J. Fish. Sci. Tech. 2(2), 155~160, 1999

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY


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

## 4. Detoxification and Toxin Composition in Paralytic Shellfish Poison-Infested Oyster during Processing

**Hyun-Jeong Jeong, Il-Shik Shin* and Young-Man Kim**

*Department of Food Science and Nutrition, Dong-eui University, Pusan 614-714, Korea*

**Faculty of Marine Bioscience and Technology, Kangnung National University, Kangnung 210-720, Korea*

(Received March 1999, Accepted October 1999)

Studies on detoxification of Paralytic Shellfish Poison (PSP)-infested oyster, *Crassostrea gigas* were carried out using available processing resources. Changes of paralytic shellfish toxin components and specific toxicity during canning process were also investigated with high performance liquid chromatography (HPLC). Toxic oysters collected at Hachŏng in Kŏje Bay were used for experimental samples. The toxicity of oysters with range of 185~778 μg/100 g was reduced below the quarantine limit of 80 μg/100 g or not detected level by the mouse bioassay after canning process. The mole % of toxin components in the shucked oyster was in the order of 25.1 mole % of gonyautoxin 1, 19.2 mole % of gonyautoxin 3, 17.2 mole % of gonyautoxin 4 and 14.6 mole % of gonyautoxin 2. This sample had tracing amounts of C1, C2, saxitoxin and neosaxitoxin. In the case of specific toxicity, the major toxins were consisted of gonyautoxin 1~4. The sum of gonyautoxin 1, 2, 3 and 4 was 80% of total toxicity of oyster. Saxitoxin and decarbamoylsaxitoxin were the more thermostable than any other toxin components.

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

## Introduction

Paralytic shellfish poisoning, a severe and occasionally fatal form of food poisoning, caused by the ingestion of certain shellfish which has taken a toxic dinoflagellates such as *Alexandrium catenella, Alexandrium tamarense, Gymnodium catenatum* and *Pyrodinium bahamense* var *compressa* (Taylor, 1985). As the causative toxins, more than 20 analogs of saxitoxin have been so far identified from several sources such as dinoflagellates and contaminated shellfish (Oshima et al., 1993).

Toxification of commercially important shellfish with paralytic shellfish poison (PSP) causes serious problems to public health and shellfish related industries. Of the several methods proposed for removal of PSP from toxic shellfish, heat treatment has been used most popularly, although a large percentage of the occurrences of PSP illness has been related to the ingestion of cooked shellfish.

In previous papers (Kim et al., 1996; Shin et al., 1996), we reported toxicity change and toxin components in PSP-infested 5 blue mussels, *Mytilus edulis* with different toxic level and only one oyster, *Crassostrea gigas* collected at Wepori, Kŏje, Korea in 1996 during canning processes. Most of papers about PSP were concentrated on toxicity change and toxin components of blue mussel.

Oyster is a favorite shellfish in Korea and is consumed as raw or cooked. It is also widely cultured for both domestic and export. However, oyster is infested by PSP in every spring and there are few papers about detoxification of PSP in oyster so far.

In this paper, we report the detoxification and change of toxin components in cultured oyster with 5 different toxic level collected at Kŏje Bay, Korea in 1997 during canning processing.

*To whom correspondence should be addressed.

## Materials and Methods

### Materials

The 5 cultured oysters, *Crassostrea gigas*, which have toxicity of 185 μg, 657 μg, 778 μg, 353 μg and 236 μg per 100 g, respectively, were collected at Kŏje Bay, Korea from 4th, Apr. to 25th, Apr. in 1997. The canning process was done at Daeil Fisheries Ltd Co. (Table 1).

### Toxicity test

The toxicity of PSP was determined by the mouse assay using ICR strain male mice weighing 19~21 g following the AOAC (Association of Official Analytical Chemists, 1990) method. Ten mice were used for each sample and the mean toxicity was expressed as μg per 100 g of edible meat or soup. The toxicities of can products were measured triplicately. The toxicities of steamed broth and canned soup were measured by concentrating under reduced pressure if their toxicities were not detected by mouse assay.

### Boiling

The 360 g of washed shell-stock oysters was cooked at 98℃ for 10 min. with two volumes of fresh water.

