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Studies for Reestabilishment of Approval Toxin Amount in Paralytic Shellfish Poison-Infested Shellfish

2. Change of Toxin Composition and Specific Toxicity in Paralytic Shellfish Toxins of Blue mussel, *Mytilus edulis* and, Oyster, *Crassostrea gigas* from Woepori, Kŏje, Korea During Canning Process

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Changes of paralytic shellfish toxin components and specific toxicity in blue mussel, *Mytilus edulis* and oyster, *Crassostrea gigas* during canning process were investigated by high performance liquid chromatography (HPLC). The mole% of the frozen shucked blue mussel were in order of 27.5 mole% of gonyautoxin 1, 23.0 mole% of gonyautoxin 8 (C1) and 23.0 mole% of *epi*-gonyautoxin 8 (C2), while those of the frozen shucked oyster were in order of 29 mole% of C1, 22 mole% of C2, 16.7 mole% of gonyautoxin 2. Both samples had minor amounts of saxitoxin and neosaxitoxin. On the other hand, in case of specific toxicity, the major toxins were consisted of gonyautoxin 1~4 in both sample. The toxicity of gonyautoxin 1~4 were 88 and 84% in blue mussel and oyster, respectively. According to the experimental results, C1, C2 and gonyautoxin 4 were very sensitive to heat treatment, while gonyautoxin 2 and saxitoxin were pretty heat resistant than any other toxin components.

Key words : paralytic shellfish toxin, toxin components, specific toxicity HPLC, gonyautoxin, saxitoxin, neosaxitoxin

Introduction

Paralytic shellfish poison (PSP) has been a serious problem for a long time in many parts of the world. The sporadic and unpredictable outbreasks usually cause serious health hazards and great losses to the seafood industry. The toxins accumulate in shellfish as a result of ingestion of toxic dinoflagellate. Saxitoxin (STX), first isolated from Alaska butter clams, *Saxidomus giganteus* and later from California mussels, *Mytilus californianus* (Schantz et al., 1957), was thought to be the only toxic principle produced by the causative organism *Gonyaulax catenella*. Recent studies, however, have shown that the toxicity is caused by a group of closely related compounds and that STX did not ever constitute the major component in many cases (Shimizu et al., 1975). More than 20 analogues of STX have been reported to occur naturally, including the deoxydecarbamoyl group recently found in the dinoflagellate *Gymnodinium catenatum* (Fig. 1, Oshima et al., 1993).

In Korea, the toxins such as gonyautoxin (GTX) 1, GTX2, GTX3 and GTX4 caused food poisoning accident in May, 1986 at Pusan (Chang et al., 1987) are the major components in blue mussel, *Mytilus edulis* and the toxins such as gonyautoxin (GTX) 1, GTX2,

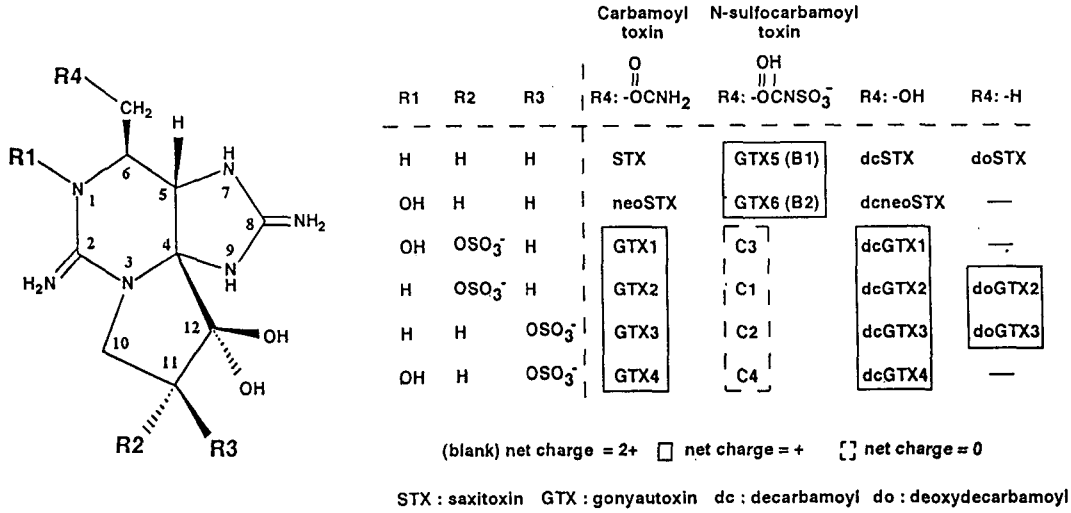

Fig. 1. Structure of paralytic shellfish toxins.

