鹿児島大学リポジトリ
Kagoshima University
Repository

Free Amino Acids, Carnosine and 5'-Inosinic Acid Contents in the Beef Loin and Beef Round

著者	KOGA Katsuya, FUKUNAGA Takao, KAWAGOE Shigeya
journal or publication title	Memoirs of the Faculty of Agriculture, Kagoshima University
volume	23
page range	121-129
URL	http://hdl.handle.net/10232/3008

Mem. Fac. Agr. Kagoshima Univ., **23**, 121-129, 1987

Free Amino Acids, Carnosine and 5'-Inosinic Acid Contents in the Beef Loin and Beef Round

Katsuya KOGA, Takao FUKUNAGA and Shigeya KAWAGOE
(Laboratory of Animal Biochemistry)
Received for Publication, September 9, 1986

Introduction

Although the price of beef is higher than those of the other animal protein sources such as pork, chicken and fishes, beef is consumed generally as a preferable food. In Japan, it is marketed as beef carcass of supreme, superior, excellent, medium, common or utility grades, the evaluation of which has been made by the public meat-quality estimating authorities with their naked eyes, basing on the degrees of marbling. Various grades of beefs described above are more expensive in that order, and furthermore the beef loin is, commercially, more valuable than the beef round. From the standpoint of chemical components, free amino acids, peptides, sugars, organic acids and nucleotides contribute to the meat flavor and palatability. Some quantitative studies on the free amino acids in aqueous beef extract have been reported (Ma et al.[11], Macy et al.[12], Field et al.[2], Wasserman et al.[19], Jarboe et al.[3] and Penet et al.[15]). The mixture of monosodium glutamate and 5'-inosinic acid or 5'-guanylic acid was found to show a surprisingly synergistic tasty effect by Kuninaka[9,10], and the synergistic relationship between two compounds was expressed as a mathematical model by Yamaguchi[20]. Nakajima et al.[13] found the presence of relatively large amount of 5'-inosinic acid and that of slight amount of 5'-guanylic acid in the marketing raw beef and pork. Terasaki et al.[17] proved the formation of 5'-inosinic acid from adenosine triphosphate in meats and its degradation to hypoxanthine having bitter taste.

Since free amino acids and 5'-inosinic acid contents were directly related to the meat flavor and taste, authors reported some studies on the free amino acids, carnosine and 5'-inosinic acid contents in the lean meats from several breeds of pigs, the strain and the strain-cross pigs (Koga et al.[4,5,6,7,8]).

No investigation on the relationship between various grades of beefs and the contents of their palatable components has been published. The present study was performed to clarify the content of components relating to the tastes in the loin and in the round of various grades of beefs.

Experimental Materials and Methods

Materials—After the progeny testing of the Japanese Black cattle, the respective loins and rounds cut off from four grades (supreme, superior, excellent and medium grades) of the dressed carcasses evaluated by the public meat-quality estimating authorities were used

in the present study. These sample meats were purchased from Minami Kyūshu Chikusan Kōgyo Ltd. Co., respectively minced and preserved at −20°C in sealed polyethylene pouches until used for the analysis of beef water extract.

Determination of moisture content—Moisture contents of sample meats were determined by the usual method (drying at 105°C).

Extraction of free amino acids and carnosine from the sample meat and their quantitative estimation—The extraction of free amino acids and carnosine from the sample meats were performed, referring to the reports of Zeika[21] and Koga et al.[5]. Five grams of the defrosted meat mixed with 5 g of fine quartz sand were thoroughly ground in a thick ceramic dish and homogenized with 30 ml of 1 % picric acid as a deproteinization agent. The homogenate was centrifuged at 8000 rpm for 10 min. The residue was extracted with 20 ml of 1 % picric acid and centrifuged. The supernatants were combined and passed slowly through the column of Dowex 2×8 (Cl form), 2×10 cm, to remove the picric acid. The effluent was evaporated to dryness under aspirator vacuum in a rotatory evaporator. The dried matter was dissolved by adding 25 ml of citrate buffer of analytical grade, pH 2.2, including 0.2N Na. One ml of this solution was used for the analysis of amino acids, employing a Model Yanagimoto LC-5S automatic recording amino acid analyzer. Because of remarkable largeness of carnosine and taurine contents, sample solutions diluted to one eleventh concentration with citrate buffer for carnosine analysis; and one sixth concentration for taurine, were separately used.

