Lebensm.-Wiss. u.-Technol., 33, 60–62 (2000)

Research Note

HPLC Determination of Carnosine in Commercial Canned Soups and Natural Meat Extracts

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(Received January 21, 1999; accepted August 24, 1999)

The skeletal muscle of vertebrates is a rich source of carnosine (β-alanyl-L-histidine). Fourteen commercially available canned soup products were screened for carnosine content by a simple and sensitive ion-exchange HPLC method. Carnosine in the soup fluid ranged from 0 to 0.35 mg/g sample. Though water soluble, carnosine was not detected in four of the nine samples of soup fluid which carried the tag 'beef' in their labels. Among the commercially available natural meat extracts with Brix values varying from 52 to 78, beef meat extract with Brix 78 was comparatively rich in carnosine (37.5–38.2 mg/g sample) compared to bonito extract with Brix 67–80, in which carnosine levels varied from 5.0 to 7.9 mg/g sample.

Keywords: carnosine; HPLC method; natural extracts; soup

Introduction

Carnosine is a dipeptide, comprising *β*-alanine and histidine as its component amino acids. It is present in high concentrations in skeletal muscle of vertebrates, including humans (1). Since the first isolation of carnosine from beef muscle extract in 1900, quantitative determination of carnosine has remained a focus of interest. Crush (2) critically reviewed the merits and demerits of methods which were prevailing until 1970 for determination of carnosine in animal tissues. The methods employed include: (a) paper chromatography followed by ninhydrin colour reaction; (b) colorimetry based on the diazotized *p*-bromoaniline reaction; and (c) isolation using ion-exchange resins followed by ninhydrin colour reaction. Since then, Wideman *et al.* (3) reported a fluorometric peptide microassay for carnosine which made use of 2-methoxy 2,4-diphenyl 3(2H)-furanone (MDPF).

During the past 25 years, carnosine has been shown to play a role in quite a number of physiological functions in vertebrates, including humans. These include buffering action in muscle (4), antioxidant action (5) and neurotransmitter function (6). Thus, potential of carnosine as a natural food supplement for health-challenged individuals is being actively studied in various laboratories. Abe (7) reported a simple and accurate HPLC method for determination of carnosine in fish muscles. A subsequent modification of this method by Abe and Okuma (8) has facilitated quick estimation of carnosine in food products. They reported on the carnosine content of fresh meats and commercially available processed meat products such as ham, sausage, corned beef, beef jerky, meat ball, chicken nugget and hamburger patty.

In this research note, we report the carnosine content in commercially available canned soups and natural meat-derived extracts to add to the meager database currently available on the carnosine composition of food products.

Materials and Methods

Materials

Samples of commercially available canned soups were randomly purchased at a supermarket in Tokyo. The samples of natural meat extracts were gifts from the Nikken Foods Co. Ltd. (Fukuroi City, Japan).

Methods

Determination of Carnosine. Standard histidine and carnosine were purchased from Sigma Chemical Co.

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0023-6438/00/010060 + 03 $35.00/0
doi:10.1006/fstl.1999.0602

(St.Louis, MO, U.S.A.). Carnosine levels in the test samples were determined using the HPLC method described by Abe and Okuma (8). The assay was performed in a Shimadzu LC-6A liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with a UV detector. The associated assay parameters were as follows: Zorbax 300-SCX column; eluent 90 mM KH_2PO_4 solution (pH 5.0) containing 10% methanol; detection temperature 55 °C; absorbance 210 nm. Ten mg/mL, solution of test sample was prepared in the eluent and 10 μL of filtered solution was injected for assay. Elution times of histidine and carnosine were 4.8 and 5.5 min, respectively.

Determination of Brix value. The Brix value of natural meat extracts was determined by a hand refractometer (Atago Co. Ltd, Tokyo, Japan).

