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Feasibility studies into the production of gamma-irradiated oyster tissue reference materials for paralytic shellfish poisoning toxins

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ABSTRACT

A study was conducted to assess the feasibility for the production of sterile, stable and homogenous shellfish reference materials containing known concentrations of paralytic shellfish poisoning (PSP) toxins. Pacific oysters were contaminated with toxins following mass culturing of toxic algae and shellfish feeding experiments. Live oysters were shucked and tissues homogenised, before measuring into multiple aliquots, with one batch subjected to gamma irradiation treatment and the other remaining untreated. The homogeneity of both batches of samples was assessed using a pre-column oxidation liquid chromatography with fluorescence detection (Pre-COX LC-FLD) method and shown to be within the limits of normal within-batch repeatability. A twelve-month stability experiment was conducted for both untreated and gamma irradiated batches, specifically examining the effects of long term storage at −20 °C, +4 °C and +40 °C. Results indicated mostly good stability of PSP toxins in both materials when stored frozen at −20 °C, but with the instability of GTX2&3 concentrations in the untreated tissues eliminated in the irradiated tissues. Analysis using a post-column oxidation (PCOX) LC-FLD method also showed epimerisation in both GTX1&4 and GTX2&3 epimeric pairs in untreated samples after only 6 months frozen storage. This issue was not present in the tissues irradiated before long term storage. Biological activity testing confirmed the absence of bacteria in the irradiated samples throughout the 12 month study period. With such results there was clear evidence for the potential of increasing the scale of the mass culturing and shellfish feeding for the production of large batches of tissue suitable for the preparation of a certified matrix reference material. Overall results demonstrated the feasibility for production of oyster reference materials for PSTs, with evidence for prolonged stability following gamma irradiation treatment and storage at −20 °C.

1. Introduction

Paralytic shellfish poisoning (PSP) toxins are produced by several species of phytoplankton which are found to bioaccumulate periodically in a wide range of filter-feeding shellfish (Hallegraeff, 2003). Consumption of contaminated shellfish can result in a variety of illnesses in the human consumer, as a result of which monitoring of PSP toxins (PSTs) in shellfish is a statutory requirement in the European Union (EU). The official EU reference method for quantifying PSP in bivalve shellfish is the mouse bioassay (MBA) (Anon., 2005a), but an alternative instrumental method is also incorporated into EU legislation (Anon., 2006). Official method (OM) AOAC 2005.06 uses

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Pre-column oxidation (Pre-COX) Liquid Chromatography with Fluorescence Detection (LC-FLD) to quantify PSTs (Anon., 2005b) and has in recent years been refined and incorporated into the UK official control monitoring programmes for the analysis of 12 different shellfish species (Turner et al., 2009, 2010a, 2011; Turner and Hatfield, 2012). More recently a post-column oxidation (PCOX) LC-FLD method (Anon., 2011a) and a receptor binding assay (RBA) (Anon., 2011b) have also been approved by the AOAC as official methods (2011.02 and 2011.27). The use of routine analytical methods requires Reference Materials (RMs) both for method validation and for quality control (QC) purposes, so there is an important requirement for availability to laboratories of PSP-contaminated materials (Hess et al., 2007). This is especially true for chromatographic methodologies where an estimation of sample toxicity is calculated following identification and quantitation of individual toxins. Both validation and routine QC require large numbers of reference materials to enable suitable numbers of analyses to be conducted, without which expensive spiking studies may be required. In the UK, naturally-occurring PSP toxic events are found to occur periodically, but there is no guarantee that these will generate enough shellfish contaminated with appropriate concentrations of toxins. Naturally-sourced contaminated shellfish will subsequently require storage, often for many years before and during use, so there is an important requirement for the reference material and the analytes it contains to remain stable under these conditions. To date a number of techniques have been tested to improve both the short term and long term stability of shellfish tissue materials, including heat treatment (Hess et al., 2007; Reeves et al., 2004), addition of antibiotics and antioxidants (McCarron et al., 2007a), freeze-drying (McCarron et al., 2007b; Louzao et al., 1994) and use of gamma irradiation (McCarron et al., 2007c). The use of gamma irradiation is attractive as the technique is carried out in the final product container once materials have been aliquoted, dispensed and sealed. A preliminary investigation into the potential use of gamma irradiation in PST-contaminated shellfish tissues demonstrated the success of the technique for removal of biological activity whilst not affecting toxin concentrations and the prevalence of matrix interferences (Turner et al., 2010b). Subsequently, work conducted in candidate oyster tissue reference materials showed additional benefits of gamma irradiation. Treatment led to improved short term stability of toxin concentrations in tissues stored under refrigerated conditions, and a reduction of toxin epimerisation (Turner et al., 2012), a process known to occur in many stored, frozen tissues (Reeves et al., 2004).

The objective of this study was therefore to determine the feasibility for production of well characterised, stable and homogenous shellfish reference materials using these techniques. PST-contaminated shellfish tissues generated following shellfish feeding experiments were used to compare the stability and homogeneity of untreated and gamma-irradiated tissues. Specifically the stability of the tissues was examined over long term storage and over a range of temperature conditions also applicable to short term transportation. The effects of irradiation were examined to confirm the ability of the treatment to remove bacterial levels over the entire study period, the effects and to assess its impacts on initial PST concentrations and long term stability. Data was generated to determine the feasibility of the gamma-irradiation of incurred shellfish tissue approach for the ultimate production of larger-scale 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 saxitoxin di-hydrochloride (STX), gonyautoxins 1 to 5 (GTX1-5), neosaxitoxin (NEO), decarbamoylsaxitoxin (dcSTX), N-sulfocarbamoyl-gonyautoxins 2 and 3 (C1&2), decarbamoylneosaxitoxin (dcNEO) and decarbamoylgonyautoxins 2 and 3 (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. Shellfish sample preparation

Contaminated Pacific oyster tissue was prepared in-house following feeding experiments of mass-cultured toxic algae as described by Higman and Turner (2010). A 12 tubular bag photobioreactor on a 14 h light:10 h dark cycle at 17 °C was utilised to produce 350 L of *Alexandrium fundyense* culture (strain CCMP 1719). This culture was subsequently fed to PSP-free Pacific oysters (*Crassostrea gigas*). Culture was added gradually over a period of 5 days to the shellfish which were being maintained in aerated recirculating seawater tanks held at 17 °C. On completion of the feeding Pacific oysters were removed and shucked. Bulk tissue samples were homogenised, separated into 6 g aliquots, dispensed into sealed polypropylene screw-topped vials and stored at −20 °C until further use.

2.3. Gamma irradiation of samples

Following evidence for the removal of biological activity from shellfish tissues without affecting concentrations of PSP toxins (Turner et al., 2010b) a proportion of tissue aliquots were randomly selected for gamma irradiation treatment. Selected samples were removed from frozen storage and transported under temperature controlled conditions to the Isotron Gamma Irradiation Processing Facility in Swindon, United Kingdom. Processing was conducted on the still-frozen vials held within clear-plastic bags, each containing a dosimeter. The samples were subjected to gamma irradiation with a Cobalt 60 source with the target dose levels of 18.0 kGy ± 10%. After processing the samples were immediately placed back into frozen storage before shipment the next day back to Cefas.