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Survey on Toxicity and Label of Dried Dressed Fish Fillet in 1998

SHIN-JUNG LIN[1], CHAU-JAN LIAO[2], SHU-KONG CHEN[3] AND DENG-FWU HWANG[2]*

[1.] *Department of Food Health, Deh Yu College of Nursing and Management, Keelung, Taiwan*

[2.] *Department of Food Science, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung, Taiwan*

[3.] *Institute of Food Hygiene, Department of Health, Executive Yuan, Taipei, Taiwan*

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ABSTRACT

Recently, dried dressed fish fillets have been reported as a cause of food poisoning. Hence, 364 samples of 58 packages of these products were collected from 9 counties; Keelung, Taipei, Hsinchu, Taichung, Tainan, Kaohsiung, Ilan, Taitung and Penghu in 1998 and their toxicities and labels were investigated. It was found that over 65% of products had no label listing the product name, manufacturing date, manufacturer, or food additives. Based on the detecting limit of 5 mouse units per gram (MU/g), the frequency of toxicity occurrence in the samples collected from Taiwan markets was 1.1%. The highest toxicity was 15 MU/g. The toxin obtained from toxic samples was partially purified by ultrafiltration and Bio-Gel P-2 column chromatography. Results of analyses by thin-layer chromatography, electrophoresis and high performance liquid chromatography showed that the toxin was composed of tetrodotoxin and anhydrotetrodotoxin.

Key words: dried dressed fish fillets, toxicity, tetrodotoxin, anhydrotetrodotoxin

INTRODUCTION

A number of human fatalities resulting from the ingestion of fish have been reported in Taiwan[1,2]. Among these poisoning incidents, tetrodotoxin (TTX) and paralytic shellfish poison (PSP) were the major fatal agents. TTX and PSP are well known as the most toxic neurotoxins. The clinical symptoms of patients in these incidents contain numbness of the extremities, severe vomiting, stupor, aphasia and respiratory difficulty. Finally, the victims fall unconscious and respiration fails. If the victim takes an excessive dose, death takes place within a maximum of approximately eight hours, most frequently within four to six hours.

In the past decade, fish implicated in episodes of TTX and PSP poisoning have usually been puffer[3-6], goby[7], purple clam[9-10] and gastropod Nassariidae[11]. Among these fish, puffer and its products, and dried dressed fish fillets (DDFF), are most often reported[12]. Hence, how to establish food safety information about puffer and its products has become crucial. Although we have pointed out[16] that a few of DDFF collected in 1988 contained a small amount of TTX, the fatal incidents due to ingesting DDFF have still occurred several times since 1988. Meanwhile, due to the increasing consumption demand of DDFF in Taiwan, the amount of these products imported from Mainland China and Vietnam has not been reported yet and seems to be increasing. To guarantee the safety of these imported products, the present study was undertaken. The samples of DDFF were collected from various counties in Taiwan and the product labeling and toxicity were investigated. Furthermore, the toxin composition of toxic samples was also identified in this study.

* Author for correspondence. Tel:02-24622192 ext. 5103; Fax:02-24626602; E-mail:dfhwang@mail.ntou.edu.tw

MATERIALS AND METHODS

I. *Materials*

Three hundred and sixty four samples of 58 packages were collected from the nine counties of Keelung, Taipei, Hsinchu, Taichung, Tainan, Kaohsiung, Ilan, Taitung and Penghu from August to December in 1998. The samples were transported to the laboratory, the contents list on the label were recorded, and the samples were kept frozen at -20˚C until use.

II. *Contents Recording*

Each package was inspected and the product name, manufacturing date, contents of food additives, manufacturer/ retailer, and DDFF with/without caudal fin were recorded.

III. *Assay for Toxicity*

The frozen DDFF were partially thawed and examined for toxicity by using the mouse method for TTX[12]. Toxicity is expressed in mouse units. One mouse unit (MU) is defined as the amount of toxin required killing a 20 g ICR strain male mouse in 30 min after a single intraperitoneal injection.

