Canning Process that Diminishes Paralytic Shellfish Poison in Naturally Contaminated Mussels (*Mytilus galloprovincialis*)

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**ABSTRACT**

Changes in toxin profile and total toxicity levels of paralytic shellfish poison (PSP)-containing mussels were monitored during the standard canning process of pickled mussels and mussels in brine using mouse bioassays and high-performance liquid chromatography. Detoxification percentages for canned mussel meat exceeded 50% of initial toxicity. Total toxicity reduction did not fully correspond to toxin destruction, which was due to the loss of PSP to cooking water and packing media of the canned product. Significant differences in detoxification percentages were due to changes in toxin profile during heat treatment in packing media. Toxin conversion phenomena should be determined to validate detoxification procedures in the canning industry.

Paralytic shellfish poison (PSP) is a group of water-soluble sodium channel blockers produced by dinoflagellates that cause human food poisoning after ingestion of toxic bivalve molluscs. Since the first PSP episodes in humans were reported (8), the incidence of PSP has increased during the last decades due to the increase in scientific knowledge, intensification of aquaculture activities, eutrophication of marine waters, and passive transport of dinoflagellates by ships and bivalve molluscs (14).

The incidence of toxic episodes has led to implementation of monitoring programs that have been effective in protecting human health in different countries. But the increase of close periods in the culture areas of bivalve molluscs has become a serious problem for the economic sectors related to the culture and canning of bivalves. Research has been focused on the study of detoxification procedures to minimize the economic impact of marine toxins (3–6, 9, 12, 21, 27, 28), but the results have not been satisfactory.

The application of high temperatures has been tested to decrease PSP toxicity in bivalve molluscs. Medcalf et al. (19) and MacDonald (18) showed the effectiveness of panfrying in decreasing PSP levels in toxic bivalves. Noguchi et al. (24, 25) studied the sterilization effects on PSP toxicity in canned scallops (*Pectinopencten yessoensis*) and showed that there is a sharp decrease in toxic levels that continued moderately during storage. Similar results were obtained by Prakash et al. (26), who demonstrated 90% detoxification during the sterilization process. Nagashima et al. (20) studied the kinetics of thermal degradation at 100°C, 110°C, and 120°C of PSP obtained from toxic bivalves. Mixtures of gonyautoxins 1 and 4 (GTX-1 and -4) and gonyautoxins 2 and 3 (GTX-2 and -3) showed first-order degradation kinetics with GTX-1 and -4 being more thermostable. Although the results indicated a significant reduction in PSP toxicity by high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and mouse bioassay, the heat treatments applied were more severe than the standard process used in the canning industry. Recently, Berenguer et al. (2) demonstrated the effectiveness of the canning process in decreasing PSP toxicity in Mediterranean cockles (*Acanthocardia tuberculatum*) (toxin levels of 800 μg saxitoxin equivalents [STX-eq]/100 g tissue) and yielding a product free from toxins. This work has brought about a change in the European Union legislation on PSP toxins (10) that authorizes the extraction of *A. tuberculatum* having toxin levels up to 300 μg STX-eq/100 g tissue when the product is destined for the canning industry. Similar regulations have been established by the Canadian government for clams (*Mya arenaria*) and mussels (*Mytilus edulis*) having toxin levels up to 160 μg STX-eq/100 g tissue. The current regulatory level for fresh bivalve molluscs in most countries is 80 μg STX-eq/100 g tissue.

In spite of decreased PSP levels during canning, the mechanisms involved have not been fully described. Factors such as changes in toxin profile during heat treatment (13, 15, 16, 22) and toxin transfer between different tissues of bivalves (23) demand a careful approach to determine the loss of PSP in toxic bivalves.

Here, we show changes in PSP levels during the standard canning process of batches of toxic mussels. Total toxicity and toxin profile changes were monitored using mouse bioassay and HPLC to clarify the mechanisms involved in decreased PSP toxicity during the canning process.
TABLE 1. PSP levels (mg STX-eq/100 g) in mussel meat during processing and determined using the mouse bioassay

<table>
<thead>
<tr>
<th>Batch</th>
<th>Raw</th>
<th>Cooked (%) reduction</th>
<th>Pickled (%) reduction</th>
<th>Brined (%) reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.012 ± 78</td>
<td>395 ± 150</td>
<td>257 ± 127</td>
<td>131 ± 72</td>
</tr>
<tr>
<td>B</td>
<td>295 ± 60</td>
<td>150 ± 44</td>
<td>127 ± 43</td>
<td>72 ± 36</td>
</tr>
<tr>
<td>C</td>
<td>186 ± 58</td>
<td>93 ± 39</td>
<td>79 ± 12</td>
<td>56 ± 15</td>
</tr>
<tr>
<td>D</td>
<td>98 ± 12</td>
<td>35 ± 5</td>
<td>36 ± 6</td>
<td>*</td>
</tr>
<tr>
<td>E</td>
<td>71 ± 21</td>
<td>34 ± 2</td>
<td>33 ± 1</td>
<td>*</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>59.5 ± 15.2</td>
<td>73.2 ± 14.4</td>
<td></td>
</tr>
</tbody>
</table>

\[ a \] % reduction is based on PSP levels found in raw tissue; \( n = 3 \).
\[ b \] *, PSP not detected by mouse bioassay (<30 mg STX-eq/100 g tissue).
\[ c \] Significantly different (P < 0.01).