Results and Discussion

Among the commercially available canned soup samples screened, six of the 14 samples tested did not contain carnosine in the soup fluid. Among the remaining eight samples, the amount of carnosine present ranged from 0.165 to 0.35 mg/g sample (**Table 1**). Among the eight types of natural meat-derived extracts examined, beef extract was found to be rich in carnosine, ranging from 37.5 to 38.2 mg/g sample (**Table 2**). Carnosine content in bonito extract ranged from 5.0 to 7.9 mg/g sample. The low values of carnosine detected for chicken extract can be attributed to the fact that anserine (methyl carnosine), in place of carnosine, is the predominant dipeptide in bird muscles (2, 9, 10). The absence of carnosine in lobster extract and short-necked clam extract is self-explanatory since dipeptides are practically absent in the muscle tissues of invertebrates (11).

During the past three decades, natural muscle and bone extracts derived from aquatic food resources, cattle, hog and poultry have become more appealing to the Oriental food industry for several reasons. One of the authors of the present study (12, 13) has previously described the production and applications of natural seafood extracts and bone extracts in Japan. The crude protein content in the seafood extracts of tuna, bonito, scallop and shrimp produced in Japan ranges from 12 to 49.3%, with Brix value (indicating total solid content) varying from 50.0 to 65.5%. Chan *et al.* (14) have discussed the preparation of a beef extract which is high in carnosine (3.79 mg/g muscle) and also low in lipid oxidation analytes. Since water content or Brix value of this particular beef extract

Table 1 Carnosine content of commercially available canned soups

Commercial Canned Soup[a]	Soup volume of the can (g)	Carnosine content in the soup fluid (mg/g sample)[b]
1. Beef broth (double rich double strength)	298	0.253
2. Fiesta chilli beef with beans	319	0.165
3. Beefy mushroom	305	0
4. Beef noodle	305	0
5. Beef with vegetables and barley	312	0
6. Beef consommé (with added gelatin)	298	0
7. Beef consommé	160	0.203
8. Potage goulache (goulash soup)	430	0.179
9. Potage ox-tail	430	0.165
10. Clear ox-tail	400	0.226
11. Beef consommé	300	0.350
12. Beef tea	160	0.179
13. Onion	160	0
14. Onion	290	0

[a] Brand details are as follows: 1–6, Campbell; 7, Heinz; 8, 9, Hero; 10, Jensen's; 11–13, MCC; 14, Blue Flag
[b] Mean of duplicate determination

Table 2 Carnosine content in natural muscle and bone extracts

Type of extract[a]	Brix value	Carnosine content[b] (mg/g sample)
Beef-muscle extracts ($n = 2$)	78	37.5–38.2
Beef-bone extracts ($n = 3$)	55–59	0.2–1.1
Chicken muscle extracts ($n = 5$)	54–67	0.7–0.9
Pork muscle extracts ($n = 6$)	53–69	1.4–1.8
Bonito (*katsuo*) extracts ($n = 3$)	67–80	5.0–7.9
Conger eel (*hamo*) extract ($n = 1$)	55	0.2
Lobster extract ($n = 1$)	52	0.1
Short-necked clam extract ($n = 1$)	43	0

[a] Number of products screened are indicated within parenthesis
[b] Mean of duplicate determinations of each product

was not reported, we cannot compare the nutritional value of the beef extract prepared by Chan *et al.* (14) with that used in the present study. However, we also have previously shown that the beef meat extract and bonito extracts analysed for this study contain high hydroxyl radical scavenging activity (15). The endogenous components of commercial meat extracts include various nitrogen-containing substances such as creatine, urea, amino acids, peptides and purines (16). Among these, it can be inferred that carnosine, when present in adequate levels, can contribute significantly to hydroxyl radical scavenging activity. Though the role of carnosine as a nutrient *per se* in the meat extracts may be limited since the volume of meat extracts used in the soups is relatively small, the accumulating evidence of the potential physiological actions of carnosine as an endogenous antioxidant (17, 18) and as an antisenescent substance in tissues (19, 20, 21) cannot be underestimated.

In summary, among the eight commercially available natural muscle and bone extracts analysed for carnosine, beef muscle extract can be inferred as a rich source of this dipeptide. This is reflected in the commercially available canned soups with beef as its major constituent.