IV. *Purification and Identification of DDFF Toxin*

After the toxicity assay, the remaining supernatants of each toxic samples were combined, concentrated under

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Table 1. Survey of product labeling and appearance of DDFF in Taiwan markets

Collection place	Sample amounts	Product title[a]		Manufacturing date[a]		Food additives[a]		Manufacturer/retailer[a]		Caudal fin[b]	
		+	–	+	–	+	–	+	–	+	–
Keelung City	6	1	5	1	5	4	2	1	5	1	5
Taipei City	8	1	7	1	7	1	7	0	8	3	5
Hsinchu City	6	1(1)*[c]	5	0	6	1	5	0	6	3	3
Taichung City	7	0	7	0	7	3	4	0	7	0	7
Tainan City	6	4(1)	2	3	3	1	5	2	4	4	2
Kaohsiung City	7	0	7	0	7	0	7	0	7	1	6
Ilan City	6	5(4)	1	2	4	2	4	5	1	0	6
Taitung City	6	3(1)	3	2	4	0	6	0	6	3	3
Penghu County	6	4(1)	2	6	0	2	4	4	2	5	1
Total	58	19(8)	39	15	43	14	44	12	46	20	38

[a] +: Labeled; –: not labeled.

[b] +: With caudal fin; –: Without caudal fin.

[c] The values in parentheses represent the numbers of incorrect labeling.

reduced pressure at 45˚C, and defatted with dichloromethane. The aqueous layer was concentrated and filtered through a Diaflo YM-1 membrane to retain substances of more than 1,000 daltons. The filtrate was applied to a Bio-Gel P-2 column (2 × 94 cm), which was developed with 0.03 M acetic acid. Toxic fractions were combined, freeze-dried, dissolved in a small amount of water, and submitted to the analyses described below. Authentic TTX and anhydrotetrodotoxin (anh-TTX), which were obtained from the liver of the puffer *Takifugu oblongus*[13], were used as the reference standards. Authentic gonyautoxins 1-4 (GTX 1-4) and saxitoxins (STXs) obtained from purple clam *Soletellina diphos*[8] and crab *Zosimus aeneus*[14], respectively, were also used as the reference standards.

(I) *Electrophoresis*

Electrophoresis was performed in 5 × 18 cm cellulose acetate strips (Chemetron) in 0.08 M Tris-HCl buffer (pH 8.7) under a constant current of 0.8 mA/cm for 1 h. TTXs were visualized as yellow or blue fluorescent spots under a UV lamp (365 nm) after spraying the strip with 10% KOH and heating at 110˚C for 10 min. GTXs and STXs were also visualized as green and blue fluorescent spots after spraying the other strip with 1% H_2O_2 and under the same UV wavelength and heating conditions as TTXs were treated.

(II) *Thin-Layer Chromatography*

Thin-layer chromatography (TLC) was performed on two 5 × 20 cm (thickness 2 mm) silica gel 60 F254 precoated plates (Merck) with pyridine-ethyl acetate-acetic acid-water (15:5:3:6) and 1-butanol-acetic acid-water (2:1:1) two separated solvent systems. Toxins were visualized in the same manner as in electrophoresis.

(III) *High-Performance Liquid Chromatography*

High-performance liquid chromatography (HPLC) was performed on a reversed-phase column (Merck Lichrospher 100 RP-18, 4 mm I.D. × 20 cm). The mobile phase for the TTX assay was sodium 1-heptane sulfonate (2 mM) in methanol (1%)-potassium phosphate buffer (0.05 M, pH 7.0). TTXs were detected by mixing the eluate with 3 N NaOH at a ratio of 1:1, followed by heating at 99˚C for 0.4 min, and monitoring the fluorescence at 505 nm with 381 nm excitation[15].

Table 2. Toxicity of dried dressed fish fillet in Taiwan markets

Place of collection	Toxic ratio	Frequency of toxicity (%)	Toxicity (MU/g)[c]
Keelung City	1/40[a]	2.5	<5.0-14.5
	(1/40)[b]	(2.5)	(14.5)
Taipei City	0/62	0	<5.0
Hsinchu City	2/48	4.2	<5.0-9.3
Taichung City	0/50	0	<5.0
Tainan City	0/40	0	<5.0
Kaohsiung City	1/50	2.0	<5.0-15.0
	(1/50)	(2.0)	(15.0)
Ilan City	0/30	0	<5.0
Taitung City	0/40	0	<5.0
Penghu County	0/40	0	<5.0
Total	4/364	1.1	<5.0-15.0
	(2/364)	(0.5)	(14.5-15.0)

[a] Toxic specimens/ total specimens.

[b] The data in parentheses were based on the limit of toxicity detected being more than 10 MU/g.

[c] The limit of toxicity detected was more then 5 MU/g.