MATERIALS AND METHODS

Raw material. Five batches (10 kg each) of fresh mussels (*Mytilus galloprovincialis*) (average size 10 cm) were collected in September 1993 from the same culture area (mussel raft) in Ría de Arosa (NW Spain) during different phases of a PSP-producing dinoflagellate bloom (batches were collected on a 2-day basis from the same rope of the mussel raft because toxicity was detected by the local monitoring authorities). After opening the bivalves under steam (5 min), whole mussel meat (average weight: 6 g/mussel) was stored at -20°C in 2-kg subsamples.

Industrial processing. Triplicate standard canning processes were used for each mussel batch in the pilot plant. After cooking in water (1:1 wt/vol) at 97°C/2 min (after reaching temperature), RR-125 cans (length, 104 mm; width, 60 mm; net weight, 120 g; drained weight, 80 g) were manufactured with two different packing media. Mussels were packed with brine (3.2 g NaCl/100 g of water, pH 6.5) or pickled (50% olive oil, 50% wine vinegar, pH 2.6). The sterilization process was 115°C/35 min and is the standard for bivalve molluscs in the Spanish canning industry. After processing, 5 × 3 batches of canned products were tested and 10 cans of each product were used.

PSP analysis. PSP toxicity was evaluated using mouse bioassays on Swiss mice (CFLP strain) of 18 to 22 g weight. Triplicate analyses were done for the following products: raw material, cooked mussels, cooking medium, canned mussels and packing medium. Mouse bioassay of mussels meat was done according to AOAC method (1). Mouse bioassay of packing medium was done by direct intraperitoneal (i.p.) injection of 1-ml aliquots of brine and water fraction of pickle.

Changes in toxin profile during the industrial process were assessed by HPLC analysis using the method of Franco and Fernández-Vila (11). HCl extracts for mouse bioassays were filtered (0.45 μm) and injected onto a chromatographic system with the following components: binary pump (model LC-250, The PerkinElmer Corp., Norwalk, Conn.), fluorescence detector (model LS-3B, Perkin-Elmer) at λex 330 nm and λem 390 nm, integrator (model 1020 Nelson Perkin-Elmer), column Merck Lichrospher 100 RP-18 (12.5 cm × 4 mm inner diameter [i.d.]) and post-column derivatization system with two Eldex A-30-SW-2 pumps, reaction chamber with teflon tubing (10 m × 0.5 mm i.d.), and temperature controller (model TCM Waters Corporation, Milford, Conn.). Certified standards of STX, neosaxitoxin (Neo-STX) and GTX-1 to -4 (PSP-1B kit, National Research Council, Halifax, Canada) were used for quantitative HPLC analysis.

Statistical analysis (t test) was done to assess the significance of the differences observed in detoxification percentages.

RESULTS AND DISCUSSION

The mouse bioassays showed large differences in the toxin levels of the five mussel batches, ranging from 71 ± 21 to 1.012 ± 78 mg STX-eq/100 g (Table 1). Chromatographic data showed the same toxin profile for the five batches, mainly composed of GTX-1 (46% of total chromatographic area), GTX-4 (14%), Cs toxins (a group of N-sulfocarbamoyl toxins that includes toxins C1, C2, C3, and C4) (24%) and decarbamoyl-STX (15%), with small amounts of STX and neo-STX. Quantitative HPLC data showed higher toxicity values than mouse bioassays (Table 2), which was due to the presence of PSP toxins with lower toxicity than STX. Furthermore, HPLC quantitative data should be carefully evaluated due to the lack of certified reference material for the whole PSP group and the differences reported (7) in postcolumn fluorescence values for these toxins.

Changes in toxin levels using mouse bioassay and HPLC throughout the different phases of processing are shown in Tables 1 and 2. Average detoxification percentages in mussel meat ranged from 59% to 87%, depending on the analytical method used (mouse bioassay/HPLC) and product obtained (brined/pickled). No significant differences were observed among mussel batches, which indicated that toxicity levels in raw material did not influence the detoxification percentages.