Extraction of 5′-inosinic acid from the sample meat and its quantitative estimation—The extraction of 5′-inosinic acid (IMP) from the sample meats was performed referring to the procedures of Nakajima et al.[13] and Ehira et al.[1] Four grams of the defrosted meat mixed with 4 g of fine quartz sand were thoroughly ground and homogenized with 10 ml of chilled 10 % perchloric acid. The homogenate was centrifuged at 3000 rpm for 5 min. The residue was reextracted with 5 ml of chilled 5 % perchloric acid and centrifuged. After repeating this process twice, the supernatants were combined and immediately neutralized with 10 N and 0.1 N potassium hydroxide to pH 6.5 and the total volume was diluted to 50 ml. The neutralized extract was centrifuged to remove the precipitate of potassium perchlorate and an aliquot of the supernatant was used as the sample for 5′-IMP analysis. The analysis of 5′-IMP was performed with a Shimadzu LC-4A type High Performance Liquid Chromatograph. Because the absence of cytosine monophosphate in the sample solution was

Table 1. Conditions for 5′-inosinic acid analysis with the high performance liquid chromatograph

Chromatograph apparatus: Shimadzu LC-4A type
Column: 4.6D×200 mm
Column adsorbent: Nucleosil 5N $(CH_3)_2$ (Chemopak conventional HPLC column)
Mobile phase: 10% (V/V) CH_3OH in 0.05M $(NH_4)_2HPO_4$, pH 3.0 with H_3PO_4
Flow rate: 1 ml per min
Column temperature: 30°C
Detection: UV 254 nm
Chart speed: 5 mm per min

confirmed in the preliminary experiment, CMP has been used as an internal standard for nucleotides analyses. In liquid chromatographic run, the appearance of IMP at the later position than the one of CMP and the ratio of the peak height of IMP to that of CMP (1.19) were already confirmed (Koga et al.[8]). Five μl from the mixture of 5 ml of sample solution and 1 ml of CMP solution including 0.4 mg were applied onto the HPLC column. Analytical conditions were as represented in Table 1.

Results and Discussion

As shown in Table 2, in all the sample meats, moisture content of the beef round was ascertained to be larger than that of the beef loin. Free amino acids and carnosine contents of various grades of beefs are represented in Table 3. In the previous study on free

Table 2. Moisture contents of the beef loin and the beef round from the Japanese Black cattles

Grade	Beef loin	Beef round
Supreme	59.20	72.49
Superior	64.85	73.56
Excellent	64.39	71.29
Medium	67.94	72.01
Average	64.10	72.34

Table 3. Free amino acids and carnosine contents in aqueous beef loin and beef round extracts (mg/100g fresh meat)

