Toxicon 90 (2014) 1–14

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Novel application of high pressure processing for the production of shellfish toxin matrix reference materials

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ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form 16 July 2014
Accepted 22 July 2014
Available online 31 July 2014

Keywords:
Paralytic shellfish poisoning
High pressure processing
Oysters
LC-FLD
Reference materials

ABSTRACT

The production of homogeneous and stable matrix reference materials for marine biotoxins is important for the validation and implementation of instrumental methods of analysis. High pressure processing was investigated to ascertain potential advantages this technique may have in stabilising paralytic shellfish poisoning toxins in shellfish tissues compared to untreated materials. Oyster tissues were subjected to a range of different temperatures and pressures, with results showing a significant reduction in biological activity in comparison to control samples, without significantly altering toxin profiles. Tissue subjected to pressures >600 MPa at 50 °C was assessed for homogeneity and stability. The sample homogeneity was determined using a pre-column oxidation LC-FLD method and shown to be within accepted levels of within batch repeatability. Short and long-term stability studies were conducted over a range of temperatures, with analysis by pre and post column oxidation LC-FLD demonstrating improved stability of toxins compared to the untreated materials and with epimerisation of toxins also notably reduced in treated materials. This study confirmed the technique of high pressure processing to improve the stability of PSP toxins compared to untreated wet tissues and highlighted its applicability in reference material preparation where removal of biological activity is of importance.

1. Introduction

Marine phycotoxins, produced by many species of naturally occurring algae, may accumulate in filter-feeding molluscs and subsequently pose a serious health risk to shellfish consumers (Hallegraeff et al., 2003). Monitoring of toxins in shellfish is an important and regulated requirement (Anon, 2005a), for which the need for suitable reference materials is essential. With recent changes to regulations and subsequent increasing use of instrumental analytical methods for shellfish toxin quantitation (Anon, 2006), the potential presence of a wide range of toxin analytes in contaminated shellfish has resulted in the need for production of a large number of different biotoxin standards. These are used primarily as instrumental calibrants for quantifying specific toxin concentrations. Commercial availability of these standards has in recent years facilitated the development of instrumental methods in replacement of animal based assays for monitoring sample toxicity. In tandem with this development is the requirement for matrix reference materials, specifically shellfish tissues containing characterised and preferably certified concentrations of the toxin analytes of relevance. However, the number of matrix CRMs available is low given the large amount of time and resources required to successfully produce such materials. A fit for purpose CRM needs to be

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http://dx.doi.org/10.1016/j.toxicon.2014.07.008
0041-0101/

homogenous across the entire production batch, and must be stable both under long term storage and transportation conditions. The stability of these materials in particular is an important issue given strong evidence to date for decomposition of tissues and associated degradation and/or transformation reactions occurring within the analyte groups present with RMs (Hess et al., 2007). To date, a number of different stabilisation methods have been investigated, many of which are designed to remove biological activity from the matrix materials before long term storage and subsequent analysis. These include steaming or other thermal processing (Hess et al., 2007; NRC-CNRC) to eliminate or reduce enzymatic activity within shellfish tissues containing domoic acid and Okadaic acid and the use of antibiotic and antioxidant additives to improve the short term stability of domoic acid RMs (McCarron et al., 2007a). Freeze drying has been investigated for the successful enhanced stabilisation of domoic acid and a range of lipophilic toxins in candidate mussel tissue RMs (McCarron et al., 2007b) and for the stability of hydrophilic toxins in a mussel RM (Louzao et al., 1994). Recently, a large scale freeze-dried tissue reference material containing domoic acid and a number of regulated lipophilic toxins was produced and characterised (McCarron et al., 2011). The use of gamma irradiation has also been investigated for the stabilisation of RMs containing lipophilic toxins (McCarron et al., 2007c).

