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Effects of cooking and heat treatment on concentration and tissue distribution of okadaic acid and dinophysistoxin-2 in mussels (*Mytilus edulis*) ☆

Pearse McCarron[a,b,*], Jane Kilcoyne[a], Philipp Hess[a]

[a] *Marine Institute, Marine Environment and Food Safety Services, Rinville, Oranmore, Co. Galway, Ireland*
[b] *University College Dublin, Conway Institute, Belfield, Dublin 4, Ireland*

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Abstract

Using high-performance liquid chromatography with mass spectrometry, the influence of conventional steaming and another heat treatment on the level of okadaic acid and dinophysistoxin-2 in mussels (*Mytilus edulis*) was investigated. Concentration increases correlated with water loss during steaming, and increased distribution of okadaic acid and dinophysistoxin-2 from the digestive glands to the remainder tissues was observed as a result of the processes examined. This suggests that the analysis of whole flesh tissues, as opposed to dissected digestive glands, is more appropriate for regulatory purposes, particularly if cooked samples are being analysed.

A systematic heat treatment experiment showed that while okadaic acid group toxins are stable during cooking processes, degradation does occur in mussel tissues after prolonged exposure to high temperatures.

The findings of the studies reported here have importance in terms of the methodology applied in regulatory phycotoxin monitoring programmes. Therefore, options for sample pretreatment are discussed.

Keywords: Shellfish toxins; Processing; Pretreatment; Diarrhetic shellfish poisoning; DSP; Food safety

1. Introduction

The accumulation in bivalve shellfish of toxins originating from marine phytoplankton has serious implications for human health. Numerous classes of toxins have been identified, which, after consumption of contaminated tissues, induce a variety of symptoms in humans including nausea, abdominal cramps, diarrhoea, memory loss, and in some extreme cases paralysis and even death.

The toxins responsible for diarrhetic shellfish poisoning (DSP) have had a severe impact on the shellfish industry internationally. The first occurrence of DSP was reported in Japan during the 1970s (Yasumoto et al., 1978). DSP is a severe gastrointestinal illness that typically follows ingestion of shellfish contaminated with toxigenic

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*Corresponding author at: Marine Institute, Marine Environment and Food Safety Services, Rinville, Oranmore, Co. Galway, Ireland.

E-mail address: pearse.mccarron@marine.ie (P. McCarron).

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dinoflagellates such as certain *Dinophysis* and *Prorocentrum* species (Quilliam, 1995). The main toxins responsible are okadaic acid (OA), which was first isolated from sponges (Tachibana et al., 1981), dinophysistoxin-1 (DTX1) isolated from mussels in Japan (Murata et al., 1982) and dinophysistoxin-2 (DTX2) initially isolated from Irish mussels (Hu et al., 1992). Additionally, a range of acyl and diol ester derivates of these three compounds have been identified (Marr et al., 1992). The European Union (EU) regulates the maximum allowable level of DSP toxins in shellfish at 160 μg/kg OA equivalents (Regulation (EC) No. 853/2004). In Ireland, the OA and DTX2 isomers are regularly detected well in excess of the regulatory levels in mussels (Hess et al., 2003), while only trace amounts of DTX1 have been reported (Carmody et al., 1995). Since summer 2006 the Marine Institute has been applying a relative toxicity factor of 0.6 for DTX2 based on the findings of Aune et al. (2007) when calculating OA equivalents.

While the official reference method for DSP toxins in the EU is the mouse bioassay, its replacement with chemical testing methods will be facilitated by the recent organisation of at least two LC–MS method validation studies for lipophilic toxins in the EU (BIOTOX 2007; CRL 2007), which are on-going. Aspects of the methodology currently used vary between the monitoring programmes of different countries. One important aspect is sample pretreatment. Most programmes analyse raw mussels, e.g. Ireland and Norway; however, some countries cook the mussels before analysis by light steaming to open the shell and stabilise the matrix, e.g. Denmark (Jorgensen and Larsen, 2004) and Germany (LFGB, 2006). Cooking, boiling or steaming are also common steps in commercial processing, as well as in the culinary preparation of molluscs and crustaceans.

Recent studies have examined the influence of cooking on various toxins in mussels. Reductions in the extractable levels of YTX from Greenshell™ mussels (*Perna canaliculus*) have been reported after steaming (Holland et al., 2006). Conventional steaming of mussels (*Mytilus edulis*) contaminated with domoic acid had a minimal effect on the result of whole mussel tissue analysis (McCarron and Hess, 2006). Work by Vieites et al. (1999) showed that a canning process resulted in a significant and reproducible reduction of PSP toxicity in naturally contaminated mussels (*Mytilus galloprovincialis*). Considerable increases in AZA toxin concentrations upon steaming fresh mussels were reported (Hess et al., 2005). This change was attributed to water loss during steaming, with the AZAs concentrating by a factor of ca. 2 in the cooked tissue as a result. No information regarding the effects of cooking on OA/DTX toxins in mussels was available for the expert consultation by FAO/IOC/WHO during 2004 (Anon 2005), and as far as the authors are aware there is no further information for OA/DTX toxins available in the literature on this topic. However, considering the lipophilic nature of these toxins, it is reasonable to assume that they would exhibit similar behaviour to AZAs.

In late July 2005, levels of OA and DTX2 significantly above the regulatory limit were detected in mussels from the northwest of Ireland. Samples were collected to examine the influence of cooking on OA and DTX2. The contribution of water loss during the cooking process to changes in the toxin concentrations was studied in detail.

2. Methods and materials

2.1. Standards and chemicals

OA standards were prepared from certified calibration solutions (NRC CRM-OA) produced by the National Research Council of Canada (NRC-IMB, Halifax, NS, Canada). A reverse osmosis purification system (Barnstead Int., IA, USA) supplied water for the mobile phase. Formic acid and ammonium formate were obtained from Sigma-Aldrich (St Louis, MO, USA). Methanol, ethylacetate and acetonitrile were purchased as pestican grade solvents from Labscan Ltd., Dublin, Ireland.

2.2. Samples

For the heat treatment and tissue distribution study two mussel samples (*Mytilus edulis*), naturally contaminated with OA and DTX2, were retrieved from Bruckless in Donegal Bay, on the northwest coast of Ireland. One sample was obtained during the first week of August and the second sample one week later. Both samples (ca. 10 kg each) were received in the laboratory within 24 h of being removed from the water.

For an additional heat treatment study examining the influence of heat-treating whole mussel tissues, without the loss of water from the tissues, a separate uncooked mussel sample obtained during 2001 from