	Beef loin				Beef round			
	Supreme	Superior*1	Excellent*2	Medium	Supreme	Superior*1	Excellent*2	Medium
Lysine	25.46	21.82	28.21	27.53	26.18	26.26	28.49	27.78
Arginine	7.67	5.32	7.68	8.12	9.58	5.63	6.40	5.93
Taurine	36.08	24.21	30.60	34.98	23.29	33.04	32.80	35.68
Aspartic A.	2.67	2.17	1.40	2.24	0.40	0.58	1.16	0.53
Threonine	40.18	25.20	38.94	25.74	22.64	21.83	19.00	21.63
Serine	7.36	5.54	7.17	6.36	9.15	6.39	6.62	6.20
Glutamic A.	11.49	8.85	8.38	5.25	11.48	7.41	7.18	6.26
Proline	1.50	1.42	1.64	0.61	2.19	1.42	1.61	2.07
Glycine	9.47	8.51	8.64	6.62	13.14	9.57	9.40	8.00
Alanine	50.47	32.30	35.15	23.40	42.24	31.38	27.85	26.42
Valine	7.95	5.88	6.41	5.98	11.92	7.40	6.73	8.32
Methionine	2.24	1.83	2.14	2.22	4.62	3.08	2.39	3.21
Isoleucine	4.07	2.84	3.31	3.11	7.35	4.55	3.48	4.59
Leucine	8.27	5.72	6.39	5.85	14.04	8.57	5.87	7.87
Tyrosine	4.35	2.90	3.49	3.41	7.00	4.47	3.67	5.71
Phenylalanine	3.77	2.97	3.15	3.28	8.43	4.51	3.47	5.62
Total FAA	223.00	157.48	192.70	164.70	213.65	176.09	166.12	175.82
Carnosine	107.00	299.92	299.06	374.56	271.07	374.53	401.98	451.34
Total	330.00	457.40	491.76	539.26	484.72	550.62	568.10	627.16

*1 Average of values as to the meats from three cattles
*2 Average of values as to the meats from four cattles

amino acids of aqueous pork extract, Koga et al.[5] observed an appearance of a high peak of unknown compound between the lysine peak and the histidine peak in the chromatogram on a short column for the analysis of basic amino acids and could identify it with carnosine. Subsequently, authors found that the amount of carnosine was the greatest in all the ninhydrin postive compounds in the water-extract from the lean meats *(Longissimus dorsi* and *Biceps femoris)* of several breeds of pigs: the amount being 400~800mg%[5,7,8].

As well as these experimental results, in all the ninhydrin positive compounds in aqueous beef extract, carnosine contents were noticeable to be remarkably large (270~400mg%, except for the supreme beef loin). The contents in the beef round were exceedingly larger than those in the beef loin in all the four grades of beefs, being 1.2~1.3 times as much as those in the latter. Although carnosine is a mild palatable dipeptide, contributing to the meat taste, the largeness of the content was in the order of medium, excellent, superior and supreme beefs in the present study, being anti-parallel to the grades of beef. On the contrary, excepting taurine, the largeness of contents of alanine, threonine, glutamic acid, glycine and serine which were palatable amino acids and contained comparatively much in beef were generally in the order of supreme, superior, excellent and medium grades of beef (Fig. 1). In all the ninhydrin positive compounds the largest compound in quantity was different among the reports of many researchers. It was carnosine in the report of Wasserman et al.[19], being coincident with our result. In other reports, it was carnosine plus anserine fraction (Macy et al.[12]), anserine (Jarboe et al.[3]) or alanine (Ma et al.[11]).

Although Field et al.[2] and Penet et al.[15] estimated quantitatively free amino acids in bovine muscles, they left the content of carnosine undetermined.

Relatively large amount of taurine has been found in the aqueous beef extract, being coincident with analytical results of Wasserman et al.[19] and Macy et al.[12] Jarboe et al.[3] detected a slight amount of taurine in the aqueous beef extract; Field et al[2]. and Penet et al.[15] detected no taurine in the same extract. Because of the lack of adenosine monophosphate deaminase in mollusks such as squid and octopus, 5'-inosinic acid has not been found in their muscles. Therefore, a considerable amount of taurine in mollusks (Ozawa et al.[14]) is supposed to contribute greatly to their palatable taste as foods. Taurine has been presumed to be a metabolizing product of cysteine (Yamaguchi)[20] and recently is noticed to be effective for recruitment, lowering blood cholesterol level and remedying of chronic hapatitis in the human (Ozawa et al.[14] and Tsuji[18]). Although taurine level in beef is much less than that in the muscle of mollusks, relatively large amount of taurine in beef must be contributing to the palatable taste of beef. The contents in the beef rounds are slightly larger than those in the beef loins excepting the supreme grade of beef. On the contrary, the contents of alanine, threonine and glutamic acid in the beef round are respectively smaller than those in the beef loin excepting the medium grade of beef. In all the free amino acids, alanine and taurine contents were comparatively large, followed by threonine, lysine, glycine and glutamic acid contents.

