藥物食品檢驗局調查研究年報. 17: 293-295 1999
Ann. Rept. NLFD Taiwan R.O.C 17: 293-295 1999

Protein and Amino Acid Profiles in Natural and Artificial Shark Fins using Capillary Electrophoresis

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ABSTRACT

The potential utility of capillary electrophoresis (CE) for differentiation between natural and artificial shark fins was established. Three major protein components (peaks I-III) of shark fins were well-separated and used as parameters for differentiation between natural and artificial shark fins. The height ratio of protein peak III to protein peak I was higher in natural shark fins than that in artificial products. Further, the profile of amino acids in natural dried shark fins was somewhat different from that in artificial samples. The level of tyrosine was especially high (5.11-8.11%) in natural dried shark fins, but low or not detectable (0-0.22%) in artificial samples.

Key Words: shark fins, elastoidin, protein, amino acids, capillary electrophoresis

INTRODUCTION

THE DORSAL, PECTORAL, ANAL AND CAUdal fins of sharks are exfoliated of scales, large central cartilage is eliminated, and the remaining small sting cartilages are dried to a product called "shark fins." This product is a fibrous protein and is called elastoidin, which is an unusual type of intracellular structural protein and includes major collagen and minor non-collagenous proteins (Kimura and Kubota, 1966; Galloway, 1985). Elastoidin fiber is distinguished from typical collagen fiber in several respects, particularly in not yielding gelatin on boiling and in being rich in tyrosine (Gross, 1963). Several reviewers (Sikorski et al., 1984; Bailey, 1987; Kimura, 1989, 1992; Yoshinaka, 1989) pointed out that the protein types and amino acid profiles of elastoidin were somewhat different from those of collagen and elastin from other animals. It has been consumed by people eating in Chinese restaurants as a seafood commonly used in soup, desired due to its elasticity and palatability. Due to its high value and limited amounts, artificial shark fins are made of other types of collagen and starch.

Analytical methods for proteins and amino acids include gel electrophoresis, ion-exchange chromatography, gel permeation chromatography, reverse-phase HPLC and capillary electrophoresis (CE). Among these, CE is a family of related techniques that employs narrow-bore (20-200 μm i.d.) capillaries to perform high efficiency separation of both large and small molecules. CE has the potential for rapid separation of proteins (Chen and Zang, 1992; de Jong et al., 1993; Lookhart and Bean, 1995) and dansyl amino acids (Jorgenson and Lukacs, 1981; Guttman et al., 1988; Su et al., 1998), with high resolution and good quantification.

Our objective was to develop a method to detect artificial shark fins, using the electropherograms of protein and amino acids in commercially dried shark fins purchased from markets and cooked shark fins from restaurants examined by CE. The differentiation between natural and artificial shark fins was evaluated to establish reliable indicators for establishing authenticity of shark fins.

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MATERIALS & METHODS

Reagents and shark fins

Standard mixture of 17 amino acids in 0.1N HCl including alanine (Ala), arginine (Arg), aspartic acid (Asp), cystine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val) was purchased from Pierce Chemical, Co. (Rockford, IL). Hydroxyproline (Hyp), norleucine (Nle, internal standard) and dansyl chloride (Dns-Cl) were from Sigma Chemical Co. (St. Louis, MO). Except for 1.25 μmole/mL for cystine, the concentration of each amino acid used was 2.5 μmole/mL. Sodium tetraborate, sodium dihydrogen phosphate, sodium hydroxide, methyl cellulose, sodium dodecyl sulfate (SDS) and 10% tetramethylammonium hydroxide (Nacakai Tesque Inc., Kyoto) were also used. Other chemicals and reagents were purchased from E. Merck (Darmstadt).

Samples of natural (18) and artificial (6) dried shark fins were purchased from Taipei markets. Among those artificial dried shark fins, one sample was collected from a vegetarian shop. Furthermore, 8 samples of cooked shark fins were also collected from restaurants in Taipei. One was obtained from a vegetarian restaurant. These samples had been cooked and seasoned in restaurants. All dried and cooked shark fins were stored at -20°C until used.

Protein analysis

After rehydrating, 100 mg each of dried and cooked shark fins was dissolved in 2 mL of 10% tetramethylammonium hydroxide solution, and heated at 70°C with stirring for 1h. The digested solution was filtered through a 0.45 μm filter, and then analyzed for protein by CE.