### Canning

Shell-stock oysters washed with fresh water were steamed at 105℃ for 10 min shucked and trimmed, respectively. The 165 g of steamed meat was packed in No. 7 can with 100 mℓ of 2% NaCl solution and retorted at 115℃ for 70 min (Boiled can). The steamed meat was smoked at 110℃ for 15 min, followed at 125℃ for 15 min. 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). The 95 g of steamed meat was packed in No. 3B of square can with 20 mℓ of acidified cotton seed oil (cotton seed oil, 1000 mℓ; paprika, 1.7 g; chilly oil, 0.8 g; Acetic acid 250 mℓ) and retorted at 115℃ for 70 min (Acidified can).

Table 1. List of tested samples

| Sample name | Sample code | Collected date | Collected area | Toxicity (μg/100 g) |
|---|---|---|---|---|
| Oyster | A | 1997. 4. 4 | Hachŏng, Koje Bay | 185 |
| 〃 | B | 1997. 4. 8 | 〃 | 657 |
| 〃 | C | 1997. 4. 10 | 〃 | 778 |
| 〃 | D | 1997. 4. 18 | 〃 | 353 |
| 〃 | E | 1997. 4. 25 | 〃 | 236 |

### HPLC analysis of toxin

Toxin analysis by HPLC was referred to Oshima (1995b)

### 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 Discussions

### Change of toxicity by boiling

Changes of toxicity in different toxic levels of the oyster by boiling were summarized in Table 2.

The toxicity of low toxic oyster, A (185 μg/100 g) and E (236 μg/100 g) was reduced below the critical limit of 80 μg/100 g after boiling. Boiling of oysters for the usual home cooking times (98℃ for 10 min) reduced their toxicity by 68~81%.

While the high toxic oyster B (675 μg/100 g), C (778 μg/100 g) and D (353 μg/100 g) have residual toxicity of 172, 228 and 89 μg per 100 g after boiling although their detoxification ratio were 74.5, 70.7 and 75.6%, respectively.

These results are similar to results of Kim et al. (1996) indicating that PSP may be detoxificated by boiling, but the boiling itself is not sufficient to detoxificate extremely high toxic shellfish. In fact, almost reported food poisoning accidents have been come from eating cooked shellfish.

Table 2. Toxicity change of oyster during boiling (98℃, 10 min.)

| Process | A | | B | | C | | D | | E | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) |
| Shell stocked oyster | 185 | | 657 | | 778 | | 353 | | 236 | |
| Boiling | | | | | | | | | | |
| Boiled meat | 59 | 68 | 172 | 74 | 228 | 71 | 89 | 75 | 45 | 81 |
| Soup | 42 | | NT* | | 86 | | 15 | | 6 | |

*ND, not detected.

Change of toxicity during canning process

Most commercial catches of shellfish are processed before marketing and their toxicities depend on the process and species of shellfish. The most common commercial processing treatments for shellfish are canning.

1. Change of toxicity during smoked canning process

Changes of toxicity in blue mussel and oyster during smoked canning process were shown in Table 3.

The toxicities of all toxic oyster were reduced below the quarantine limit of 80 μg/100 g after retorting. Especially, the high toxic oyster C of 778 μg/100 g in which toxicity was about 10 times greater of the critical limit, the toxicity of 205 μg/100 g remained after smoking process, but the toxicity was also reduced to 51~60 μg/100 g, below the critical limit after retorting process. These results are similar to the results of Kim et al. (1996).

2. Change of toxicity during boiled canning process

Changes of toxicity in oysters during boiled canning process were shown in Table 4.

There was no difference in the detoxification effects between boiled canning process and smoked canning process. Especially, the detoxification ratio (89~100%) and the residual toxicity (ND~60 μg/100 g) by boiled can process were almost the same as smoking canning process although the boiled can process had not smoking process.

Above results are similar to the tests with sea scallop and oyster in which toxicity was reduced to 90% (Noguchi et al., 1980a and 1980b; Takata et al., 1994).