This profile is similar to that of free amino acids in the aqueous pork extract obtained by us[7,8].

Total amounts of free amino acids in the beef loins and rounds were larger in the supreme grade beef than in the other grades of beefs, but a significant difference was not observed among superior, excellent and medium grades of beefs. These total amounts are

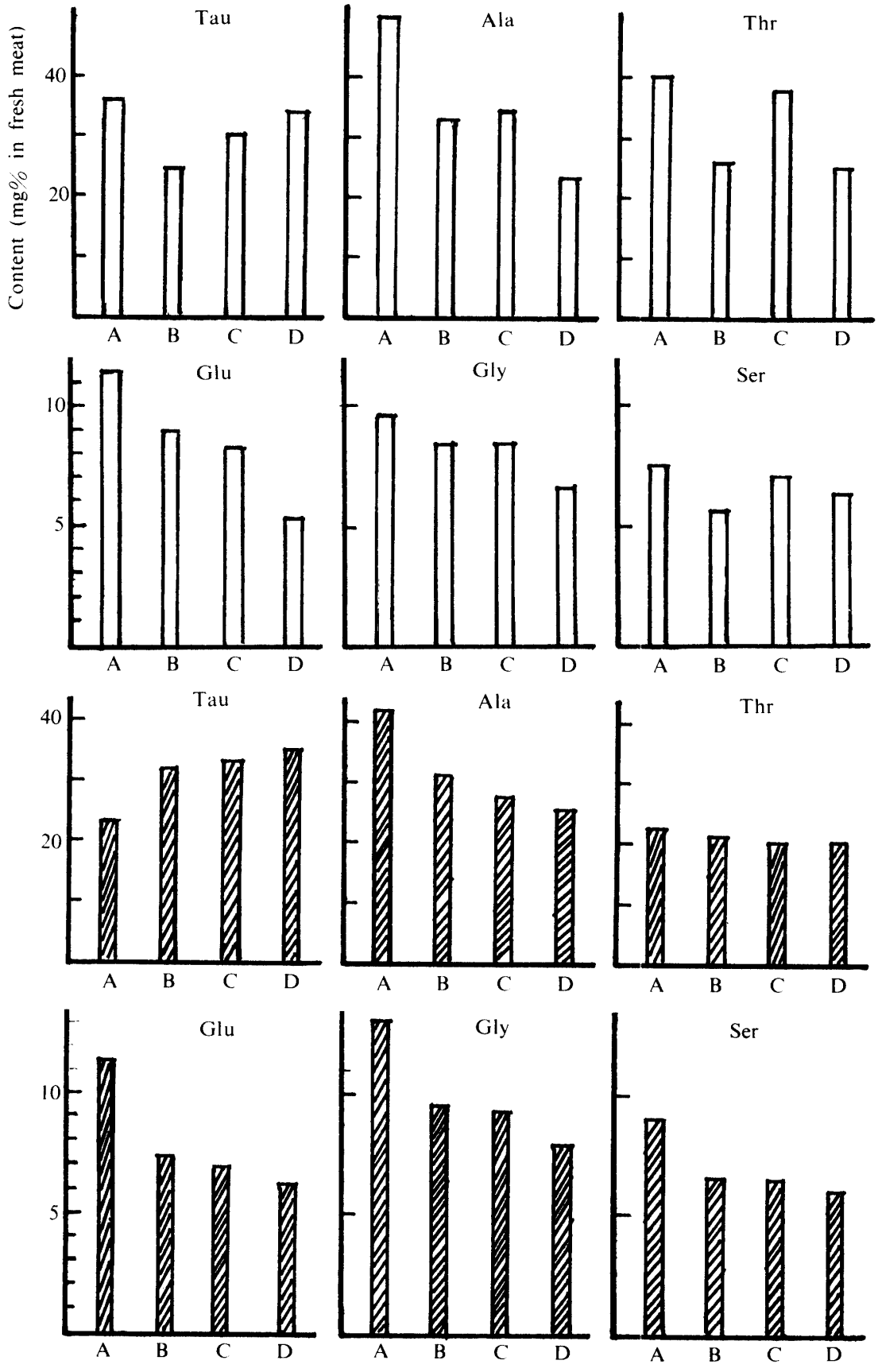

Fig. 1. Comparison among some palatable amino acids contents in various grades of beefs.
□ : Beef loin, ▨ : Beef round
A : Supreme grade, B : Superior, C : Excellent, D : Medium

