Discrimination of Meat Species in Processed Meat Products Based on the Ratio of Histidine Dipeptides

Hiroki ABE* and Emiko OKUMA*

* *Department of Food Science and Nutrition, Kyoritsu Women's University,
1-710 Motohachioji, Hachioji, Tokyo 193*

Meat species discrimination was examined on heat processed meat products by the analysis of histidine dipeptides, carnosine, anserine, and balenine, using high-performance liquid chromatography. The distribution of these dipeptides and their ratios, carnosine/anserine and balenine/anserine, were specific to each meat species of beef, pork, horse, deer, chicken, and turkey. Products made from single meat species of beef, pork, or chicken were almost always correctly identified from the dipeptide ratios. This discrimination technique was unaffected by the contents of moisture, fat, and/or other ingredients. The meat proportion in most of the mixed meat products made from any two meat species of beef, pork, and chicken was able to be semi-quantitatively determined using the standard curves of dipeptide ratio versus meat proportion.

Many investigations have been carried out to develop the analytical method for the discrimination of meat species of fresh meat and in processed meat products. Several methods such as protein electrophoreses, immunoassays, and profile analyses of certain proteins have been known to be valuable for the determination of fresh meat species¹⁾²⁾. For processed meat products, however, most of the methods are not applicable because of the denaturation of muscle proteins during the curing and heat processing of raw meat. Therefore, for the discrimination of meat species in heat-processed meat products, it is necessary to use species specific and heat stable components such as heat stable proteins¹⁾³⁾⁴⁾, fatty acids or fat species¹⁾⁵⁾ and histidine dipeptides¹⁾⁵⁾ or to use DNA hybridization^{6)~8)} or polymerase chain reaction⁹⁾ methods.

Of these methods for processed meat products, histidine dipeptides: *i.e.*, carnosine (β -alanyl-L-histidine), anserine (β -alanyl-1-methyl-L-histidine), and balenine (ophidine; β -alanyl-3-methyl-L-histidine), were first used by TINBERGEN and SLUMP¹⁰⁾ for the detection of chicken meat in heated pork product. CARNEGIE *et al.*^{11)~13)} later confirmed the applicability of this method to various meat products.

More recently, FLOWMAN and CLOSE¹⁴⁾ also suggested the possibility of this method for the identification of meat species used in processed meat products. We have already reported a simple, sensitive, and time-saving determination method of these dipeptides using ion-exchange high-performance liquid chromatography (HPLC)¹⁵⁾. Thus, we intended to apply the method to meat species discrimination in processed meat products.

In this paper, we describe the distribution of histidine dipeptides in meat, meat species discrimination in processed meat products made from one meat species, and the semi-quantitative discrimination in those made from two meat species.

Materials and Methods

Materials

Horse and Japanese deer meats were obtained from local suppliers in Aomori and Iwate Prefecture, respectively. The other fresh meats were purchased from a wholesale food market in Hachioji (Tokyo). Samples of commercially available processed meat products were randomly purchased at supermarkets, groceries, and fast food shops in Tokyo and Los Angeles.

Preparation of meat extract

After hand-trimming of the visible fat or removal of other ingredients from meats or meat products, about 50 g of the sample was minced using a meat grinder and mixed well in a mortar. From the sample, a 1 g portion was homogenized with 7 ml of 8% perchloric acid using a Polytron homogenizer (Kinematica, Zurich). After centrifugation at $20\,000\times g$ for 15 min at 0°C , 5 to 20 μl of the supernatant was injected into the HPLC.

Determination of histidine dipeptides

Histidine dipeptides were determined using a previously described HPLC method¹⁵⁾ with some modifications. Separation was carried out on a Shimadzu LC-6A liquid chromatograph equipped with a column ($4.6\times 250\text{ mm}$) packed with Nucleosil-5 SA cation exchange resin (Macherey-Nagel, Duren). Elution was isocratic using 80 mM potassium phosphate, pH 5.1, containing 10% methanol at 55°C and at a flow rate of 1 ml/min. Dipeptides were monitored at UV 210 nm with 0.01–0.04 absorbance units at full scale.

Standard curves for calculating meat proportions in mixed meat samples

After two meat species from minced beef, pork, and chicken were mixed in various proportions (10% interval) in a mortar, meat balls weighing about 3 g were made and heated for 1 min in an oven preheated at 200°C .