Table 3. Toxicity change of oyster during smoked canning process

| Process | A | | B | | C | | D | | E | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) |
| 1. Shucked oyster | 185 | | 657 | | 778 | | 353 | | 236 | |
| 2. Steaming | | | | | | | | | | |
| Steamed meat | 59 | 68 | 172 | 74 | 228 | 71 | 89 | 75 | 45 | 81 |
| Broth | 41 | | NT | | 86 | | 15 | | 6 | |
| 3. Smoked meat | 53 | 71 | 216 | 67 | 205 | 74 | 90 | 75 | 43 | 82 |
| 4. Canning | | | | | | | | | | |
| Canned meat | ND | 100 | 43 | 93 | 45 | 94 | 40 | 89 | ND | 100 |
| | ND | 100 | 43 | 93 | 39 | 95 | 43 | 88 | ND | 100 |
| | ND | 100 | 43 | 93 | 59 | 92 | 40 | 89 | ND | 100 |
| Juice layer | ND | | 22 | | 23 | | 18 | | ND | |
| Oil layer | ND | | ND | | ND | | ND | | ND | |

ND, not detected: NT, Not tested

Table 4. Toxicity change of oyster during boiled canning process

| Process | A | | B | | C | | D | | E | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) |
| 1. Shucked oyster | 185 | | 657 | | 778 | | 353 | | 236 | |
| 2. Steaming | | | | | | | | | | |
| Steamed meat | 59 | 68 | 172 | 74 | 228 | 71 | 89 | 75 | 45 | 81 |
| Broth | 41 | | NT | | 86 | | 15 | | 6 | |
| 3. Canning | | | | | | | | | | |
| Canned meat | ND | 100 | 47 | 93 | 45 | 94 | 40 | 89 | ND | 100 |
| | ND | 100 | 43 | 93 | 48 | 94 | 38 | 89 | ND | 100 |
| | ND | 100 | 50 | 92 | 60 | 92 | 39 | 89 | ND | 100 |
| Soup | ND | | 25 | | 25 | | 16 | | ND | |

ND, not detected: NT, Not tested

3. Change of toxicity during acidified canning process

Change of toxicity in oysters during acidifying canning process was shown in Table 5.

The toxicity of all oysters was not detected or reduced below the critical limit after retorting same as in smoked canning and boiled canning. The detoxification ratio (76~100%), however, was lower than that (89~100%) of smoked canning or boiled canning process. Kim et al. (1996) reported that detoxification effect of acidfying canning process was lower than that of smoked canning or boiled canning process.

PSP was thermostable at range of pH 2~4 (Chang et al., 1988). The residual toxicity of oyster in acidified can was supposed to be higher than taht of smoked can or boiled can because pH of acidified can was about 4.5.

From above results, commercial canning process of oyster reduced its toxicity by more than 76%. Steaming reduced 68~81% of total toxicity and retorting produces a further small drop in toxicity (equivalent to 5~30% of toxicity of raw shellfish). We found that toxicity of raw oysters affected toxicity of their canned products and showed that toxin-free canned oysters were regularly obtained when toxicity of raw oyster were below 236 μg/100 g and that canned oysters below the quarantine limit were regularly obtained when toxicity of raw shellfish was below 778 μg/100 g.

These results are very similar to the results of detoxification of PSP-infested blue mussels or oyster during canning process (Kim et al., 1996), suggesting that the quarantine limit can be also level up more than raw scores of 80 μg/100 g for canning in Korea. But if toxicity still exceed 80 μg/100 g, the cans must be destroyed.

**Changes of toxin composition during canning process**

Analysis of toxic components in oysters during canning process was shown in Table 6. GTX 1 (25.1 mole %) was predominant components in the shucked oyster, followed GTX 3 (19.1 mole %) and GTX4 (17.2 mole %). It also contained the minor amounts of C1, C2, STX and neoSTX. After

Table 5. Toxicity change of oyster during acidfied canning process

| Process | A | | B | | C | | D | | E | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) | Toxicity (μg/100 g) | Reduction rate (%) |
| 1. Shucked oyster | 185 | | 657 | | 778 | | 353 | | 236 | |
| 2. Steaming | | | | | | | | | | |
| Steamed meat | 59 | 68 | 172 | 74 | 228 | 71 | 89 | 75 | 45 | 81 |
| Broth | 41 | | NT | | 86 | | 15 | | 6 | |
| 3. Canning | | | | | | | | | | |
| Canned meat | 43 | 77 | 63 | 90 | 51 | 93 | 44 | 88 | ND | 100 |
| | 42 | 77 | 54 | 92 | 60 | 92 | 42 | 88 | ND | 100 |
| | 44 | 76 | 58 | 91 | 51 | 93 | 44 | 88 | ND | 100 |
| Juice layer | ND | | 22 | | 23 | | 18 | | ND | |
| Oil layer | ND | | ND | | ND | | ND | | ND | |

