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Species Identification of a Causative Moray Eel Meat by SDS-PAGE

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ABSTRACT

Attempts were made to identify the species of food poisoning moray eel. Besides the causative processed moray eel meat, eight other raw commercial moray eel meats including *Gymnothorax favagineus*, *G. fimbriatus*, *G. flavimarginatus*, *G. meleagris*, *G. pseudothyrsoideus*, *G. undulates*, *G. albimarginatus* and *G. javanicus* were heated at 100℃ for 30 min and then assayed using the electrophoresis method of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE patterns for eight processed commercial moray eel meats showed species-specific protein bands < 30 kD. The species of causative processed moray eel implicated in food poisoning case was identified as *G. javanicus* in comparison to the 2% SDS and 8 M urea extracted protein revealed by SDS-PAGE patterns.

Key words: moray eel, species identification, processed fish products, electrophoresis

INTRODUCTION

In April 2004, a food poisoning incident occurred due to ingesting cooked moray eel meat at Fugil fishing port, Taipei, Taiwan. A 47-year-old patient showed clinical symptoms of pricking sensation of lips, tongue and throat, vomiting, abdominal cramps, diarrhea, headache, dizziness, vertigo and paralysis. These symptoms disappeared gradually, and they were similar to those of ciguatera poisoning[(1)]. Although ciguatera poisoning has been reported occasionally in Taiwan, the toxicity and the species of toxic fish samples implicated were rarely elucidated, because the causative residues had not been retained for study and the morphological characterization of the retained was difficult.

Moray eels are commercially important fish species in Taiwan, they are usually cut into fillets before selling. It is hard to identify the species by examining only the fillet morphology. These fishes are commonly found in the tropical and subtropical coral reef area around the world they are carnivorous and important edible species[(2)]. There are more than 20,000 cases of food poisoning per year[(3)]. In 2001, one of the ciguateric toxins, ciguatoxin, has been isolated from moray eel *Gymnothorax javanicus*[(2)]. Ciguatoxin is produced by *Gambierdiscus toxicus*, which is usually attached on coral[(4-6)]. The toxin and related compounds are first accumulated by herbivorous fish and then transferred into carnivorous fish via food chain. When people consume these toxic herbivorous or carnivorous fish, they may be intoxicated[(7)].

Since 2001, it has been shown that analysis of mitochondrial DNA (mtDNA) was successful in differentiating species of fish[(8)]. The cytochrome *b* (*cyt b*) gene has been used in species identification[(9)] and in taxonomic phylogenetic studies[(10)]. The *cyt b* gene is considered one of the most useful genes for phylogenetic work and is probably the best-known mitochondrial gene with respect to structure and function of protein products[(11,12)]. Meanwhile, several electrophoretic methods have been employed to differentiate species of seafood or seafood products[(13)]. Other feasible methods have been subsequently applied in identifying fish species, including sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[(14-16)], urea-isoelectric focusing (urea-IEF)[(17)], two-dimensional (2D) gel electrophoresis[(18,19)], high performance liquid chromatography (HPLC)[(20)], immunoassay[(21)] and

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capillary electrophoresis (CE)[22].

The aims of this study were to establish basic data of SDS-PAGE patterns and to quantify the amount of SDS-extracted and urea-extracted proteins in eight commercial moray eel species in Taiwan. The species of causative processed moray eel fillet might then be identified compared to these commercial moray eel species.

MATERIALS AND METHODS

I. *Fish Samples*

Samples of eight moray eel species, *Gymnothorax favagineus* (GFA), *G. fimbriatus* (GFI), *G. flavimarginatus* (GFL), *G. meleagris* (GME), *G. pseudothyrsoideus* (GPS), *G. undulates* (GUN), *G. albimarginatus* (GAL) and G. *javanicus* (GJA) were purchased from seafood markets in Keelung, Taiwan. Each species was represented by at least three specimens. The body weight and body length of tested moray eel samples are as follows: GFA, 541-1,313 g, 72-90 cm; GFI, 1,169-1,359 g, 87-90 cm; GFL, 938-2,617 g, 79-105 cm; GME, 1,054-1,363 g, 72-81 cm; GPS, 541-823 g, 67-74 cm; GUN, 1,022-1,303 g, 79-89 cm; GAL, 173-980 g, 49-103 cm; GJA, 554-3,350 g, 71-107 cm. Among them, the meat of each moray eel was collected from whole specimen and homogenized. A half of mixed meats were repeatedly sampled and rehomogenized. Finally 10 g of mixed meats was directly taken into centrifuge tubes and cooked at 100°C for 15 and 30 min, and 121°C for 15 min. After cooling, these heated samples and the causative samples (CS) of cooked moray eel fillet (250 g) provided by the victim were stored at -20°C until use.