Dipeptides were extracted from the cooked meat balls and determined as previously described. For every combination, beef-pork, pork-chicken, and beef-chicken, experiments were repeated 8 to 16 times. Standard curves of histidine dipeptide ratios versus meat proportions were obtained from the average and standard deviations of the carnosine/anserine and balenine/anserine ratios for every meat proportion.

Results and Discussion

Distribution of histidine dipeptides in meats

The concentration of histidine dipeptides and their ratios were determined in raw beef, pork, horse, deer, chicken, and turkey (Table 1). The distribution of these dipeptides was species specific in these meats. Variation between different beef (individual variation) was small as seen in the small standard deviations. This was also the case with pork and chicken. Our early results on various muscle portions of beef and pork suggested the small differences in the dipeptide contents (unpublished data). No large sample variation of histidine dipeptides was also found in deer. The sample variation, however, was large in horse and two bird species. Horse meat contained the highest carnosine level which was in the range of 26.3 to $57.1\text{ }\mu\text{mol/g}$ in leg muscles. In case of birds, the data were divided into two typically

Table 1 The content and the ratios of histidine dipeptides in meat

Meat/Muscle	Sample No.	Contents ($\mu\text{mol/g}$ wet wt.)			Dipeptide ratio	
		Car	Ans	Bal	Car/Ans	Bal/Ans
Beef/leg	5 ^{*1}	26.1 ± 3.7	5.94 ± 1.75	0.103 ± 0.026	4.79 ± 1.48	0.018 ± 0.005
Pork/leg	6 ^{*1}	29.5 ± 9.6	1.42 ± 0.29	1.77 ± 0.71	20.5 ± 3.4	1.20 ± 0.27
Horse/leg	3 ^{*2}	42.6 ± 12.6	0.176 ± 0.030	0.019 ± 0.004	260 ± 111	0.107 ± 0.009
Deer/leg	5 ^{*2}	3.35 ± 0.68	13.9 ± 1.93	3.91 ± 0.74	0.253 ± 0.080	0.281 ± 0.021
Chicken/leg	3 ^{*1}	5.7 ± 1.7	17.1 ± 3.7	0.055 ± 0.028	0.33 ± 0.03	0.003 ± 0.001
/breast	4 ^{*1}	10.4 ± 1.3	32.0 ± 1.4	0.197 ± 0.031	0.33 ± 0.04	0.003 ± 0.001
Turkey/leg	3 ^{*2}	4.53 ± 0.68	20.5 ± 1.9	0.077 ± 0.009	0.22 ± 0.03	0.004 ± 0.001
/breast	2 ^{*2}	11.2 ± 1.3	46.0 ± 0.8	0.810 ± 0.023	0.24 ± 0.02	0.018 ± 0.004

Mean $\pm$ SD.

^{*1} Average from the same muscle from different animals.

^{*2} Average from different muscles of one animal.

Abbreviations : Car, carnosine ; Ans, anserine ; Bal, balenine.

different muscles, the leg and breast muscles (Table 1). They clearly differed from each other in dipeptide contents. These differences, however, were eliminated when the ratios of carnosine /anserine and balenine/an-serine were compared as seen in Table 1. These results were almost identical to those previously reported^{12) 14)}.

CARNEGIE *et al.*¹¹⁾ proposed the ratio of carnosine/an-serine for the estimation of meat species in processed meat products. Table 2 shows the ranges of the ratios in all meats examined in the present experiment. The carnosine/an-serine ratio appeared to be highly specific in each meat. Although the ratio of carnosine/an-serine was almost the same in deer and turkey, the balenine/an-serine ratio was distinctly different between the two species. The balenine/an-serine ratio was a very small value and similar between chicken and turkey, but the carnosine/an-serine ratio was different between these birds. Based on our preliminary experiment, the carnosine/an-serine and balenine/an-serine ratios in lamb meat were found to be 1.6 and 0.01, respectively, which were also different from those found in the other species shown in Table 2 (unpublished data). These ratios were almost similar to those previously reported^{12) 14)}. Thus, these ratios may be independent of individuals of one animal species, muscle portions of an animal, and of water or fat contents. These data indicate that the raw meat species can be correctly discriminated from these ratios. Preliminary experiments revealed that these dipeptides were stable during post-

mortem conditioning at 4°C for 2 weeks, low temperature storage at 1°C for 9 days after conditioning, and heating for 5 h at 120°C (unpublished data). The contents of these dipeptides shown in Table 1 were not changed at all after cooking for 1 min at 200°C. CARNEGIE *et al.*^{12) 14)} also reported that heating beef and pork for 30 min at 180°C produced no loss of these dipeptides.