exceedingly larger than the analytical values of Macy et al.[12], Jarboe et al.[3] and Penet et al.[15] It demonstrates the sufficient extraction of free amino acids from sample beefs. In all the four grades of beefs, total amount of ninhydrin positive compounds was ascertained to be considerably greater in the beef round than in the beef loin. According to the classification of the taste of free amino acids described by Takahashi et al.[16], in all the sample beefs, the total amounts of good-tasting amino acids such as taurine, glutamic acid, aspartic acid, threonine, serine, glycine and alanine were confirmed to be surprisingly larger than those of bitter-tasting amino acids (proline, arginine, valine, methionine, isoleucine, leucine and phenylalanine) (Table 4).

In the beef loin, the amounts of the former were about four times as much as those of the latter and in the beef round, about three times. In the pork loin and the pork round, the ratios of the amount of good-tasting amino acids to that of bitter-tasting amino acids were respectively 4 : 1 and 5～6 : 1[7,8]. The ratio calculated from the data of Jarboe et al.[3], who employed the bovine *semimembranosus* muscle as experimental materials, was amounting to 1.1 : 1. The difference between their value and ours must be attributable to the extracting degree of amino acids. Additionally, quantitative differences among the literature data and the present data may be due to the fluctuation of beef composition caused by numerous intrinsic and extrinsic factors as described by Jarboe et al.[3].

Nakajima et al.[13] found the presence of 107mg% and 123mg% of 5'-inosinic acid in the marketing raw beef and pork. Terasaki et al.[17] revealed that the time and the amount reaching maximum content of inosinic acid in meats were varied with the species of animals and the slaughtering methods. Upon the storage at 4°C after slaughter, the maximum content in chicken breast meat, (210～270mg%) and that in pork (110～180mg%) were observed

Table 4-a. Good-tasting and bitter-tasting amino acids contents in aqueous beef loin extract

(mg/100g fresh meat)

Grade	Tasty A. A.[a]	Bitter A. A.[b]	a/b
Supreme	157.7	35.5	4.4
Superior	106.8	26.0	4.1
Excellent	130.3	30.7	4.2
Medium	105.0	29.2	3.6

Table 4-b. Good-tasting and bitter-tasting amino acids contents in aqueous beef round extract

(mg/100g fresh meat)

Grade	Tasty A. A.[a]	Bitter A. A.[b]	a/b
Supreme	122.3	58.1	2.1
Superior	110.2	35.2	3.1
Excellent	104.0	30.3	3.4
Medium	104.7	37.6	2.8

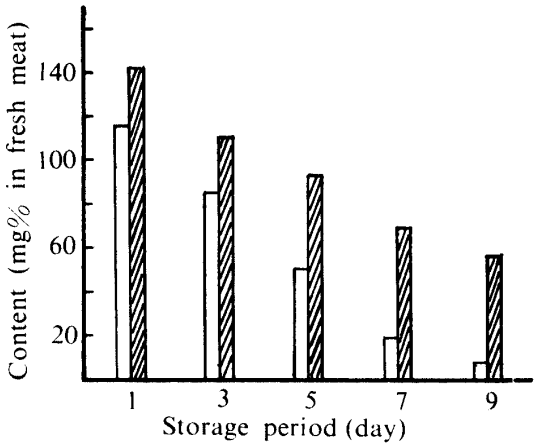

Fig. 2. Variation of inosinic acid content in beef during storage at 5°C.