ND, not detected: NT, Not tested

Table 6. Change of toxin composition in oyster collected at 1997 during smoked canning process

| Process | Toxin composition (Mole%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | GTX1 | GTX2 | GTX3 | GTX4 | dcGTX2 | dcGTX3 | neoSTX | dcSTX | STX |
| 1. Shucked oyster | 4.1 | 4.2 | 0.0 | 0.0 | 25.1 | 14.6 | 19.1 | 17.2 | 1.7 | 3.7 | 7.1 | 1.2 | 2.0 |
| 2. Steaming | | | | | | | | | | | | | |
| Steamed oyster | 7.8 | 2.4 | 0.0 | 0.0 | 26.1 | 21.0 | 6.9 | 8.6 | 8.0 | 2.3 | 6.8 | 1.3 | 8.8 |
| 3. Smoked oyster | 5.9 | 1.7 | 0.0 | 0.0 | 25.6 | 16.5 | 5.0 | 7.8 | 12.5 | 3.9 | 4.3 | 3.2 | 13.3 |
| 4. Canning | | | | | | | | | | | | | |
| Canned oyster | 0.0 | 0.0 | 0.0 | 0.0 | 3.9 | 2.5 | 0.7 | 0.0 | 2.6 | 0.8 | 0.0 | 47.1 | 42.5 |
| Soup | 0.0 | 0.0 | 0.0 | 0.0 | 3.7 | 2.5 | 0.0 | 0.0 | 1.4 | 1.7 | 0.0 | 48.6 | 42.1 |

4. Detoxification and Toxin Composition in Paralytic Shellfish Poison-Infested Oyster during Processing

Table 7. Change of toxicity and toxin composition in oyster collected at 1997 during smoked 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. Shucked oyster | 0 | 4 | 0 | 0 | 240 | 50 | 118 | 120 | 0 | 10 | 26 | 64 | 6 | 20 | 660 |
| 2. Steaming | | | | | | | | | | | | | | | |
| Steamed oyster | 0 | 0 | 0 | 0 | 66 | 20 | 12 | 16 | 0 | 16 | 4 | 16 | 2 | 22 | 174 |
| 3. Smoked oyster | 0 | 0 | 0 | 0 | 76 | 18 | 10 | 18 | 0 | 24 | 8 | 12 | 4 | 40 | 212 |
| 4. Canning | | | | | | | | | | | | | | | |
| Canned oyster | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 24 | 40 |
| Soup | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 12 | 22 |

steaming, it was found that the mole % of C2:C1, GTX4:GTX1 and GTX3:GTX2 were changed close to 1:3 ratio. These results indicate that epimerization between β-epimer (GTX3, GTX4, C2, C4) and α-epimer (GTX2, GTX1, C1, C3) occured during steaming. Shin et al. (1996) reported that the same epimerization occurred in blue mussel during steaming.

The change of specific toxicity in oyster during canning process was shown in Table 7. The majority of the toxin was consisted of GTX1, 2, 3 and 4. The sum of GTX1, 2, 3 and 4 was 80% of total toxicity. STX was increased after steaming. These results showed that N-sulfocarbamoyl toxin group was easily hydrolyzed at neutral pH, but yielded carbamoyl derivatives at a different position (Oshima, 1995a). Shin et al. (1996) also reported that the increasing of STX's specific toxicity occurred in blue mussel after steaming. The 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).

In Korea, the toxins such as GTX1, 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*. The toxins such as GTX1, GTX2, GTX3 and GTX4 are the major components and C1, C2, STX and neoSTX are the minor components in blue mussel, *Mytilus edulis*, at Jinhae Bay (Lee et al., 1992). Jeon and Han (1998) reported that the major toxin component in wild mussels (*Mytilus corsucus*) collected at Koje island were GTXs in spring. From above results, we conclude that the main toxic components of oysters were identical to those of blue mussels.

