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Effect of industrial process for canning on OA toxins detection/quantification by different techniques

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Summary

During a long-lasting DSP episode due to *Dinophysis acuminata* and *D. acuta*, much higher than expected DSP toxins levels were detected on industrial steamed and canned mussels when estimated by LC-MS/MS. The mussels had been collected from production areas authorised for harvesting (using LC-MS/MS). An experiment was designed to study the effects of the main steps of the canning process and the differences in the detection of okadaic acid group toxins between three analytical methods: Mouse Bioassay, LC-MS/MS and a phosphatase inhibition test. The experiment was conducted with mussels from two production areas of the Galician coast. LC-MS/MS technique was found to be unsuitable for detection of toxicity in fresh mussels being the mouse bioassay more efficient. However, mouse bioassay probably underestimated the actual toxicity in canned mussels. The increase of toxicity in steamed mussels (~ 147 %) by LC-MS/MS was higher than that expected by dehydration (~ 30 %), because an increase in toxins content was observed. During the canning process there was a decrease in toxin content that was almost compensated by the dehydration (~ 20 %) maintaining a concentration of toxins similar to that detected in steamed mussels. The results obtained with the phosphatase inhibition tests were similar to those by LC-MS/MS.

Introduction

Accumulation of toxins of the okadaic acid (OA) group in bivalve molluscs is a problem for the public health and then for the aquaculture and exploitation of wild populations all around the world. On 1st of july 2011, mouse bioassay was replaced by LC-MS/MS, as the reference method for the control of lipophilic toxins in the European Union. Since then, in the Galician canning and steaming factories sector, it has been observed an increase in the cases of mussels that showing toxicities clearly below the regulatory limit for lipophilic toxins, become above those limits after industrial cooking process. During autumn 2013, these increments in toxicity after industrial cooking would seem higher than those explainable by the expected dehydration. In this study, changes in DSP toxicity, DSP toxin content and toxin concentration in mussels (*Mytilus galloprovincialis*), during canning process were studied by mouse bioassay, LC-MS/MS and a phosphatase inhibition test.

Materials and Methods

The design of the study was a two-way ANOVA with the industrial treatment (raw, steamed and canned) and mussel batch as factors (four batches: 2 in Ría de Arousa and 2 in Ría de Pontevedra). Four groups of 40 individuals were used for each combination treatment-batch, so 12 samples were analysed unprocessed, 12 samples were steamed at 130 ºC during 70 seconds and other 12 samples, after the steaming treatment were packed in oval containers, brine added, closed hermetically and sterilized in autoclave at 115ºC for 40 minutes. Each sample (n= 36) was analysed by: mouse bioassay (Yasumoto et al.,

1978; Fernández et al., 2002), LC-MS/MS (EU-RL-MB, 2011; Gerssen et al., 2009; Regueiro et al., 2011) and a phosphatase inhibition test (Smienk et al., 2013).

Results and Discussion

The weight of mussels decreased with the steaming approximately 31 %, in average. Canning produced an additional dehydration of 19 %. Only OA, DTX2, unidentified derivates of both toxins (detected by alkaline hydrolysis) and low levels of 13-desmethyl SPX C, were detected by LC-MS/MS.

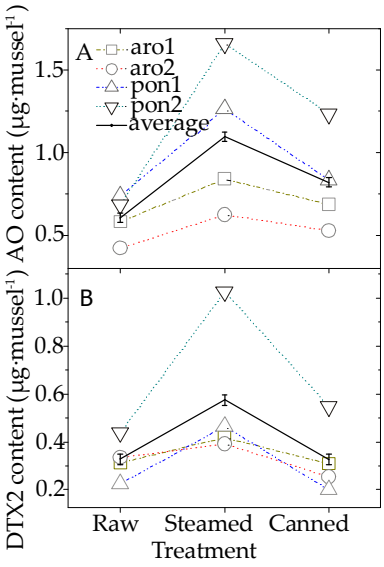

Figure 1. Effect of the treatment on AO toxin content (A) and DTX2 toxin content (B). Different lines with their symbols are the different batches.

By MBA it was observed a decrease of the toxicity with each step of the canning process. With raw mussels 14 out of 16 samples gave a positive result (above the legal limit), while for steamed and canned mussels only 12 and 4 samples were positive, respectively.

Estimated toxicities by LC-MS/MS increased with the steaming process and decreased (relative to the steamed mussels) after canning. All the raw samples analysed by LC-MS/MS were below the legal limit (65-150 µg OA equiv.·Kg^{-1}). By the contrary all the steamed and canned samples were well above the legal limit (194-291 and 188-317 µg OA equiv.·Kg^{-1}, respectively). The average increase with steaming was around 150 %, but the estimated toxicity reached levels above twice that in the raw mussels.

By the phosphatase inhibition test 12 out of 16 raw samples and all the steamed and canned samples analysed were above the legal limit. The relationship between LC-MS/MS and phosphatase inhibition test toxicity results was good ($r^2 = 0.92$).

A high increase in the toxin content with the steaming of the mussels was observed. Later the canning process produced a decrease, in such a way that the toxin content in canned mussels was almost the same that that observed for raw mussels for DTX2 and a slightly higher for OA (Fig. 1).

Conclusions

1.- During industrial steaming there were changes in the mussel matrix or in the toxins derivatives in such a way that an important part of the toxins that were not detected in raw mussels were detected after steaming, apparently increasing toxin content of the mussels and consequently their toxicity.

2.- During canning process there was a decrease in toxins content probably due to the thermal treatment.

References

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