□: Beef loin, ▨: Beef round

Table 5. 5'-Inosinic acid contents in various grades of beefs

(mg%)

Grade	Beef loin	Beef round
Supreme	38.2	20.0
Superior	43.2	65.0
Excellent	47.7	78.5
Medium	56.1	92.8

after 8 hr and 3 days, respectively. From the sensory evaluation, they considered that the content of inosinic acid was directly related to the flavor and taste of meat. Our experiment concerning the variation of inosinic acid content in one beef sample after being defrosted revealed a decreasing of the content with the time of storage at 5°C, as shown in Fig. 2. The extraction of inosinic acid and its analysis were performed on the sample meats after storage for 1 day at 5°C. The results were shown in Table 5. Excepting the supreme grade of beef, inosinic acid contents in the beef round were ascertained to be larger than those in the beef loin, being 1.5～1.7 times as much as those in the latter. The largeness of the content was anti-parallel to the grades of beef.

Judging from the experimental results described above, it was not supposed that the contents of the palatable compounds including free amino acids and inosinic acid were directly related to the grades of beef.

Summary

Quantitative analyses of free amino acids, carnosine and 5'-inosinic acid were performed on the beef loins and rounds from various grades of dressed carcasses of the Japanese Black cattles evaluated by the public meat-quality estimating authorities. The results were

as follows:

1. Of all the ninhydrin positive compounds in aqueous beef extracts, carnosine contents were noticed to be remarkably large and the comparatively large amount was ascertained in the following compounds, namely, alanine, taurine, threonine, lysine, glycine and glutamic acid, in this order. The largeness of the carnosine content was anti-parallel to the grades of beef and the contents in the beef round were exceedingly larger than those in the beef loin, in all the four grades of beefs.

Excepting taurine, the largeness of the respective contents of alanine, threonine, glutamic acid, glycine and serine which are palatable amino acids was generally in the order of supreme, superior, excellent and medium grades of beef, being proportional to the grades of beef. While taurine contents in the beef round were slightly larger than those in the beef loin, the contents of alanine and glutamic acid in the beef round were respectively smaller than those in the beef loin.

2. Total amounts of free amino acids in the beef loins and rounds were larger in the supreme grade beef than in the other grades of beef, but any significant difference was not observed among the other grades of beefs. In all the four grades of beefs, the total amounts of ninhydrin positive compounds were ascertained to be considerably greater in the beef round than in the beef loin.

3. In all the sample meats, the total amount of the good-tasting amino acids was confirmed to be surprisingly greater than that of the bitter-tasting amino acids.

In the beef loin, the amount of the former was about four times as much as that of the latter, and in the beef round, about three times.

4. Excepting the supreme grade of beef, inosinic acid content in the beef round was ascertained to be greater than that in the beef loin. The largeness of the contents was anti-parallel to the grades of beef.

Acknowledgment

This study was supported by a Grant-in-Aid for Special Project Research from the Ministry of Education, Science and Culture of Japan (1984), for which the authors are very grateful. High Performance Liquid Chromatograph was purchased by the grant.

References

1) Ehira, S., Uchiyama, H., Uda, F. and Matsumiya, H.: A rapid method for determination of the acid-soluble nucleotides in fish muscle by concave gradient elution. *Bull. Jap. Soc. Sci. Fisheries,* **36**, 491-496 (1970)

2) Field, R. A. and Chang, Y.: Free amino acids in bovine muscles and their relationship to tenderness. *J. Food Sci.,* **34**, 329-331 (1969)

3) Jarboe, J. K. and Mabrouk, A. F.: Free amino acids, sugars and organic acids in aqueous beef extract. *J. Agr. Food Chem.*, **22**, 787-791 (1974)

4) Koga, K., Fukunaga, T. and Inao, H.: Studies on the content of free amino acids, inosinic and lactic acids in the lean meat from pigs of different body weights at slaughtering. *Bull. Fac. Agr., Kagoshima Univ.,* **No. 25**, 95-102 (1975)

5) Koga, K., Fukunaga, T., Shimotamari, T. and Kawaida, H.: Free amino acids and carnosine content in the lean meats from several varieties of pigs fed on the feedstuff containing sweet potato meal. *Bull. Fac. Agr., Kagoshima Univ.,* **No. 33**, 91-97 (1983)

