Toxicon 112 (2016) 1–7

Contents lists available at ScienceDirect

Toxicon

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

Effect of the industrial canning on the toxicity of mussels contaminated with diarrhetic shellfish poisoning (DSP) toxins

Juan Blanco [a, *], Fabiola Arévalo [b], Jorge Correa [b], M. Corina Porro [c], Ana G. Cabado [c], Juan M. Vieites [c], Angeles Moroño [b]

[a] CIMA (Centro de Investigacións Mariñas), Pedras de Corón s/n, Vilanova de Arousa, 36620, Spain
[b] Intecmar (Instituto Tecnolóxico para o Control do Medio Mariño de Galicia), Peirao de Vilaxoán s/n, Vilagarcía de Arousa, 36611, Spain
[c] ANFACO-CECOPESCA, Carretera de Colexio Universitario, 16, Vigo, 36310, Pontevedra, Spain

ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
13 January 2016
Accepted 20 January 2016
Available online 21 January 2016

Keywords:
DSP toxins
Okadaic acid
DTX2
Canning
Prickled sauce
Sterilization
Mussel

ABSTRACT

The effect of canning in pickled sauce and autoclaving on weight, toxin content, toxin concentration and toxicity of steamed mussels was studied. Weight decreased by 25.5%. Okadaic acid (OA) and DTX2 content of mussel meat decreased by 24.1 and 42.5%, respectively. The estimated toxicity of the mussel remained nearly unchanged (increased by 2.9%). A part of the toxins lost by the mussels was leached to the sauce but the remaining part should have been thermally degraded. DTX2 underwent more degradation than OA and, in both toxins, free forms more than conjugated ones. This process, therefore, cannot be responsible for the large increments of toxicity of processed mussels —relative to the raw ones-sometimes detected by food processing companies. The final product could be monitored in several ways, but analysing the whole can content or the mussel meat once rehydrated seems to be the most equivalents to the raw mussel controls.

1. Introduction

Marine phycotoxins, especially those of the okadaic acid group which are the causative agent of the syndrome known as Diarrethic Shellfish Poisoning (DSP) (Murata et al., 1982; Yasumoto et al., 1978) are a worldwide problem for food safety and public health and consequently for aquaculture and for the activities of food transforming companies.

Galicia (NW Spain) is one of the main mussel producers in the word, with an annual production of about 240,000 *t* (235,459 in 2014, (Pesca de Galicia, 2015). More than 40% of the produced mussels are transformed by food processing industries, mostly to canned mussels in pickled sauce but also to other canned products, to vacuum packed steamed mussels or to other minor derivatives. In the European Community and other countries, bivalves must not contain toxins above a regulatory level in order to be harvested, marketed or processed. Notwithstanding, during the required processing, the toxins or the mussels can undergo changes that could lead to modifications of their toxin content or their potential toxicity (which depends on toxin concentration and on the power of the involved toxins). Assessing the safety of the transformed mussels, therefore, should not rely on the analyses made on the harvested product and require controls after each processing step.

Posing a risk for public health, many countries have implemented monitoring systems for these compounds, coupled to management policies that ban mollusc harvesting when the natural or cultured populations exceed a toxicity threshold. Traditionally, mouse bioassays have been used to monitor this group of toxins (EURLMB, 2013; Fernández and Cembella, 1995; Fernández et al., 2002, 2003) but, in recent years, other methodologies, as liquid chromatography coupled to mass spectrometry through electrospray interfaces (Gerssen et al., 2009; Quilliam, 2003; Regueiro et al., 2011), or phosphatase inhibition assays (Eberhart et al., 2013; Garibo et al., 2012; Smienk et al., 2013), have been widely used to protect the consumers.

Until 2013, in Galicia (NW Spain), all official toxicity determinations were carried out by means of mouse bioassay (MBA) but during 2013 and 2014 progressively more determinations were carried out by LC-MS/MS, as this is the reference technique in the

* Corresponding author.
E-mail address: juan.carlos.blanco.perez@xunta.es (J. Blanco).

http://dx.doi.org/10.1016/j.toxicon.2016.01.061
0041-0101/

EU since July 2011 (European Commision, 2011), until December 31st 2014, when LC-MS/MS replaced completely MBA. During this transition, the mussel processing companies (steaming and canning companies), in their own-checks, have observed a substantial increase in the number of mussel batches that, being the raw samples below the banning threshold, after the usual steaming process increased the concentration even over the legal limit. When this happens, some mussel batches must be discarded once processed and consequently important economic losses are generated.

Steaming is one of the simplest processing methods. Typically, mussels are subjected to high temperature (around 130 °C) for a short period of time (approx. 30 s). Canning typically requires steaming as a first step, followed (sometimes with other additional treatments) by autoclaving sealed cans containing mussels in an acidic sauce. Both processing methods have an important component of thermal treatment. It is known that cooking or, in general, thermal processing of molluscs produces dehydration of the meat and consequently a weight decrease, while the degradation of lipophilic toxins is null or very limited (EFSA Panel on Contaminants in the Food Chain, 2009b; Hess et al., 2005; McCarron et al., 2008, 2009; Picot et al., 2012). Therefore, an increase of toxin concentration proportional to the weight loss should be expected after steaming or canning. Notwithstanding, some of the observed increments by the food processing industries, mainly at the end of 2013, were too high as to be explained only by dehydration.