GTX3 and GTX4 are the major components and GTX8 (C1), *epi*-GTX8 (C2), STX and neoSTX are the minor components in blue mussel, *Mytilus edulis*, at Jinhae Bay (Lee et al., 1992). However, comparison of toxin components in blue mussel and oyster, and change of toxin components during canning processing have not been reported so far. In this study, we report the toxin components in blue mussel and oyster causing the death accident in May, 1996 at Woepori, Kŏje, Korea (Sin-Kyengnam Ilbo, 1996), and change of toxin components during canning processing were investigated.

Materials and Methods

Materials

Blue mussel, *Mytilus edulis* (toxicity, 8,924 μg/100 g) and oyster, *Crassostrea gigas* (toxicity, 856 μg/100 g) caused food poisoning accident were collected at Woepori, Kŏje, on 18th, May, 1996.

Canning

Shell-stock blue mussel and oyster washed with fresh water were steamed at 105℃ for 10 min., shucked and trimmed, respectively. The steamed meat were smoked at 110℃ for 15 min., followed at 125℃ for 15 min., next. The 60 g of smoked meat was packed in No. 3B of square can with 50 ml of cotton seed oil and retorted at 115℃ for 70 min. (Smoked can).

Preparation of toxin extracts for HPLC analysis

Toxin extracts from blue mussel and oyster were prepared according to the standard mouse bioassay (heat homogenate with equal volume of 0.1 N HCl for 5 min. and centrifuge or filter). The toxin extracts were passed through a Sep-Pak C-18 cartridge column (Waters) which had been washed and equilibrated previously with 10 ml each of methanol and distilled water. The first 1.5 ml of eluate was discarded, the next 0.5 ml was collected in reservoir of an ultrafiltration kit (Waters Ultrafree C3GC, 10,000 dalton cut-off) and centrifuged at 5,000×g for 5 min.

HPLC analysis of toxin

Toxin analysis was carried out with post column derivatization HPLC system of Ohsima (1995b). Three mobile phases were used for different toxin groups. Details of analytical HPLC conditions were shown in Table 1.

Table 1. Operating conditions for HPLC analysis of paralytic shellfish poisoning toxins

Parameter	Condition of description
HPLC pump	Hitachi L-6000 with a syringe-loading sample injector (Rheodyne 7125)
Column	Reversed-phase, C8-bonded silica gel, Develosil C8~5 , 4.6×150 mm (Nomura Chemical Co.)
Mobile phase	
Flow rate	0.8 ml/min.
(a) For C1~C4toxins	Tetrabutylammonium phosphate (1 mM) adjusted to pH 5.8 with acetic acid
(b) For GTX1 to GTX6, dcGTX2 and dcGTX3	Sodium 1-heptanesulfonate (2 mM) in 10 mM ammonium phosphate, pH 7.1
(c) For STX1, neoSTX and dcSTX	Sodium 1-heptanesulfonate (2 mM) in 30 mM ammonium phosphate, pH 7.1-acetonitrile (100×5)
Oxidizing reagent	
Flow rate	0.4 ml/min.
Composition	Periodic acid (7 mM) in 50 mM potassium phosphate buffer, pH 9.0
Reaction	10 m Teflon tubing (0.5 mm id) at 65℃ in a water bath and at 85℃ in a dry oven
Acidifying reagent	
Flow rate	0.4 ml/min.
Composition	0.5 M acetic acid
Detector	Fluoromonitor (Hitachi F-1050) with a 150-W xenon lamp
Excitation	330 nm
Emission	390 nm

PSP standard toxin

The standard STX, neoSTX, dcSTX, GTX1~5, dcGTX2~3 and C1~4 were obtained from Ph. D. Yasukatu. Oshima (Tohoku University, Sendai, Japan).