Meat species discrimination in processed meat products made from one meat species

Table 3 shows the contents and ratios of histidine dipeptides in various commercially available meat products made from one meat species. These data were obtained from three separate extracts from one product. The dipeptide content significantly differed among the products, which may reflect the difference in the moisture and fat contents and/or other ingredients of each product. The ratios of these dipeptides, however, were almost consistent with the ratios of each meat (Tables 1 and 2). This indicates that the ingredients other than meat will have almost no effects on the ratios. Thus, the determination of moisture content as described by the other workers^{12) 14)} can be omitted in the present method.

In the meat products shown in Table 3, lachsschinken (No. 7), for instance, showed a rather high carnosine/an-serine ratio compared with the pork value. This reason was unclear, but the product was estimated to be made from pork muscle judging from the balenine/an-serine value. Canned sausage (No. 11) and frozen meat ball (No. 15) which were labeled as only pork showed rather low ratios compared with the pork value. These ratios were rather similar to those of the pork products mixed with chicken (Table 4). Thus, these samples were considered to contain other meat such as chicken or non-skeletal muscle of pork such as heart muscle which contains only trace amounts of these dipeptides¹²⁾. All the other samples were correctly identified to be made from corresponding meat specified on the label. From these data, it is apparent that these meat species are easily discriminated in the processed meat products, if they are made from only one

Table 2 Ranges of the ratios of histidine dipeptides in meat

Meat	Car/Ans	Bal/Ans
Beef	4-15	0.012-0.068
Pork	16-26	0.83-2.1
Horse	102-394	0-0.21
Deer	0.13-0.35	0.25-0.31
Chicken	0.28-0.81	0.005-0.015
Turkey	0.18-0.27	0.003-0.022

Table 3 Histidine dipeptide contents and the ratios in various processed meat products made from single meat species

No.	Meat products	Raw materials labeled	Contents ($\mu\text{mol/g}$ wet wt.)			Dipeptide ratio	
			Car	Ans	Bal	Car/Ans	Bal/Ans
Ham							
1.	Beef ham	beef	14.3	3.03	0.094	4.7	0.03
2.	Pork ham A	pork inside ham	10.0	0.445	1.43	23	3.2
3.	B	pork	9.42	0.618	1.05	15	1.7
4.	Boneless ham	pork inside ham	14.5	0.792	1.29	18	1.6
5.	Chicken ham A	chicken	3.39	7.38	0.038	0.46	0.005
6.	B	chicken	8.54	21.5	0.143	0.40	0.007
7.	Lachsschinken A	pork loin	24.4	0.616	1.08	40	1.8
8.	B	pork loin	26.3	1.35	2.94	19	2.2
Sausage							
9.	Frankfurter	beef	5.63	0.728	0.049	7.7	0.067
Canned products							
10.	Corned beef	beef	2.05	0.368	0.010	5.6	0.027
11.	Sausage	pork	4.20	1.06	0.18	4.0	0.17
12.	Meat paste	chicken	3.62	8.49	0.046	0.43	0.005
Dried products							
13.	Beef jerky A	beef	48.5	8.95	0.397	5.4	0.044
14.	B	beef	50.6	9.85	0.328	5.1	0.033
Frozen products							
15.	Meat ball	pork	0.820	0.110	0.087	7.5	0.79
16.	Chicken nugget	chicken	4.94	13.7	0.084	0.36	0.006
Hamburger patty							
17.	Brand A	beef	9.27	1.34	0.063	6.9	0.047
18.	B	beef	5.81	1.03	0.030	5.6	0.029
19.	C	beef	8.91	1.18	0.063	7.6	0.053
20.	D	—	7.29	1.11	0.044	6.6	0.040

meat species.