In a previous work, using the same mussel batches (Blanco et al., 2015), we studied the effect of steaming on toxin content, toxin concentration and estimated toxicity of the mussels (naturally exposed to the toxin for a long time). In that study we found that an important part of the unexpectedly high increases of toxicity with processing were not due to the processing method itself, but to the underestimation of the toxicity in raw mussels. The precise effect of the second step of the typical canning process (autoclaving in pickled sauce) has not been studied.

In this work, we evaluate (i) the effect of autoclaving mussels in pickled sauce on the weight, toxin content, toxin concentration and estimated toxicity of mussels that had been exposed to okadaic acid and/or DTX2 for a long time; and (ii) the migration of toxins from the meat to the sauce.

2. Material and methods

2.1. Experimental design

The aim of this experiment was to determine the effect of canning (sterilization) mussels previously steamed on toxin content, toxin concentration and toxicity. It was designed assuming that two main factors might affect the toxin concentration in canned mussels: 1) canning process; and 2) the characteristics of the mussels (profile of toxins and biometry). Therefore, a two-way ANOVA was chosen, equivalent to the one used in our previous study (Blanco et al., 2015), with canning as one factor with two levels (steamed and canned mussels) and mussel batch as the second factor (four batches chosen to differ in okadaic acid/DTX2 ratio and meat weight/total weight ratio).

2.2. Sampling, biometry and industrial canning

Two mussel batches were collected on January 22nd 2014 and the other two on January 27th, from both the Ría de Pontevedra (PON 1 and PON2) and the Ría de Arousa (ARO 1, ARO 2) (Galicia, NW Spain) (Figs. 1 and 2 and Tables I and II Suppl. Material). All collected mussels were at the final step of a series of three long-lasting toxic episodes, during which those from the Ría de Pontevedra had been more affected than those from the Ría de Arousa. Before sampling, it was known that their toxicity levels were close to the regulated limit for the toxins of the okadaic acid group. The last episode was originated by a bloom of two species of *Dinophysis*, *Dinophysis acuta*, which in this area is known to produce OA, DTX2 and some diol-esters, among the toxins of the OA group (Pizarro et al., 2008, 2009; Reguera et al., 2014), and *D. acuminata*, which produces OA (Blanco et al., 1995; Reguera et al., 2014).

Once obtained, the mussels were kept refrigerated until the next day when they were cleaned and individually separated, in order to prepare adequately randomized samples. After individualization, the average sizes of the different batches were estimated, and the mussels whose size deviate from the average by more than 20% were discarded. The remaining mussels in each batch were randomly distributed into 8 groups of 40 mussels. Each one of those groups constituted a pooled sample that was considered an individual sample in the experiment. Four out of the 8 samples from each batch were subjected to the process of industrial steaming (treatment = steamed, n = 16) and the remaining 4 were steamed and subjected to the canning/autoclaving process (treatment = canned, n = 16).

The weight of the mussels after each treatment was recorded and the fresh weight and size were reported in a previous study (Blanco et al., 2015).

The samples corresponding to the steaming treatment were heated at 130 °C for 70 s in a continuous industrial steamer. Once steamed, shells were discarded and the meat of the 40 mussels that constitute each sample was weighed. The samples corresponding to the canning treatment were steamed, placed in cans filled with pickled sauce, the cans sealed and then autoclaved at 115 °C for 40 min. Pickled sauce contained mainly sunflower oil, vinegar, paprika and other minor components.

2.3. Toxin extraction and alkaline hydrolysis

2.3.1. Mussels

The pooled meat of the 40 mussels that constitute each sample −after rinsing the sauce in the case of canned mussels-was homogenized with a blade homogenizer. The extraction and hydrolysis were carried out following the standardized operating procedure (SOP) of the EU-RL for the determination of marine lipophilic biotoxins in molluscs (EURLMB, 2011). Briefly, a 2-g aliquot of the homogenate was extracted by vortexing it twice with 9 mL of MeOH for 30 s. The extracts were clarified by centrifugation at 2000 g (4 °C) for 10 min. The obtained supernatants were combined and the total volume adjusted to 20 mL using MeOH. An aliquot of this extract was filtered through a 0.22 μm syringe filter, diluted to ½ using MeOH and 5 μL were injected in the LC-MS/MS system.

In order to determine the total concentration (free + conjugated forms) of the toxins of the okadaic acid group present in the samples, one 5-mL aliquot of each methanolic extract was subjected to alkaline hydrolysis by adding 625 μL of 2.5 M NaOH, vortexing for 30 s, and heating at 76 ± 4 °C for 40 min. The hydrolysate was allowed to reach room temperature, weighed in order to check that there were no solvent losses by evaporation, neutralized by adding 625 μL of 2.5 M HCl and vortexed again. An aliquot of the neutralized hydrolysate was filtered through a 0.22 μm syringe filter, and diluted 5/8 (with MeOH). 5 μL of this solution were injected in the LC-MS/MS system.

2.3.2. Pickled sauce

Free toxins were extracted from pickled sauce with MeOH. For that purpose, 2 mL of MeOH were added to 1 mL of sauce and