A P/ACE 5500 CE system with UV detector (Beckman Instrument, Inc., Fullerton, CA) and Beckman eCap capillary tubing 27 cm (20 cm to detector window) x 20 μm i.d. was used for protein separation. The P/ACE system was used on-line at 214 nm. During electrophoresis, the capillary tube was maintained at ambient temperature (usually 25°C) with circulating coolant surrounding the capillary tube. Diluted samples were introduced by pressure injection for 5s, and electrophoresis was performed with 0.01% methyl cellulose in 0.1M sodium tetraborate solution (pH 10.0) at 8 KV. Between runs, the capillary tube was sequentially washed with 2 column volumes of 0.1N sodium hydroxide and water, followed by reconditioning with 10 volumes of 0.1M sodium tetraborate solution.

Amino acid analysis

All samples were dried for 24h under vacuum and ground to powder. Each sample (20 mg) was hydrolyzed for 24h at 110°C in twice distilled constant boiling HCl (6N) under nitrogen in flame sealed Pyrex tubes.

Hydrolyzed samples as well as standard amino acid mixture aliquots were dried by rotary evaporation under vacuum. The residue was then diluted with deionized water and the evaporation was repeated twice. Complete removal of HCl was accomplished by keeping the sample overnight under reduced pressure over NaOH. The residue was dissolved in 100 μL of deionized water, then 100 μL of 0.5M $NaHCO_3$ in deionized water and 100 μL of 0.02M Dns-Cl in acetone were added to each sample. Samples were reacted

原載於Journal of food science. 1998.63(5):782-784

in the dark for 40 min at 65 °C. Then 50 μL of derivatized samples were diluted with 150 μL of deionized water and 50 μL of 0.1M SDS in 0.02M sodium tetraborate and sodium dihydrogen phosphate buffer (pH 9.0) for amino acid analysis. Dns-Cl solution was always freshly prepared.

The above CE system was used with Beckman eCap capillary tubing 57 cm long (50 cm to detector window) × 50 μm i.d. for amino acids analysis. Electrophoresis was performed with a mixed solution including 1 volume of methanol and 9 volumes of 0.1M SDS in 0.02M sodium tetraborate and sodium dihydrogen phosphate buffer (pH 9.0) at 27 KV. Other procedures were the same as for protein analysis.

RESULTS & DISCUSSION

THE ELECTROPHEROGRAMS OF PROTEINS from natural and artificial dried shark fins were compared (Fig. 1). The protein components of natural and common artificial dried shark fins were well separated in 10 min total run time with good peak resolution between 7–8 min. There were no proteins in the vegetarian samples. Among the peaks of dried shark fins proteins, three peaks (I–III) showed better resolution, sharpness and symmetry. To compare the differentiation of protein profile in natural and artificial dried shark fins, the respective peak height ratio of protein peaks II and III to protein peak I were calculated (Table 1). The respective average value of peaks II and III to peak I were 2.78±0.63 (mean±S.D.) and 3.04±0.60 in natural dried shark fins, but 0.16±0.05 and 0.31±0.03 in artificial samples. There were differences ($p<0.05$) between natural and artificial dried shark fins. Among the artificial dried shark fins, one vegetarian sample showed no protein component and may have been formed from nonproteineous materials.

Table 1—Peak height ratio of proteins in commercial dried shark fins

Source	No. of samples	Peak height ratio of protein	
		Peak II/peak I	Peak III/peak I
Natural	18	2.78±0.63[a] (1.69-3.94)[b]	3.04±0.60 (1.71-3.99)
Artificial	6	0.16±0.05 (0-0.21)	0.31±0.03 (0-0.42)

[a]Mean±S.D.
[b]Data represent the range of values, and zero represents no detectable data.

The peak height ratios of protein in cooked samples collected from restaurants were compared (Table 2). Samples A-D showed higher peak height ratios of proteins, especially the ratio of peak III to I. But the samples E–H showed lower peak height ratio or no detectable level of protein component. This indicated that samples E–H may be artificial shark fins. Of these, sample H was from a vegetarian restaurant and showed no detectable protein.

Except Dns-Thr and Dns-Ser, 16 Dns-amino acids were well separated in 32 min total run time with good peak resolution, sharpness and symmetry. Standard curves of 16 amino acids were separately prepared in the range of 7.5-50 nmole and peak area vs amount of amino acid was plotted. Data for standard curves were subjected to linear regression analysis. The correlation coefficient for every curve was 0.99. This indicated a definite linear relationship between amino acid concentration and detector response. The minimum detectable amount of Dns-amino acids was in the range of 0.01–0.03 pmole. We concluded that the CE system for amino acid analysis was satisfactory. Recovery of all 16 amino acids by the sample digestion procedure was 99.3±0.6% (mean±S.D.) according to the method described (Su et al., 1998).