Paralytic shellfish poisoning (PSP) toxins are one group of marine biotoxins for which monitoring in shellfish is a statutory requirement in the European Union (EU). Currently one matrix CRM is available commercially for PSP toxins, which was produced in oyster tissue, with homogeneity and stability enhanced through a variety of mechanisms including water and pH adjustment, use of chemical additives and gamma irradiation of the post-production samples (Cefas Certificate of Anal, 1101; Turner et al., 2012, 2010a). Other feasibility studies conducted have demonstrated the potential for use of freeze drying for a PSP oyster tissue (Turner et al., 2013a) and the use of targeted chemical (Turner et al., 2012) and enzymatic transformation (Turner et al., 2013b) reactions to stabilise toxin concentrations over short and long term storage.

High Hydrostatic Pressure Processing (HHP) or High Pressure Processing (HPP) is a pasteurisation technique which has been employed for over a hundred years as a non-thermal method of producing food products free from microbiological contamination (Rendueles et al., 2010). The process has been applied to a wide range of foodstuffs with a high water content including seafood (Campus, 2010) and is of great interest due to the potential for decontaminating food whilst minimising the impact of the processing on the physical and nutritional characteristics of the food products. Foods are subjected to pressures in the region of 150 MPa–600 MPa and held at this pressure for a set period of time, typically 1–15 min (Murchie et al., 2005). Water is used as a pressure transmitting medium so that the pressure is evenly distributed throughout the product, consequently not resulting in any form of crushing to the load. As a processing technique which increases the safety of the food products, there has been considerable interest in the application of HPP to bivalve molluscs such as oysters which are typically eaten raw, given the known concentration of microbial activity as a consequence of the filter-feeding activity of the animals. Benefits of the processing in oysters include the removal of total viable counts (TVC) [e.g.19] in addition to specific microbiological contaminants including *Escherichia coli*, *Vibrio* and norovirus (Murchie et al., 2005; Cruz-Romero et al., 2007; He et al., 2002; Ye et al., 2012; Kingsley et al., 2007).

With these benefits, the technique was investigated in this study for a novel application involving the removal of biological activity from homogenised oyster tissue. This work focussed specifically on the potential application of HPP to the elimination of microbial activity in shellfish tissue homogenates and the subsequent production of the stable, homogenous matrix reference materials required for method validation and quality assurance. Work was conducted to assess the effects of varying HPP parameters on biological activity, PSP toxin concentrations and matrix components potentially forming in the tissues. Analysis was conducted using the AOAC Official Method 2005.06, pre-column oxidation liquid chromatography with fluorescence detection (Pre-COX LC-FLD) which enabled the quantitation of individual PSP toxins and toxin epimeric pairs (Anon, 2005b). The quantitation of individual PSP epimers was achieved using AOAC Official Method 2011.02, post-column oxidation liquid chromatography with fluorescence detection (PCOX LC-FLD) (Anon, 2011). The within-batch repeatability of the process was assessed through the treatment of replicate samples under different processing conditions and the between-batch repeatability examined through the treatment of a second larger volume of tissue also subsequently subjected to analysis to assess material homogeneity and stability. Data was ultimately generated to determine the feasibility of HPP of incurred shellfish tissue for the ultimate production of certified reference materials.

2. Materials and methods

2.1. Chemicals and standards

Analytical grade chemicals and HPLC-grade solvents were used throughout the study. Certified reference materials (GTX1&4, NEO, dcSTX, GTX2&3, GTX5, C1&2, STX dihydrochloride (di-HCl), dcNEO and dcGTX2&3) were obtained from the Institute for Biotoxin Metrology, National Research Council Canada (NRCC, Halifax, Nova Scotia, Canada). For Pre-COX LC-FLD analysis, primary toxin standards were diluted in 0.1 mM acetic acid to produce instrument calibration standards. For PCOX analysis, instrumental calibrations for GTX and STX analysis were prepared in 0.3 mM HCl, with C1&2 standards diluted in pH5 water.

2.2. Contaminated oyster preparation

Two batches of PSP-positive Pacific oyster tissues (*Crassostrea gigas*) were prepared following feeding experiments using mass-cultured toxic *Alexandrium* algae as described by Higman and Turner, 2010 (Higman and