RESULTS AND DISCUSSION

The results of survey of DDFF product labeling in the Taiwan market are shown in Table 1. It was found that over 65% of products had no labeling, and that 39, 43, 44, and 46 out of 58 DDFF products contained no labeling of product name, manufacturing date, food additives, or manufacturer/retailer name, respectively. The caudal fins of DDFF in 38 out of 58 products were not preserved, indicating that 8 out of 58 product names were not properly labeled. These results showed that some of the DDFF manufacturers were neither supervised nor obeyed the Food Sanitation Law in Taiwan.

The toxicities of DDFF products surveyed in this study are shown in Table 2. Based on the detecting limit of 5 MU/g,

the frequency of toxicity of DDFF collected from Taiwan markets was 1.1% (4/364). The toxic specimens were found in Hsinchu and Kaohsiung cities with toxicity ranging from 7.2 to 15.0 MU/g. However, the frequency of toxicity of DDFF was 0.5% (2/364), and there was only one toxic specimen found in each of Hsinchu and Kaohsiung cities, when 10 MU/g was used as the detecting limit of toxicity in Japan. No matter which limit was used, the frequency of toxicity of DDFF collected in 1998 was lower than that collected in 1988[16]. However, the highest toxicity of DDFF collected in 1998 was similar to that collected in 1988. Both had low toxicity levels (<20 MU/g).

The minimum lethal dose of TTX for a human in oral administration is assumed to be 10,000 MU[18]. Therefore, in this study, people who ingest toxic DDFF over 1,000 g may risk death. Hwang *et al.*[19] have indicated that there was only one species in Taiwan, green toadfish (*Lagocephalus lunaris*), found to be highly toxic in their muscle (ingesting over 100 g toxic muscle caused death). However, other species might be found to be highly toxic in their ovary and liver (ingesting over 10 g toxic viscera caused death). Hence it appeared that DDFF made from puffer fish muscle might be contaminated by the toxic viscera during processing. Therefore, the handling of raw materials should be highly supervised to avoid contamination. Besides, the morphology of toxic green toadfish is similar to that of non-toxic brown-backed toadfish (*Lagocephalus wheeleri* and *L. gloveri*). It is easy to be confused with and mistaken for toxic puffer and used as raw material.

After toxicity assay, the toxins were extracted, concentrated and purified from the remaining toxic specimens. The crude toxins (8.3 mg) with toxicity 120 MU/mg were obtained. As shown in Figure 1, DDFF toxin in whichever systems exhibited one spot, which coincided with TTX and/or anh-TTX. According to Figure 2, electrophoresis of DDFF toxin showed three spots. Among them, two spots were undistinguished from TTX and anh-TTX, respectively, both in migration distances (7.1 and 4.6 cm) and in fluorescent colors (yellow and blue). The other spot with migration distance 1.9 cm was judged as tetrodonic acid-like substance according to previous paper[13]. Figure 3 shows the HPLC patterns of DDFF toxin. The toxin gave rise to three peaks whose retention times (13.5 and 17.4 min) coincided well with those of TTX and anh-TTX, respectively. The other peak with retention time of 8.0 min was also judged as a tetrodonic acid-like substance according to a previous paper[20].

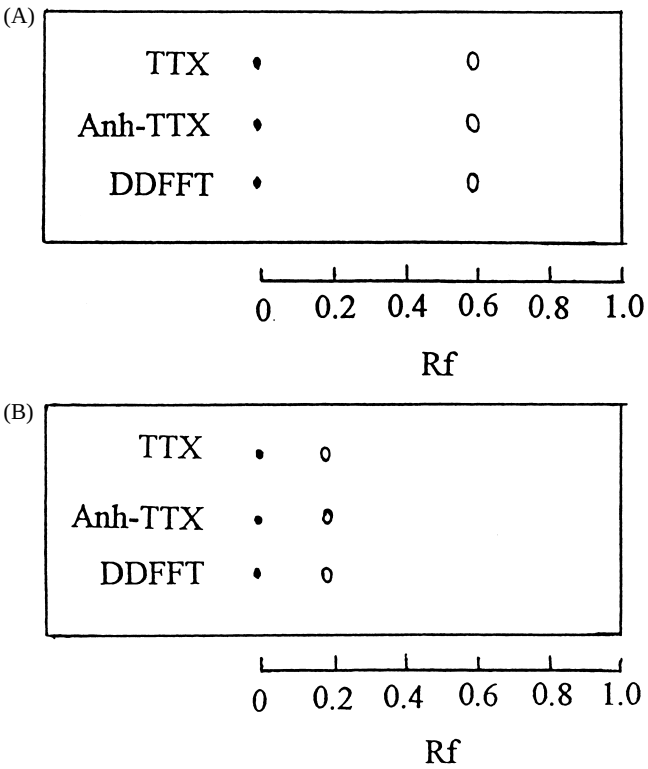

Figure 1. TLC of the toxin (DDFFT) occurred in dried dressed fish fillet, along with authentic TTX and anh-TTX.
Solvent system: A) pyridine-ethyl acetate-acetic acid-water (15:5:3:6); B) 1-butanol-acetic acid-water (2:1:1). The plate was sprayed with 10% KOH or with the Weber reagent.