II. *Extraction Procedures*

Two reagents (2% SDS and 8 M urea) were used. All extracts were supplemented with 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.01% (w/v) NaN_3 to inhibit proteases and microbial growth. The extracts of fish proteins were prepared according to the procedures described by Eitenne *et al.*[23] All operations were performed at room temperature.

For the SDS extracts, 1.0 g of heated samples were homogenized with 5 mL of the extraction solution using a Polytron-Aggregate (setting 4.0, Kinenatica Littau/Luzern, Switzerland) at low speed for 1 min. After homogenizing, the mixture was boiled for 2 min in a water bath at 100°C, and then homogenized again. The homogenates were kept at room temperature for 30 min and then centrifuged at 10,000 ×g for 15 min at 10°C using Himac CF 15D2 with rotor RT15A6 (Hitachi, Japan). The supernatants were collected and stored at room temperature (maximum one week) before analyzed by electrophoresis.

For the urea extracts, the same procedure for homogenizing was followed. After homogenizing, the homogenates were kept at room temperature for 30 min, and then centrifuged as described before. The supernatants were collected, and then stored as SDS extracts.

III. *Determination of Protein Concentration*

The protein concentration was determined by bicinchoninic acid method[24]. The protein extracts were adjusted with sample buffer to 2 mg/mL for electrophoresis.

IV. *SDS-PAGE*

SDS-PAGE was performed according to the modified procedure of Laemmli[25] and O'Farrell[26], using a Mini-Protean unit (Bio-Rad, Richmond, CA, USA). Slab gels consisted of a separating gel (15.0%), which was polymerized for 1 hr, and a stacking gel (4.0%), which was poured for 30 min before sample application. The 10 μL of samples (20 μg of extracted protein) were applied in the wells of the gel. Electrophoresis was carried out at constant voltage of 150 volts when the tracking dye reached the separating gel. Electrophoresis was completed when the dye front reached the bottom of the gel.

V. *Gel Stain and Destain*

Gels were stained either with Coomassie blue reagent, 0.1% (w/v) Coomassie blue R-250 in 40% methanol and 10% acetic acid. After staining, gels were destained in 10% methanol and 10% acetic acid. Molecular weights were determined by comparing relative mobilities of protein bands to standard proteins[27]. Protein standards were obtained from Bio-Rad (Broad range kit: myosin 211 kD, β-galactosidase 121 kD, bovine serum albumin 100 kD, ovalbumin 54.4 kD, carbonic anhydrase 38.7 kD, soybean trypsin inhibitor 29.8 kD, lysozyme 20.0 kD, aprotinin 7.3 kD).

VI. *Image Analysis*

The gels were scanned by Gel Dox® (Bio-Rad) and the acquired images were analyzed with software Quantity One® (Bio-Rad). Images of protein profiles were stored in computer and molecular weights were estimated by comparing Rf values on the gel with those of the protein standards. The relative amount of each protein band in SDS-PAGE patterns was the optical density of each protein band to total optical densities of all protein bands in the same lane.

RESULTS AND DISCUSSION

The amounts of SDS-extracted proteins and urea-extracted proteins from moray eel meat were shown

in Table 1. There were significant differences between the raw fish and the ones heated at 100°C for 15 min, 100°C for 30 min and 121°C for 15 min in SDS-extracted proteins, showing the decrease of 7.4%, 21.3% and 35.0%, respectively. The results of urea-extracted proteins showed greater decrease in protein amount with 19.5%, 36.9% and 47.3%, respectively. Thus the increase in heating temperature and time both reduced the extractable proteins from raw moray eel meat. It also showed that the protein concentration of SDS-extracted was higher than that of urea-extracted. It indicated that 2% SDS was more suitable than 8 M urea to extract denatured protein from processed eels. Figure 1 shows the SDS-PAGE patterns of SDS-extracted proteins (Figure 1A) and urea-extracted proteins (Figure 1B) from *G. javanicus* stained with Coomassie blue after different processed conditions. At the heat-processing temperature of 100°C for 15 min and 30 min, the protein bands of SDS-extracted and urea-extracted proteins were clear and remained differentiable. However, the processed moray eel meats heated at 121°C for 15 min were degraded seriously and could not be used in species identification (Figure 1).