Results and Discussion

Changes of toxin components during canning process

Analysis of toxin components from blue mussel during canning process were shown in Table 2. GTX 1 (27.5 mole%) was predominant toxin components in the frozen shucked blue mussel, followed GTX 8 (C1, 23.0 mole%) and *epi*-GTX 8, (C2, 23.0 mole%). While the frozen shucked oyster contained large proportion of C1 (29 mole%) and C2 (22 mole%), followed GTX 2 (16.7 mole%) as shown in Table 3. Both samples showed the presence of minor amounts of STX and neoSTX. After steaming, it was found that the mole% of C2 : C1, GTX4 : GTX1 and GTX3 : GTX2 were changed close to 1 : 3 ratio. These results indicated that epimerization between β-epimer (GTX3, GTX4, C 2, C4) and α-epimer (GTX2, GTX1, C1, C3) occured during steaming. Toxins having a hydroxysulfate moiety at position 11 undergo epimerization through keto-enol equilibration (Shimizu, 1984). The equilibration was accelerated at higher pH and higher temper-

Table 2. Change of toxin composition of blue mussel during smoking canning process

Process	Toxin composition (Mole %)												
	C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	dcGTX2	dcGTX3	neoSTX	dcSTX	STX
1. Frozen shucked blue mussel	23.0	23.0	0.0	0.2	27.5	3.9	3.6	14.5	0.8	1.1	1.8	0.0	0.8
2. Thawing													
Thawed blue mussel	23.7	19.4	0.0	0.2	30.8	3.8	2.9	14.6	0.8	1.0	2.0	0.0	0.8
Drip	23.2	17.8	0.0	0.2	29.3	5.7	4.6	13.4	1.7	1.9	1.4	0.2	0.5
3. Steaming													
Steamed blue mussel	33.7	8.6	0.0	0.0	16.5	20.3	6.3	4.0	4.4	1.9	1.2	0.3	2.7
Broth	26.8	6.3	0.0	0.0	25.9	13.0	4.3	7.2	4.4	6.6	3.3	0.1	1.5
4. Smoked blue mussel	30.0	7.0	0.0	0.0	16.5	22.1	6.9	4.0	4.1	1.8	1.4	0.5	5.6
5. Canning													
Canned blue mussel	0.0	0.0	0.0	0.0	4.7	3.4	1.6	0.0	1.1	1.8	0.0	44.5	43.0
Soup	0.0	0.0	0.0	0.0	7.2	4.1	1.4	0.0	1.4	2.7	0.0	42.0	41.3

Table 3. Change of toxin composition oyster during smoking canning process

Process	Toxin composition (Mole %)												
	C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	dcGTX2	dcGTX3	neoSTX	dcSTX	STX
1. Frozen shucked oyster	29.0	22.0	0.0	0.0	10.3	16.7	12.0	4.9	1.9	1.8	0.4	0.0	1.0
2. Thawing													
Thawed oyster	30.9	23.1	0.0	0.0	9.2	16.0	12.1	4.9	1.0	1.0	0.6	0.0	1.3
Drip	13.4	8.7	0.0	0.0	16.1	20.8	14.7	6.8	5.6	4.9	4.8	0.0	4.2
3. Steaming													
Steamed oyster	56.5	16.3	0.0	0.0	3.6	10.9	3.8	1.2	2.5	1.1	1.1	0.0	3.0
Broth	42.7	14.6	0.0	0.0	8.1	12.7	5.5	3.1	6.9	2.5	1.8	0.0	2.2
4. Smoked oyster	52.3	14.6	0.0	0.0	3.0	9.5	4.2	0.8	3.0	3.6	0.9	1.5	6.5
5. Canning													
Canned oyster	0.0	0.0	0.0	0.0	0.0	3.9	0.8	0.0	3.4	1.2	0.0	47.5	42.9
Soup	0.0	0.0	0.0	0.0	0.0	5.8	0.0	0.0	0.6	5.1	0.0	51.0	37.5

Table 4. Change of toxicity and toxin composition of blue mussel during smoking canning process

Process	Specific toxicity (μg/100 g)														Total toxicity (μg/100 g)
	C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	GTX5	dcGTX2	dcGTX3	neoSTX	dcSTX	STX	
1. Frozen shucked blue mussel	26	400	0	0	4,950	466	490	1,908	0	90	146	308	0	140	8,924
2. Thawing															
Thawed blue mussel	20	262	0	0	4,304	348	308	1,490	0	74	102	264	0	106	7,278
Drip	16	190	0	0	3,210	410	378	1,072	0	126	154	146	10	54	5,766
3. Steaming															
Steamed blue mussel	26	104	0	0	2,066	1,668	600	370	0	360	180	140	20	334	5,868
Broth	2	8	0	0	366	120	46	74	0	40	70	42	2	30	800
4. Smoked blue mussel	22	84	0	0	2,016	1,766	640	354	0	328	168	162	28	692	6,260
5. Canning															
Canned blue mussel	0	0	0	0	28	12	8	0	0	4	8	0	132	250	442
Soup	0	0	0	0	4	2	0	0	0	0	0	0	10	18	34