Acknowledgment

We thank Dr. H. Abe for advice regarding the method for determination of carnosine in food samples.

References

1 Boldyrev, A. A. and Severin, S. E. The histidine-containing dipeptides; carnosine and anserine: distribution, properties and biological significance. *Advances in Enzyme Regulation*, **30**, 75–194 (1990)
2 Crush, K. G. Carnosine and related substances in animal tissues. *Comparative Biochemistry and Physiology*, **34**, 3–30 (1970)
3 Wideman, J., Brink, L. and Stein, S. New automated fluorometric peptide microassay for carnosine in mouse olfactory bulb. *Analytical Biochemistry*, **86**, 670–678 (1978)
4 Okuma, E. and Abe, H. Major buffering constituents in animal muscles. *Comparative Biochemistry and Physiology*, **102A**, 37–41 (1992)
5 Kohen, R., Yamamoto, Y., Cundy, K. and Ames, B. Antioxidant activity of carnosine, homocarnosine and anserine present in muscle and brain. *Proceedings of National Academy of Sciences USA*, **85**, 3175–3179 (1988)
6 Margolis, F. L. Carnosine in the primary olfactory pathway. *Science*, **184**, 909–911 (1974)
7 Abe, H. Determination of L-Histidine related compounds in fish muscles using high performance liquid chromatography. *Bulletin of the Japanese Society of Scientific Fisheries*, **47**, 139 (1981)
8 Abe, H. and Okuma, E. Discrimination of meat species in processed meat products based on the ratio of histidine dipeptides. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **42**, 827–834 (1995)
9 Davey, C. L. The significance of carnosine and anserine in striated skeletal muscle. *Archives of Biophysics and Biochemistry*, **89**, 303–308 (1960)
10 Brown, C. E. Interactions among carnosine, anserine, ophidine and copper in biochemical adaptation. *Journal of Theoretical Biology*, **88**, 245–256 (1981)
11 Boldyrev, A. A. Carnosine: Biological role and potential applications in medicine. *Biochemistry* (trans of *Biokhimiya*), **57**, 898–904 (1992)
12 Ochi, H. Production and applications of natural seafood extracts. *Food Technology*, **34**(11), 51–53, 68 (1980)
13 Ochi, H. Production and applications of bone extracts. *Food Technology*, **35**(7), 58–59 (1981)
14 Chan, K. M., Decker, E. A. and Means, W. J. Extraction and activity of carnosine, a naturally occurring antioxidant in beef muscle. *Journal of Food Science*, **58**, 1–4 (1993)
15 Sri Kantha, S., Wada, S., Takeuchi, M., Watabe, S. and Ochi, H. A sensitive method to screen for hydroxyl radical scavenging activity in natural food extracts using competitive inhibition ELISA for 8-hydroxy deoxyguanosine. *Biotechnology Techniques*, **10**, 933–936 (1996)
16 Yudkin, J. *The Penguin Encyclopedia of Nutrition*, New York: Viking Penguin Inc., p.236 (1985)
17 Boldyrev, A. A. Does carnosine possess direct antioxidant activity? *International Journal of Biochemistry*, **25**, 1101–1107 (1993)
18 Chan, K. M. and Decker, E. A. Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science and Nutrition*, **34**, 403–426 (1994)
19 McFarland, G. A. and Holliday, R. Retardation of the senescence of cultured human diploid fibroblasts by carnosine. *Experimental Cell Research*, **212**, 167–175 (1994)
20 Sri Kantha, S., Wada, S., Tanaka, H., Takeuchi, M., Watabe, S. and Ochi, H. Carnosine sustains the retention of cell morphology in continuous fibroblast culture subjected to nutritional insult. *Biochemical and Biophysical Research Communications*, **223**, 278–282 (1996)
21 McFarland, G. A. and Holliday, R. Further evidence for the rejuvenating effects of the dipeptide L-Carnosine on cultured human diploid fibroblasts. *Experimental Gerontology*, **34**, 35–45 (1999)