Table 2—Peak height ratio of proteins in cooked shark fins from restaurants

Sample[a]	Peak height ratio of protein	
	Peak II/peak I	Peak III/peak I
A	1.75±0.08[b]	4.13±0.07
B	1.80±0.11	4.47±0.13
C	1.57±0.14	3.88±0.10
D	1.05±0.03	2.87±0.15
E	0.33±0.06	0.24±0.02
F	0.08±0.01	0.33±0.03
G	0.92±0.15	0.97±0.04
H	0[c]	0

[a]Samples A through G were collected from common restaurants and sample H was from a vegetarian restaurant.
[b]Mean±S.D., n=3.
[c]Zero indicates no detectable protein.

The levels of amino acids in all samples were summarized (Table 3). Among these amino acids, the levels of Tyr, Cys, Ile and Met in natural dried shark fins were higher than those in artificial samples, especially for Tyr. The average levels of Tyr in natural and artificial dried shark fins were 6.92±0.91% and 0.19±0.03%, respectively, significantly different from each other ($p<0.05$). The amino acid levels of cooked shark fins from restaurants were compared (Table 4). Also the level of Tyr in sample A–D was higher than that of sample E–G. Judging from high Tyr level, samples A–D may be from natural shark fins, but samples E–G are from artificial samples. Therefore, the high peak height ratio of protein peak III to I and high Tyr level are two additional indicators for identification of authenticity of shark fins.

The protein components of shark fins have been reported to contain collagen and noncollageneous protein (Gross, 1963; Galloway, 1985). The collagen component of elastoidin from the shark spurhound is assumed to comprise three identical α-chains (Woodhead-Galloway et al., 1978). Kimura et al. (1986) also reported that elastoidin of great blue shark contained α-chain and a homotrimer in the collagen. In our results, the proteins from elastoidin could be easily digested and dissolved with 10% tetramethylammonium hydroxide solution. Natural dried shark fins are rich in peak II and III proteins, but the peak II protein in cooked shark fins degrades. The effect of cooking on the amount of each protein component in elastoidin should be further studied. Meanwhile, the characteristics of these protein components and variations of protein and

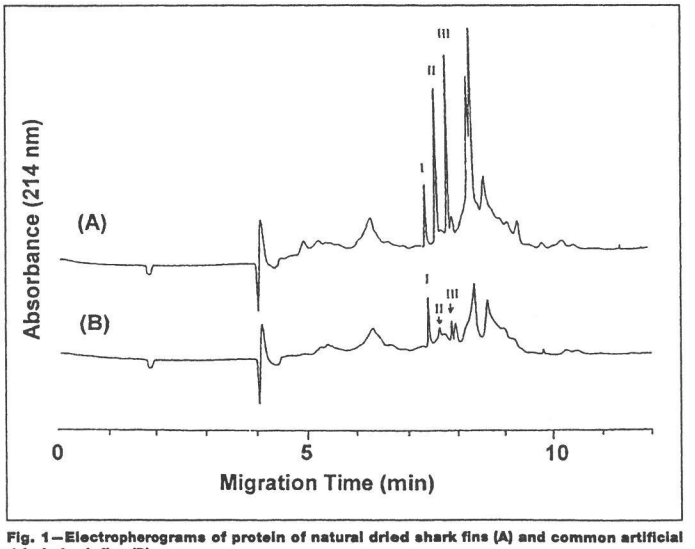

Fig. 1—Electropherograms of protein of natural dried shark fins (A) and common artificial dried shark fins (B).

Table 3—Amino acid levels in commercial dried shark fins

Amino acid	Amino acid levels in dried shark fins (%)	
	Natural	Artificial
Hyp	5.58±0.43[a] (4.76–5.98)[b]	7.77±0.51 (6.85–8.21)
Ala	9.72±2.41 (7.07–13.52)	7.72±1.26 (6.52–9.45)
Gly	18.40±3.15 (14.21–24.33)	18.11±3.03 (14.30–21.41)
Val	2.32±0.55 (1.69–3.14)	1.51±0.32 (1.22–1.96)
Pro	11.92±2.26 (9.26–15.40)	11.09±1.44 (9.52–12.35)
Met	1.25±0.20 (1.05–1.50)	0.23±0.12 (0–0.45)
Glu	8.78±1.52 (6.51–10.92)	9.18±0.81 (8.25–10.23)
Ile	1.99±0.30 (1.55–2.40)	0.95±0.08 (0.87–1.06)
Asp	5.21±1.01 (3.89–6.69)	4.69±0.47 (3.98–4.97)
Leu	2.31±0.41 (1.87–3.07)	2.30±0.51 (1.67–2.75)
Phe	2.19±0.30 (1.85–2.66)	1.56±0.38 (1.22–2.11)
Cys	0.66±0.13 (0.43–0.81)	0
Arg	7.00±0.13 (5.75–8.34)	5.38±1.28 (4.27–7.09)
Lys	3.12±0.43 (2.75–3.87)	2.83±0.72 (2.09–3.50)
His	1.32±0.24 (1.05–1.69)	0.42±0.16 (0.25–0.62)
Tyr	6.92±0.91 (5.11–8.11)	0.19±0.03 (0.16-0.22)