Table 5. Change of toxicity and toxin composition of oyster during smoking canning process

Process	Specific toxicity (μg/100 g)														Total toxicity (μg/100 g)
	C1	C2	C3	C4	GTX1	GTX2	GTX3	GTX4	GTX5	dcGTX2	dcGTX3	neoSTX	dcSTX	STX	
1. Frozen shucked oyster	4	46	0	0	220	232	194	76	0	26	30	8	0	20	856
2. Thawing															
Thawed oyster	4	52	0	0	210	238	208	80	0	16	18	12	0	30	868
Drip	0	2	0	0	40	34	28	12	0	10	10	12	10	10	168
3. Steaming															
Steamed oyster	6	28	0	0	66	130	52	16	0	30	16	18	20	56	438
Broth	2	10	0	0	56	56	28	16	0	30	12	12	0	16	238
4. Smoked oyster	4	12	0	0	64	118	36	14	0	28	12	14	28	64	394
5. Canning															
Canned oyster	0	0	0	0	0	2	0	0	0	2	2	0	28	48	82
Soup	0	0	0	0	0	4	0	0	0	0	4	0	24	36	68

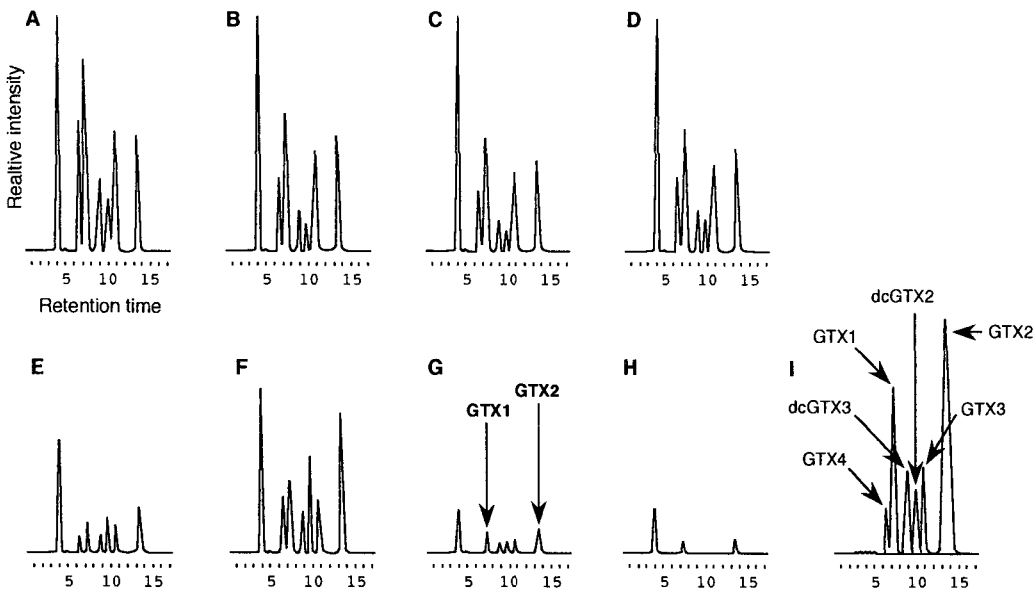

Fig. 2. HPLC chromatograms of GTX fractions in blue mussel during smoking canning process.
A, shucked blue mussel; B, thawed blue mussel; C, drip;
D, steamed blue mussel; E, steamed broth; F, smoked blue mussel;
G, canned blue mussel; H, soup; I, standard GTX toxins

ature, and N-sulfocarbamoyl toxin group equilibrated much faster than the carbamate group. The biosynthesis product of the dinoflagellates was thought to be β-epimer (GTX3, GTX4, C2, C4), since only this epimer group was detected in active growing cells (Oshima et al., 1992). In toxins transmitted to shellfish, epimerization proceeds gradually, until it reaches equilibrium at a $\beta : \alpha$ ratio close to 1 : 3. Thus, the relative ratio of epimers provide information on how long toxins have been retained by shellfish (Oshima, 1995a).