## Acknowledgments

The authors wish to express their gratitude to Mr J. T. Lee, the representative and the staff of Daeil Fisheries Co. Ltd. for making available the canning system. This study was supported by grants from R&D Promotion Center for Agriculture, Forestry and Fishery of Korea Rural Economic Institute.

## References

AOAC. 1990. Paralytic shellfish Poison. In Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Ed (Kenneth, H ed) Association of Official Analytical Chemists Inc. Virginia, pp. 881~882.

Chang, D.S., I.S. Shin, H.Y. Goo, E.G. Oh, J.H. Pyun and Y.H. Park. 1988. Studies on distribution, characterization and detoxification of shellfish toxin in Korea. 3. Detoxification of paralytic shellfish poison of Sea mussel, *Mytilus edulis*. J. Korean Fish. Soc. 21 (5), 297~302 (in Korean).

Jeon, J.K. and M.S. Han. 1998. Monitoring of intoxication and toxin composition on wild mussels (*Mytilus corsucus*) from coastal waters near Koje Island, Korea in 1996 and 1997. Bull. Korean Fish. Soc. 31 (6), 817~822 (in Korean).

Kim, Y.M., S.H. Choi, S.J. Kim, S.B. Suh, H.S. Pyun, D.S. Chang and I.S. Shin. 1996 Studies for Reestabilishment of Approval Toxin Amount in Paralytic Shellfish Poison-Infested Shellfish 1. Toxicity Change in Paralytic Shellfish Poison-Infested Blue mussel, *Mytilus edulis* and Oyster, *Crassostrea gigas* during Boiling and Canning Processes. J. Korean Fish. Soc. 29 (6), 893~899.

Lee, J.S., J.K. Jeon, M.S. Han, Y. Oshima and T. Yasumoto. 1992. Paralytic shellfish toxins in the mussel *Mytilus edulis* and dinoflagellate *Alexandrium tamarense* from Jinhae Bay, Korea. J. Korean Fish. Soc. 25 (2), 144~150.

Noguchi, T., Y. Ueda, Y, Onoue, M. Kono, K. Koyama, K. Hashimoto, Y. Seno and S. Mishima. 1980a.

Reduction in Toxicity of PSP Infested Scallops during Canning Process. Bull. Japan. Soc. Sci. Fish. 46 (10), 1273~1277 (in Japanese).

Noguchi, T., Y. Ueda, Y, Onoue, M. Kono, K. Koyama, K. Hashimoto, T. Takeuchi, Y. Seno and S. Mishima. 1980b. Reduction in Toxicity of Highly PSP-Infested Scallops during Canning Process and Storage. Bull. Japan. Soc. Sci. Fish. 46 (11), 1339~1344 (in Japanese).

Oshima, Y. 1995a. Chemical and enzymatic transformation of paralytic shellfish toxins in marine organisms. In Harmful Marine Algal Blooms. (Lassua, P., Arzul, G., Erard, E., Gentien, P. and Marcaillou, C. ed) Lavoisier, Intercept Ltd. 475~480.

Oshima, Y. 1995b. Postcolumn derivatization liquid chromatographic method for paralytic shellfish toxins. J. AOAC Inter. 78 (2), 528~532.

Oshima, Y., S.I. Blackburn and G.M. Hallegraeff. 1993. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate *Gymnodium catenatum* GRAHAM from three different countries. Marine Biol., 116, pp. 471~476.

Prakash, A., J.C. Medcof and A.D. Tennant. 1971. Paralytic shellfish poisoning in eastern Canada. Fish. Res. Bd. Canada. 177, 1~87.

Shin, I.S., S.H. Choi, T.S. Lee, H.J. Kim, J.S. Lee and Y. M. Kim. 1996 Studies for reestablishment 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, Koje, Korea during boiling and canning process. J. Korean Fish. Soc. 29 (6), 900~908.

Takata, K., M. Mizuta and T. Monden. 1994. Reduction in Toxicity of PSP-infested Oyster by heat treatment. J. Food Hygiene. 35 (6), 624~630 (in Japanese).

Taylor, F.J.R. 1985. The taxonomy and relationship of red tide flagellates, In toxic dinoflagellate )D. M. Anderson, A. W. White and D. G. Baden, eds.). Elsevier. New York. pp. 11~26.