Semi-quantitative discrimination of meat species in mixed meat products made from two meat species

Carnegie *et al.*¹²⁾ previously tried to estimate the pork proportion in a mixture with other meat species such as beef, mutton, and chicken meat. Using the dipeptide ratios of each meat species, they made theoretical curves for the dipeptide ratios versus pork proportions and determined the pork proportion in several meat products. If a calibration curve including standard deviations is constructed from known mixtures of any two meat species, it may be possible to semi-quantitatively deter-

mine the proportion of a given meat in a meat product made from two meat species.

Figure 1 represents the standard curves for cooked meat mixtures from any two combinations of beef, pork, and chicken meat. These curves were in good agreement with the theoretical ones calculated from the dipeptide ratios for each meat shown in Table 1. As the curve was rather hyperbolic for the pork and chicken mixture, both dipeptide ratios would give a broad range for the chicken proportion if the chicken proportion was above 50%. This was also the case for the beef and chicken mixture. For the carnosine/anserine ratio of 1.0 in beef and chicken mixture, for instance,

chicken proportion was estimated to be 25-70% based on the standard curve in Fig. 1 because of the relatively large standard deviations. Thus, the estimation was considered to be semi-quantitative as has been previously stressed¹²⁾.

Table 4 shows the estimation of the chicken proportion in several commercially available meat products which were labeled pork and chicken. These products largely differed in their dipeptide contents, which showed the difference in the contents of moisture, fat, and/or other ingredients. In the dipeptide ratios, however, the variations were eliminated and all the values fell into the range between pork and chicken (Table 1). From the ratios and

the standard curves shown in Fig. 1, the chicken proportion was able to be estimated as shown in the parentheses in Table 4. The proportions estimated from the carnosine/anserine and balenine/anserine ratios were in good agreement in all samples. The range of the chicken proportion was rather broad in Chinese shao-mai (No. 7) in which the meat content was considered to be low from the dipeptide contents.

The beef proportion in the various meat products labeled pork and beef was estimated (Table 5). These products differed in dipeptide contents as was seen in the other products (Tables 3 and 4). Beef proportions obtained from each dipeptide ratio, however, were

Fig. 1 Standard curves for calculating meat proportion in processed meat products made from two meat species from any two of beef, pork, and chicken

Thick line represents the average value from 8 to 16 determinations, and the thin line represents the standard deviation.

Table 4 Estimation of chicken proportion in the processed meat products made from pork and chicken

No.	Meat products	Contents ($\mu\text{mol/g}$ wet wt.)			Dipeptide ratio	
		Car	Ans	Bal	Car/Ans	Bal/Ans
1.	Wiener A	12.2	1.78	0.735	6.85 (11~17)	0.412 (7~17)
2.	B	3.2	4.65	0.076	0.69 (54~87)	0.016 (55~89)
3.	C	3.5	7.42	0.064	0.47 (77~96)	0.009 (79~100)
4.	D	4.8	9.64	0.133	0.50 (75~95)	0.014 (66~95)
5.	E	4.5	6.46	0.198	0.70 (57~86)	0.031 (40~80)
6.	Retort hamburg	3.0	7.28	0.054	0.41 (82~98)	0.007 (86~100)
7.	Shao-mai	1.0	0.89	0.044	1.12 (36~70)	0.049 (30~70)

Chicken meat proportions (%) were estimated from the dipeptide ratios and the standard curves in Fig. 1 and are shown in parentheses.

Table 5 Estimation of beef proportion in the processed meat products made from pork and beef

No.	Meat products	Contents ($\mu\text{mol/g}$ wet wt.)			Dipeptide ratio	
		Car	Ans	Bal	Car/Ans	Bal/Ans
1.	Wiener A	9.2	0.89	2.23	10.3 (22~45)	2.49 (-)
2.	B	8.2	1.09	0.385	7.5 (40~76)	0.354 (30~55)
3.	C	9.4	1.51	0.359	6.2 (52~98)	0.239 (43~59)
4.	D	4.8	0.73	0.106	6.6 (48~92)	0.146 (54~75)
5.	E	15.6	1.39	0.536	11.2 (18~39)	0.385 (24~44)
6.	F	19.8	1.68	0.665	11.8 (16~35)	0.396 (22~42)
7.	Canned wiener A	4.4	2.19	0.086	2.0 (-)	0.039 (78~95)
8.	B	4.4	2.53	0.172	1.7 (-)	0.068 (69~89)
9.	Frankfurter	8.4	0.41	0.404	20.5 (0~10)	0.974 (0~12)
10.	Retort hamburg	6.1	0.27	0.214	22.6 (0~5)	0.794 (2~12)