Change of specific toxicity during canning process

STX (497μg/μmole) had the highest specific toxicity, followed GTX1 (494 μg/μmole) and neoSTX (459 μg/μmole). The specific toxicity and its change of toxin components from blue mussel and oyster during canning process were shown in Table 4, 5 and Fig. 2~5. The majority of the toxin present consisted of gonyautoxin 1~4 in both sample. The toxicity of gonyautoxin 1~4 were 88% and 84% in blue mussel and oyster, respectively. On the other hand, dcSTX was appeared newly, and the specific toxicities of dcGTX2 and dcGTX3 were increased after steaming and retorting (Table 4 and 5). These results seemed to N-sulfocarbamoyl toxin group was easily hydrolyzed at neutral pH, but yielded decarbamoyl derivatives at a different position (Oshima, 1995a). Increase of STX's specific toxicity was due to the decrease in the N-OH toxin group (GTX1, GTX4 and neoSTX) accompanied by an increase in the N-H toxin group (GTX2, GTX3 and STX). It was found that the specific toxicity of C 1, C2 and GTX4 were decreased first with retorting, followed by GTX 1 and GTX 3 (Fig. 2 and 3). STX and dcSTX, however, kept the high specific toxicity after retorting (Fig. 4 and 5).

From above results, In case of mole%, the major components of PSP toxin in blue mussel and oyster in Weopori, Kŏje, Korea, were C1, C2, GTX1 and GTX 2. While, in case of specific toxicity, the major toxin components were GTX1~4 in both sample. We, the-

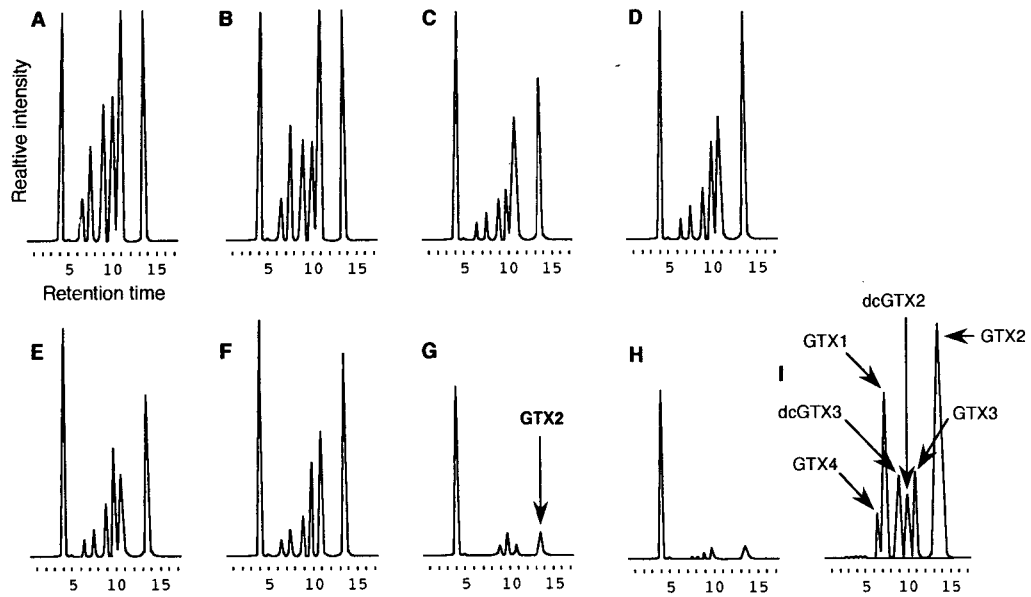

Fig. 3. HPLC chromatograms of GTX fractions in oyster during smoking canning process.
A, shucked oyster; B, thawed oyster; C, drip;
D, steamed oyster; E, steamed broth; F, smoked oyster;
G, canned oyster; H, soup; I, standard GTX toxins