Judging from the high molecular weight region (> 30.0 kD) of two extracted proteins stained with Coomassie blue, there were no species-specific protein bands among

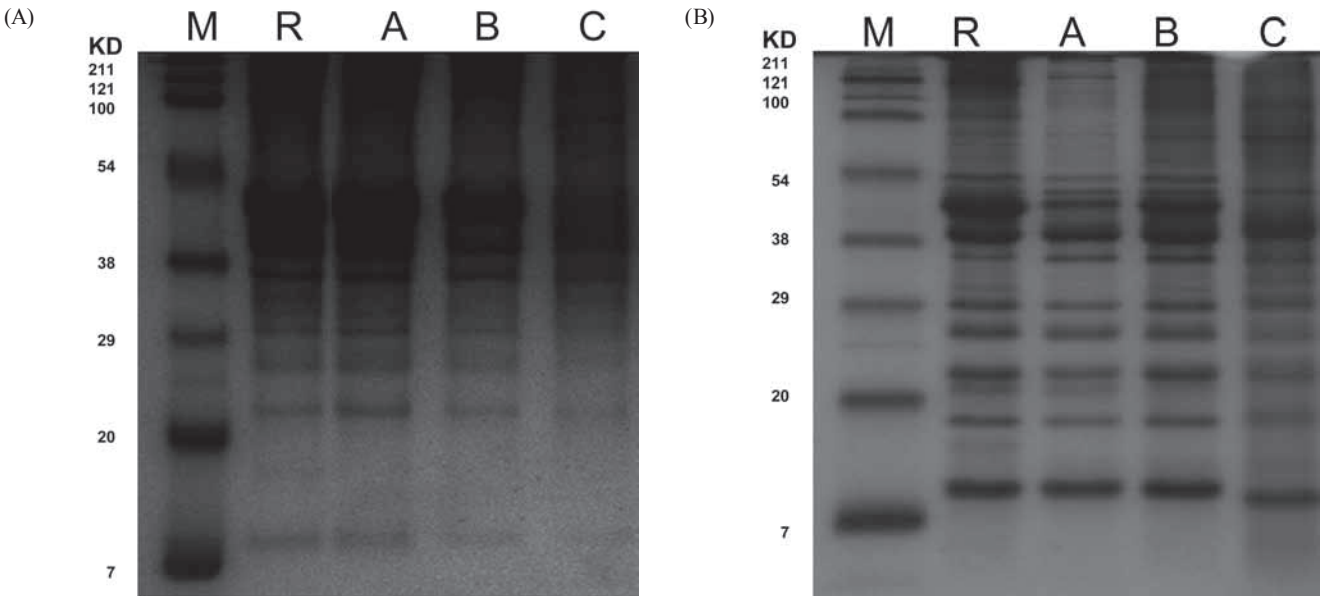

Figure 1. SDS-PAGE patterns of moray eel protein extracted with SDS (A) and urea (B) from *G. javanicus* meat with different heating processes. A: 100°C, 15 min; B: 100°C, 30 min; C: 121°C, 15 min. M: protein standards; R: raw-meat of *G. javanicus.*

Table 1. The protein amounts (n = 3) of SDS and urea extracts from raw and processed moray eel meat

	SDS extract (mg)				Urea extract (mg)			
	Raw	Processed meat			Raw	Processed meat		
Sample		100°C, 15 min	100°C, 30 min	121°C, 15 min		100°C, 15 min	100°C, 30 min	121°C, 15 min
GFA	117.5 ± 0.1	107.7 ± 0.1	88.0 ± 0.1	68.5 ± 0.1	111.5 ± 0.1	77.0 ± 0.1	63.5 ± 0.1	51.5 ± 0.1
GFI	119.0 ± 0.1	106.0 ± 0.1	84.5 ± 0.1	71.5 ± 0.1	104.0 ± 0.1	87.5 ± 0.1	61.5 ± 0.1	56.0 ± 0.1
GFL	109.5 ± 0.1	103.0 ± 0.1	90.5 ± 0.1	80.5 ± 0.1	104.0 ± 0.1	80.5 ± 0.1	68.0 ± 0.1	47.5 ± 0.1
GME	114.5 ± 0.1	106.5 ± 0.1	87.5 ± 0.1	66.5 ± 0.1	97.0 ± 0.1	66.5 ± 0.1	52.0 ± 0.1	52.5 ± 0.1
GPS	114.0 ± 0.1	105.0 ± 0.1	89.5 ± 0.1	73.0 ± 0.1	91.0 ± 0.1	85.5 ± 0.1	49.0 ± 0.1	50.0 ± 0.1
GUN	114.0 ± 0.1	103.0 ± 0.1	98.5 ± 0.1	76.0 ± 0.1	93.0 ± 0.1	87.0 ± 0.1	48.5 ± 0.1	55.0 ± 0.1
GAL	100.0 ± 0.1	95.5 ± 0.1	84.0 ± 0.1	65.5 ± 0.1	94.0 ± 0.1	74.5 ± 0.1	61.0 ± 0.1	49.0 ± 0.1
GJA	113.0 ± 0.1	107.7 ± 0.1	86.0 ± 0.1	83.5 ± 0.1	95.5 ± 0.1	74.5 ± 0.1	94.5 ± 0.1	53.0 ± 0.1
CS*			63.0 ± 0.1				49.5 ± 0.1	

*CS (causative sample) was the moray eel fillet provided by the victim.