Comparative analysis of quantitative data did not show significant differences in detoxification percentages determined by HPLC and mouse bioassay for both cooked mussels and mussels in brine. In pickled mussels, detoxification percentages determined using mouse bioassays were significantly lower (P < 0.01) than those using HPLC.
TABLE 2. PSP levels (µg STX-eq/100 g) in mussel meat during processing and determined using HPLC

<table>
<thead>
<tr>
<th>Batch</th>
<th>Raw</th>
<th>Cooked (% reduction)*</th>
<th>Pickled (% reduction)*</th>
<th>Brined (% reduction)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3,693 ± 954</td>
<td>2,168 ± 351</td>
<td>845 ± 238</td>
<td>414 ± 195</td>
</tr>
<tr>
<td>B</td>
<td>1,932 ± 344</td>
<td>835 ± 399</td>
<td>412 ± 287</td>
<td>226 ± 125</td>
</tr>
<tr>
<td>C</td>
<td>1,443 ± 783</td>
<td>524 ± 181</td>
<td>248 ± 106</td>
<td>145 ± 89</td>
</tr>
<tr>
<td>D</td>
<td>701 ± 260</td>
<td>332 ± 264</td>
<td>152 ± 100</td>
<td>165 ± 11</td>
</tr>
<tr>
<td>E</td>
<td>511 ± 217</td>
<td>105 ± 43</td>
<td>43 ± 32</td>
<td>85 ± 40</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>299 ± 129</td>
<td>135 ± 65</td>
<td>139 ± 52</td>
</tr>
</tbody>
</table>

*% reduction is based on PSP levels found in raw tissue; n = 3.

was due to conversion of Cs toxins to the more toxic GTX toxins during heat treatment in an acid medium (7, 17) and confirmed by HPLC analysis. This conversion process produced a lower detoxifying effect by mouse bioassay in pickled mussels, which indicated that toxin profile in raw material should be determined to assess the effect of thermal treatment on PSP toxicity.

Changes in toxin profile were also reflected in the comparative analysis of detoxification percentages obtained for pickled mussels and mussels in brine using mouse bioassay. The results obtained were significantly \( P < 0.01 \) lower in pickled mussels, which supported the Cs → GTX conversion observed during heat treatment in acid packing medium. As might have been expected, HPLC data did not show significant differences in detoxification percentages for brined and pickled mussels.

In spite of reduced PSP levels in mussel meat, the loss of toxins in cooking water and packing medium should be quantitated to calculate the real destruction of PSP during thermal processes. Toxin losses in cooking water (Table 3) ranged from average percentages of 31% (mouse bioassay) to 39% (HPLC) of total toxicity in raw mussels. According to these values, the real destruction of PSP during cooking (Table 3) was estimated at 19.7% (HPLC) and 25.1% (mouse bioassay) of total toxicity in raw material.

PSP analysis in canned products also showed significant transfer of toxins to the packing medium (Fig. 1), and ranged from 29.8 ± 4.9% (mouse bioassay) to 28.6 ± 6.7% (HPLC) for pickled mussels (percentages referred to total toxicity in cooked mussels). In brined mussels, the average losses in packing medium ranged between 19.6 ± 6.7% (mouse bioassay) and 23.7 ± 9.4% (HPLC). Based on the average net and drained weight for cans, and PSP levels in the different fractions of canned product (mussel meat and packing medium), the PSP destruction percentages for the sterilization process ranged from 33.3 ± 20.1% (mouse bioassay) to 35.4 ± 21.4% (HPLC) of total toxicity in cooked mussel meat for mussels in brine. In pickled mussels, the values ranged from 26.8 ± 8.8% (HPLC) to 0% (mouse bioassay). No toxicity reduction was observed for pickled mussels using mouse bioassays, which was associated with changes in toxin profile previously described.

### CONCLUSIONS

Although some limitations could be associated with the examination of a single PSP bloom (different toxic profiles), at least for the toxin profile studied, the standard canning process (which included three thermal processes: steaming, cooking, and sterilization) produced a significant and reproducible reduction of PSP toxicity in mussel meat (over 50% of toxicity level in raw material in all cases). The decrease in toxicity levels was not dependent on toxin levels of raw material. During the sterilization process, the detoxification percentages could be affected by changes in toxin profile due to toxin conversion. Conversion from Cs to GTXs yielded a relative increase in PSP toxicity, and these changes should be determined to validate detoxification procedures. Detoxification percentages in mussel meat...
FIGURE 1. Percentages of PSP loss to packing medium and PSP destruction during the sterilization process of pickled mussels (A) and mussels in brine (B). Percentages are based on PSP levels found in cooked mussels; missing values for mouse bioassay of batches D and E indicate PSP levels under the limit of detection of the method; n = 3.

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REFERENCES