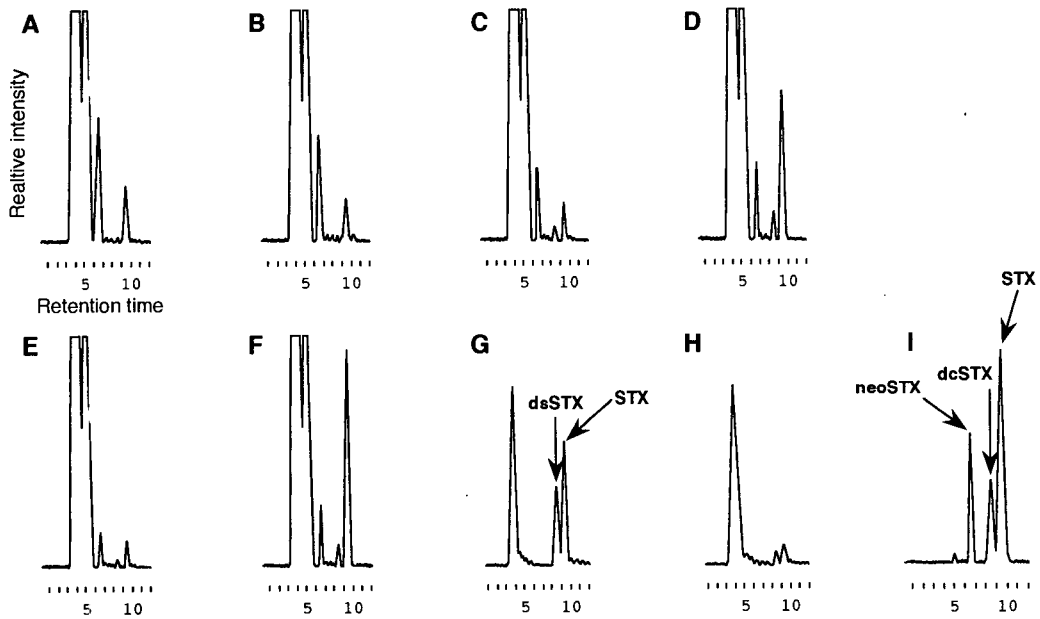

Fig. 4. HPLC chromatograms of GTX fractions in blue mussel during smoking canning process.
A, shucked blue mussel; B, thawed blue mussel; C, drip;
D, steamed blue mussel; E, steamed broth; F, smoked blue mussel;
G, canned blue mussel; H, soup; I, standard GTX toxins

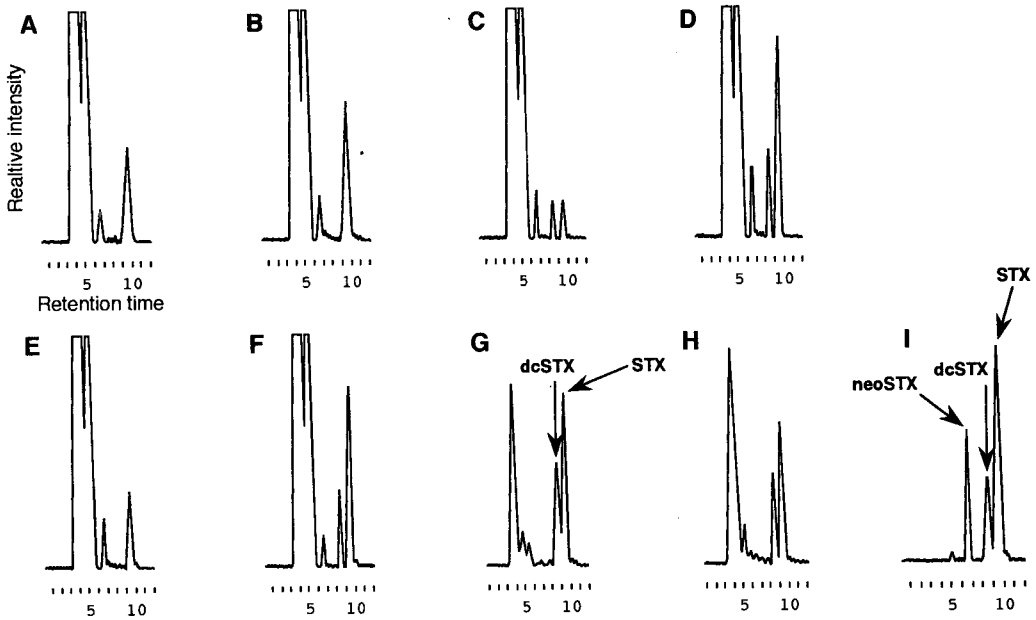

Fig. 6. HPLC chromatograms of GTX fractions in oyster during smoking canning process.
A, shucked oyster B, thawed oyster; C, drip;
D, steamed oyster; E, steamed broth; F, smoked oyster;
G, canned oyster; H, soup; I, standard GTX toxins

refore, found that the origins of toxins causing the death accident were GTX1~4.

We found also that STX and dcSTX were the most thermostable toxin among all toxin components from retaining the high specific toxicity after retorting.

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