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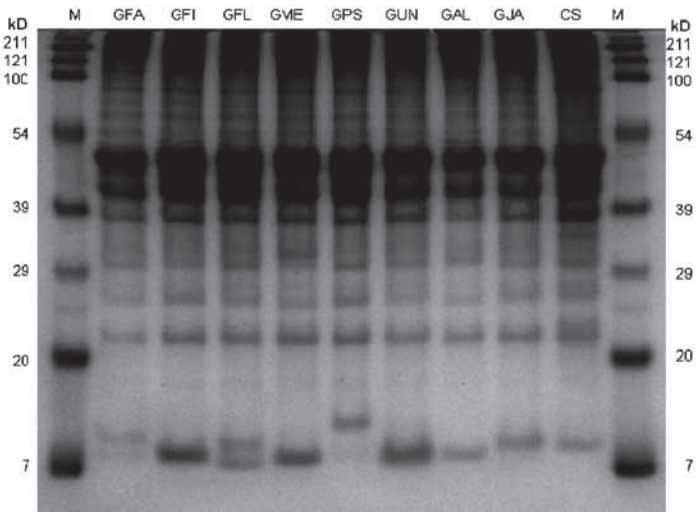


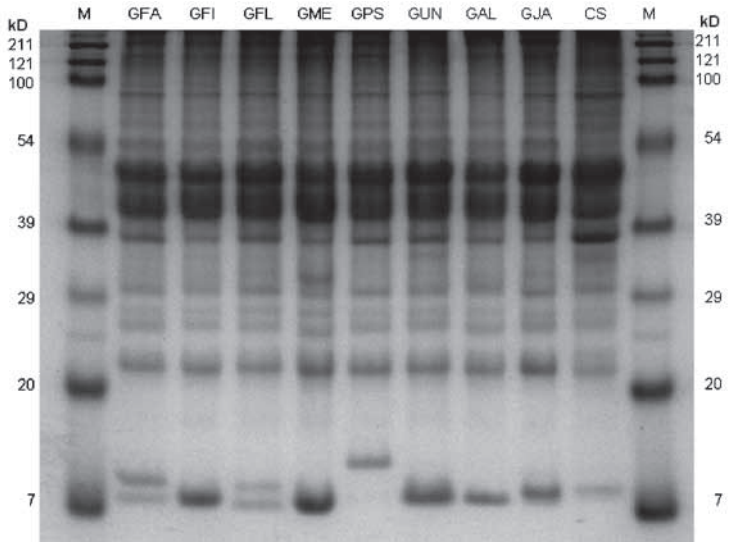
MW kD	GFA	GFI	GFL	GME	GPS	GUN	GAL	GJA	CS
29.0							2.58 ± 0.07		
28.7			0.57 ± 0.04	0.84 ± 0.03		3.95 ± 0.07			
28.4					2.05 ± 0.04				
27.7			0.58 ± 0.03						
26.9				2.23 ± 0.03			1.01 ± 0.05		
26.7	2.36 ± 0.07								
26.2		0.67 ± 0.06							
25.9								4.80 ± 0.03	4.75 ± 0.02
25.7						4.60 ± 0.03			
25.4							2.55 ± 0.03		
25.1					4.60 ± 0.03				
23.0			0.73 ± 0.05	2.10 ± 0.07					
22.4							3.33 ± 0.08	4.45 ± 0.06	4.62 ± 0.05
22.1	4.10 ± 0.07	2.65 ± 0.07			4.01 ± 0.02	6.00 ± 0.02			
20.5			0.50 ± 0.03						
16.4				0.81 ± 0.02					
14.5						3.09 ± 0.03			
14.2		0.47 ± 0.04					0.81 ± 0.03	1.25 ± 0.04	0.82 ± 0.05
13.5					0.73 ± 0.02				
9.3					3.39 ± 0.05				
8.9	4.67 ± 0.03		0.83 ± 0.03						
8.5				5.20 ± 0.07					
8.3		7.01 ± 0.02							
8.0	1.50 ± 0.03		0.91 ± 0.04						
7.7								8.71 ± 0.04	4.95 ± 0.03
7.3						6.14 ± 0.08	3.72 ± 0.04		
6.1					2.15 ± 0.04				