Beef proportions (%) were estimated from the dipeptide ratios and the standard curves in Fig. 1 and are shown in parentheses.

almost in agreement with the expected values (Table 2) except for several products such as Nos. 1, 7, and 8. It was impossible to determine the beef proportion in sample No. 1 which showed a much higher balenine/anserine ratio than its range in only pork. Canned wiener sausages, Nos. 7 and 8, on the other hand, showed a lower carosine/anserine ratio than beef, though the balenine/anserine ratio suggested a high proportion of beef. In these sausages, it is probable to be adulterated chicken meat or visceral muscle of cattle or pig.

Very close estimations from both ratios were obtained in the other beef and pork proportions such as Nos. 5, 6, 9, and 10. Products with a low pork proportion such as Nos. 2-4, however, showed rather broad and different

proportions estimated from the two ratios, because of the large standard deviations of the carnosine/anserine ratio within the high beef proportion in the standard curve shown in Fig. 1. The indication of "55% beef" on the label in No. 2 product almost coincided with the estimated beef proportion of 40-55% obtained from the overlapped range between the two estimated ranges, 40-76% from the carnosine/anserine ratio and 30-55% from the balenine/anserine ratio. Thus, the meat proportion may be estimated more precisely when the overlapped value obtained from the carnosine/anserine and balenine/anserine ratios is compared.

Meat products containing beef and chicken were not able to be analyzed in the present

experiment, since it was expected to give almost similar results to the other combinations shown above. Many techniques have been developed to discriminate meat origin in uncooked meat and heat processed meat products. Most of the methods, however, were developed for the detection of meat adulteration qualitatively. The semi-quantitative determination of meat species in heat processed products has not yet been successful except for enzyme-linked immunosorbent assays¹⁾ and the DNA hybridization method⁷⁾. As proposed by CARNEGIE *et al.*¹²⁾, it was confirmed by the present experiments that the histidine dipeptide analysis was a simple and fast method for the semi-quantitative discrimination of meat species.

Judging from the above data, this method may lead to a wrong conclusion when the product contains meat extract or non-skeletal muscle and contains only a small amount of meat or small amount of adulteration of other meats. The method may also be useless for products made from more than three meat species. In spite of these disadvantages, the proposed method has some advantages as follows : (1) easy sample preparation with no special reagent, (2) high-sensitivity for detection, especially for balenine, (3) fast (25 min) and simple isocratic HPLC, (4) no necessity for the determination of the other components such as moisture content, (5) substantially independent of the ingredients contained in the products, (6) stability of these compounds even in cooked meat, and (7) availability of two different complementary factors, the carnosine/anserine and balenine/anserine ratios. In order to more precisely estimate the meat proportion in mixed meat products, further determination of these dipeptide ratios would be needed on large populations and various muscle samples of each animal.

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ヒスチジン関連ジペプチド比による食肉加工製品中の肉種の判別

阿部宏喜*・大熊恵美子*

* 共立女子大学家政学部食物学科
(〒193 東京都八王子市元八王子町 1-710)

高速液体クロマトグラフィーによりヒスチジン関連ジペプチド、すなわちカルノシン、アンセリンおよびバレンインを分析し、食肉加工製品中の肉種の判別の可能性を検

討した。牛, 豚, 馬, 鹿, 鶏および七面鳥肉におけるこれらジペプチドの分布およびジペプチドの比率, すなわちカルノシン/アンセリンおよびバレニン/アンセリンは種特異的であった。これらの比率から, 牛, 豚あるいは鶏肉を単独で用いたほとんどの食肉加工製品の肉種の判別が可能であった。水分, 脂質含量あるいはその他の添

加物は肉種の判別に影響を与えなかった。また, これらの肉の内, 二種を混合した食肉加工製品については, 混合比率とジペプチド比の検量線を用いることにより, ほとんどの製品で半定量的に肉種を判別することが可能であった。