluble intramolecular collagen characteristics from stretched and aged muscle. *J. Food Sci.* 36:1114-1117.
- Kuroda, M., and T. Harada. 2000. Incorporation of histidine and β -alanine into the macromolecular fraction of beef soup stock solution. *J. Food Sci.* 65:596-603.
- Kuroda, M., R. Ohtake, E. Suzuki, and T. Harada. 2000. Investigation on the formation and the determination of γ -glutamyl- β -alanyl-histidine and related isopeptide in the macromolecular fraction of beef soup stock. *J. Agric. Food Chem.* 48:6317-6324.
- Nagaraj, R. H., M. Portero-Otin, and V. M. Monnier. 1996. Pyrraline ether cross-link as a basis for protein crosslinks by the advanced Maillard reaction in aging and diabetes. *Arch. Biochem. Biophys.* 325:152-158.
- Otterburn, M. S. 1983. Isopeptides: The occurrence and significance of natural and xenobiotic crosslinks in proteins. *ACS Symp. Ser.* 234:221-232.
- Otterburn, M., M. Healy, and W. Sinclair. 1977. The formation, isolation and importance of isopeptides in heated proteins. *Adv. Exp. Med. Biol.* 86b:239-262.
- Pongor, S., P. C. Ulrich, F. A. Bencsath, and A. Cerami. 1984. Aging of proteins: Isolation and identification of a fluorescent chromophore from the reaction of polypeptide with glucose. *Proc. Natl. Acad. Sci. USA* 81:2684-2688.
- Sell, D. R., and V. M. Monnier. 1989. Structure elucidation of a senescence cross-link from human extracellular matrix. *J. Biol. Chem.* 264:21597-21602.
- Spanier, A. M., K. W. McMillin, and J. A. Miller, 1990. Enzyme activity levels in beef: Effect of postmortem aging and endpoint cooking temperature. *J. Food Sci.* 55:318-322.
- Tajima, M., T. Mitsuhashi, A. Mega, and N. Arakawa. 1989. Some origin of soluble protein from heating meat into soup. *J. Home Econ. Jpn.* 40:121-125.
- Tajima, M., T. Mitsuhashi, A. Mega, and N. Arakawa. 1991. Heat-induced effect on soluble proteins in meat soup stock. *J. Home Econ. Jpn.* 42:967-971.
- Uchida, K., and E. R. Stadtman. 1992. Modification of histidine residues in proteins by reaction with 4-hydroxynonenal. *Proc. Natl. Acad. Sci. USA* 89:4544-4558.
- Uchida, K., and E. R. Stadtman. 1993. Covalent attachment of 4-hydroxynonenal to glyceraldehyde-3-phosphate dehydrogenase. A possible involvement of intra- and intermolecular cross-linking reaction. *J. Biol. Chem.* 268:6388-6393.
- Weder, J. K. P., and U. Scharf. 1981a. Model studies on the heating of food proteins - Heat-induced oligomerization of ribonuclease. II. Isolation of oligomers and comparative studies. *Z. Lebensm. Unters. Forsch.* 172:104-109.
- Weder, J. K. P., and U. Scharf. 1981b. Model studies on the heating of food proteins - Heat-induced oligomerization of ribonuclease. III. On the location of acid-labile crosslinking peptides. *Z. Lebensm. Unters. Forsch.* 172:185-189.
- Yonemitsu, M., T. Okamura, A. Nishikawa, and H. Ohmura, 1997. Manufacture of concentrated extract. Patent assignee: Ajinomoto Co., Inc. Japan Kokai Tokkyo Koho. Appl. No. JP 97-135673.