Figure 2. SDS-PAGE patterns of SDS extracted protein from eight moray eel species (100°C, 30 min) stained with Coomassie blue (n = 3). M: protein standards, GFA: *Gymnothorax favagineus*, GFI: *G. fimbriatus*, GFL: *G. flavimarginatus*, GME: *G. meleagris,* GPS: *G. pseudothyrsoideus*, GUN: *G. undulatus*, GAL: *G. albimarginatus,* GJA: *G. javanicus*, and CS: causative sample. Data shown as Mean ± STD.

these moray eel species (Figure 2). In addition, the amounts of high molecular weight region (> 30.0 kD) were too large to be differentiated between moray eel species. The bands at the high molecular weight region were apparently not useful in identifying moray eel species.

All tested moray eel species could be differentiated using the SDS-PAGE patterns of SDS-extracted proteins in the low molecular weight region (< 30.0 kD) (Figure 2). The causative moray eel meat established the same SDS-PAGE patterns of the SDS-extracted proteins of *G. javanicus*. Therefore, SDS-PAGE patterns of the SDS-extracted protein stained with Coomassie blue would be useful for moray eel species identification.

The low molecular weight region (< 30.0 kD) of the SDS-PAGE patterns of urea-extracted proteins showed species-specific protein patterns (Figure 3). The percentage compositions of urea-extracted proteins of the low molecular weight (< 30.0 kD) are shown in Figure 3.



MW kD	GFA	GFI	GFL	GME	GPS	GUN	GAL	GJA	CS
27.4		0.65 ± 0.03							
27.1					2.74 ± 0.04	0.41 ± 0.02	0.50 ± 0.04		
26.1			1.43 ± 0.06					0.79 ± 0.11	1.42 ± 0.11
25.5	1.92 ± 0.02								
23.1		5.93 ± 0.05					4.50 ± 0.03		
22.9					5.73 ± 0.04	3.38 ± 0.08			
22.2			4.27 ± 0.07	5.53 ± 0.07				4.77 ± 0.04	5.81 ± 0.15
21.9	9.97 ± 0.06								
16.7		3.20 ± 0.08					0.57 ± 0.06		
16.3					2.51 ± 0.07				
15.7						0.53 ± 0.05			
15.4			1.84 ± 0.01	3.67 ± 0.10					
13.3	3.46 ± 0.02								
10.4					5.65 ± 0.04				
8.7	5.34 ± 0.01		5.33 ± 0.05						
8.3		7.97 ± 0.03			2.21 ± 0.03		4.66 ± 0.07	7.75 ± 0.12	5.77 ± 0.08
8.1						8.16 ± 0.09			
7.8	3.50 ± 0.05		4.03 ± 0.09	7.19 ± 0.12					

Figure 3. SDS-PAGE patterns of urea extracted proteins from eight moray eel species (100°C, 30 min) stained with Coomassie blue (n = 3). The symbols refer to moray eel species and the footnote detailed in Figure 2.

The moray eel species could be identified by the pattern of the low molecular weight region in the urea-extracted proteins. The causative moray eel meat showed similar SDS-PAGE pattern of the urea-extracted proteins to that of G. *javanicus*. Hence, the species of the causative moray eel was identified as *G. javanicus*.

The low molecular weight region of the SDS-PAGE patterns of solvent extracted proteins which shows species-specific protein patterns were similar to other reports[4,15,17]. In this study, the individual mobility variation between fish of the same species was not measured. Protein band density appeared somewhat variable and this may have a slight effect on our ability to discriminate moray eel species.

Moray eels have relatively higher price in Taiwan. Therefore, some fraudulent retailers use cheaper fish to substitute for moray eels. Because moray eel products are usually heavily processed, the proteins in the processed moray eel products have usually degraded and almost all proteins can be denatured and damaged, such as by heating at 121°C for 15 min. Then, the protein analysis methods for fish species identification are inappropriate. In this situation, polymerase chain reaction (PCR) amplification and restriction enzyme analysis of the cytochrome *b* gene may be applied for identification of fish species[11,28].

CONCLUSIONS

Species identification of 8 tested moray eel species can be achieved by examining the lower molecular weight protein bands following SDS-PAGE of 2 % SDS or 8 M urea extractants along with the densities of the characteristic protein bands using staining. The below 30.0 kD protein bands in SDS-PAGE pattern were more useful than those of higher molecular weight proteins (> 30.0 kD). Moreover, the causative heated moray eel fillet implicated in the food poisoning was identified as *G. javanicus*.

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