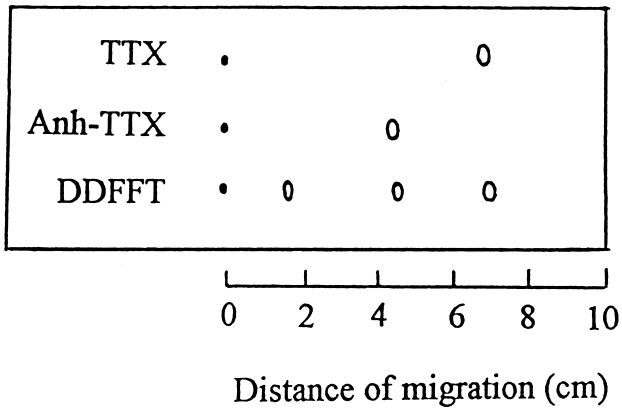

Figure 2. Electrophoresis of the toxin (DDFFT) occurred in dried dressed fish fillet, along with authentic TTX and anh-TTX.

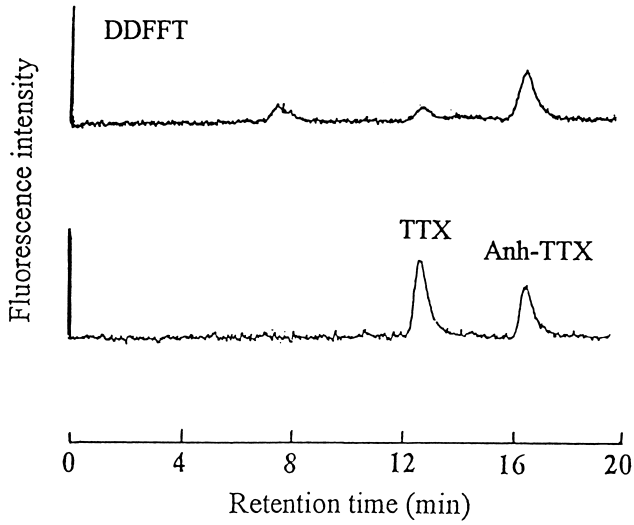

Figure 3. HPLC of the toxin (DDFFT) occurred in dried dressed fish fillet, along with authentic TTX and anh-TTX.

It was concluded from the results that the DDFF samples, detected to be toxic, contained TTX and anh-TTX.

In conclusion, according to the results of survey of DDFF product labeling in Taiwan markets, most of the DDFF manufacturers or retailers were not well supervised and did not obey the Food Sanitation Law in Taiwan. In addition, the DDFF toxin was identified as TTX, which might be from toxic puffer fish. More attention should be paid while raw puffer was chosen and handled in the processing of DDFF.

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1998年市售香魚片之毒性及產品標示調查

林欣榮[1]　廖昭然[2]　陳樹功[3]　黃登福[2*]

1. 德育醫管專校　食品衛生科
2. 國立台灣海洋大學　食品科學系　基隆市北寧路2號
3. 行政院衛生署食品衛生處

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摘　要

由於香魚片最近在國內偶爾會造成食物中毒事件，因此為知台灣市販香魚片之標示和毒性，乃自1998年8月-12月,自基隆、台北、新竹、台中、台南、高雄、宜蘭、台東、澎湖等九地區，共採得58包香魚片，經調查產品名稱、製造日期、食品添加物製造者等標示，發現產品有65%以上均未符合食品衛生法之標示。產品毒性經檢查香魚片364個樣品，若以5 MU/g檢出界限，則發現4個樣品有毒，有毒檢體發現率為1.1%，毒性最高值為15 MU/g，有毒香魚片中之毒素，經抽出超過濾和層析管精製，粗毒經薄層層析電泳分析和高效能液相層析儀分析，得知毒素含有河魨毒和脫水河魨毒。

關鍵詞：香魚片，毒性，河魨毒，脫水河魨毒