6) Koga, K., Fukunaga, T., Uchiyama, K. and Kawaida, H.: Estimation of 5'-inosinic, 5'-guanylic and lactic acids in the lean meats from several breeds of pigs fed on the feedstuff containing sweet potato meal. *Bull. Fac. Agr., Kagoshima Univ.,* **No. 34**, 67-74 (1984)

7) Koga, K., Fukunaga, T., Ohki, Y. and Kawaida, H.: Free amino acids and carnosine contents in the lean meats from the strain and the strain-cross pigs. *Bull. Fac. Agr., Kagoshima Univ.,* **No. 35**, 65-73 (1985)

8) Koga, K., Fukunaga, T., Shinkura, T. and Kawaida, H.: Free amino acids carnosine and 5'-inosinic acid contents in the lean meats from the strain and the triple-cross pigs. *Bull. Fac. Agr., Kagoshima Univ.,* **No. 36**, 111-119 (1986)

9) Kuninaka, A.: Studies on taste of ribonucleic acid derivatives. *Nippon Nōgei-Kagaku Kaishi,* **34**, 489-492 (1960)

10) Kuninaka, A.: The taste and manufacturing procedure of 5'-nucleotides. *Protein, Nucleic Acid, Enzyme,* **6**, 403-410 (1961) Kyoritsu Shuppan Ltd. Co..

11) Ma, R. M., Matlack, M. B. and Hiner, R. L.: A study of the free amino acids in bovine muscles. *J. Food. Sci.,* **26**, 485-491 (1961)

12) Macy, R. L. Jr., Naumann, H. D. and Bailey, M. E.: Water-soluble flavor and odor precursors of meat. II. Effects of heating on amino nitrogen constituents and carbohydrates in lyophilized diffusates from aqueous extracts of beef, pork and lamb. *J. Food Sci.,* **29**, 142-148 1964)

13) Nakajima, N., Ichikawa, K., Kamada, M. and Fujita, E.: Food chemical studies on 5'-ribonucleotides. Part II. On the 5'-ribonucleotides in food (2), 5'-Ribonucleotides in fishes, shellfishes and meats. *Nippon Nōgei-Kagaku Kaishi,* **35**, 803-808 (1961)

14) Ozawa, A., Aoki, S., Suzuki, K., Sugimoto, M., Fujita, T. and Tsuji, K.: Taurine content in fish and shells. *Nippon Eiyō Shokuryo Gakkaishi,* **37**, 561-567 (1984)

15) Penet, C. S., Worthington, R. E., Phillips, R. D. and Moon, N. J.: Free amino acids of raw and ground beef and pork. *J. Food Sci.,* **48**, 298-299 (1983)

16) Takahashi, M. and Tazaki, R.: How does seasoning mean? *Bunseki-Kagaku* (Japan Analyst), **23**, 965-970 (1974)

17) Terasaki, M., Kajikawa, M., Fujita, E. and Ishii, K.: Studies on the flavor of meats, Part I. Formation and degradation of inosinic acids in meats. *Agr. Biol. Chem.* (Tokyo), **29**, 208-215 (1965)

18) Tsuji, K.: Taurine and cholesterol metabolism. *Kagaku to Seibutsu (Chemistry and Biology),* **23**, 217-218 (1985)

19) Wasserman, A. E. and Spinelli, A. M.: Sugar-amino acid interaction in the diffusate of water extract of beef and model systems. *J. Food Sci.,* **35**, 328-332 (1970)

20) Yamaguchi, K.: Metabolism and nutrition of sulphur-containing amino acid, especially of taurine. *Kagaku to Seibutsu (Chemistry and Biology),* **23**, 299-308 (1985)

21) Zeika, L. L.: Meat flavor. Method for rapid preparation of the water-soluble low molecular weight fraction of meat tissue extract. *J. Agr. Food Chem.,* **37**, 893-895 (1969)