[a]Mean±S.D.
[b]Data represent the range of values, and zero represents no detectable amino acids.

Table 4—Amino acid levels in cooked shark fins from restaurants

Amino acid	Amino acid levels in sample (%)						
	A[a]	B	C	D	E	F	G
Hyp	5.02±0.29[b]	4.78±0.15	5.12±0.24	5.05±0.41	7.20±0.54	7.01±0.38	7.25±0.87
Ala	10.37±0.16	13.48±0.54	7.79±0.11	8.92±1.24	7.52±0.45	9.45±1.12	8.78±0.84
Gly	18.71±0.24	18.41±1.56	15.44±0.68	17.22±1.47	15.42±1.16	17.37±1.34	14.56±0.63
Val	2.80±0.14	1.73±0.12	2.04±0.28	1.84±0.31	1.24±0.09	1.47±0.11	1.22±0.15
Pro	11.18±0.07	10.26±1.13	9.48±0.82	10.56±0.45	10.25±1.12	9.52±0.87	9.88±0.43
Met	0.12±0.05	0.20±0.04	0.19±0.06	0.31±0.06	0.14±0.03	0[c]	0
Glu	10.88±0.54	10.21±1.02	10.26±0.57	10.23±0.87	8.23±0.87	10.21±0.89	9.16±0.56
Ile	2.14±0.22	1.86±0.12	1.78±0.54	1.64±0.27	0.97±0.04	1.06±0.07	0.93±0.13
Asp	7.76±0.38	7.13±0.65	7.01±0.41	7.41±0.82	3.94±0.32	4.88±0.23	3.96±0.72
Leu	2.38±0.41	1.90±0.18	2.24±0.22	2.31±0.14	1.75±0.37	1.68±0.41	1.87±0.33
Phe	2.22±0.11	1.86±0.08	2.30±0.14	2.07±0.35	1.32±0.06	1.42±0.23	1.26±0.06
Cys	0.14±0.02	0.21±0.04	0.15±0.05	0.30±0.11	0	0	0
Arg	8.34±0.61	7.36±0.87	6.81±0.28	6.97±0.15	4.31±0.57	4.52±0.28	4.54±0.25
Lys	3.39±0.18	2.87±0.47	2.89±0.07	3.02±0.26	2.12±0.42	2.33±0.31	2.74±0.08
His	1.44±0.09	1.05±0.04	1.24±0.12	1.52±0.28	0.47±0.05	0.58±0.12	0.60±0.13
Tyr	7.20±0.24	6.43±0.10	7.77±0.09	7.10±0.17	0.18±0.05	0.16±0.03	0.21±0.04

[a]Samples A through G were collected from common restaurants.
[b]Mean±S.D., n=3.
[c]Zero represents no detectable amino acids.

amino acid components in shark fins from different species also need further study.

We found that the level of Tyr in natural shark fins was especially higher than that of artificial samples. This confirmed other report (Gross, 1963). The product of common artificial shark fins was assumed to be made of these materials because the profiles of amino acids were similar to general types of collagen and elastin (Negro et al., 1987). There were no proteineous components and amino acids in vegetarian shark fins, so those products may be prepared from polysaccharides.

CONCLUSIONS

THE HIGH PEAK RATIO OF PEAK III PROTEIN to peak I protein and high tyrosine level in shark fins determined by capillary electrophoresis were two sensitive indicators for identifying the authenticity of shark fins. Both measurements can be good indicators to differentiate natural and artificial shark fins in food products.

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Ms received 11/25/97; revised 3/11/98; accepted 4/1/98.

This study was supported by the Council of Agriculture, R.O.C. We thank Dr. W.H. Chang, National Taiwan University and Dr. B.S. Pan, National Taiwan Ocean University, for their kind suggestions and help.