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SCIENTIFIC OPINION

Influence of processing on the levels of lipophilic marine biotoxins in bivalve molluscs[1]

Statement of the Panel on Contaminants in the Food Chain

(Question No EFSA-Q-2009-00203)

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PANEL MEMBERS

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SUMMARY

The European Food Safety Authority (EFSA) published an opinion on Marine Biotoxins: Okadaic acid and its analogues in November 2007. The European Commission asked EFSA to elaborate further on the influence of processing (cooking, steaming, autoclaving) on the levels of lipophilic marine biotoxins e.g. okadaic acid (OA) and related toxins. EFSA was also asked to assess if the initial level of marine biotoxins belonging to the group of lipophilic toxins can be modified by processing.

Steaming of mussels caused a 30 % to 70 % increase in the concentration of OA-group toxins (expressed as µg OA equivalents/kg) in the whole flesh. After autoclaving, this increase was between 70 % and 84 %. Water loss was identified as the cause of these increases in concentration. In addition there was some evidence that the redistribution of OA-group toxins from the digestive gland to the remaining tissues might occur during processing. This indicates that the analysis of whole shellfish flesh, as opposed to the digestive gland, might be more appropriate for regulatory purposes, particularly when processed shellfish is analysed.

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Steaming of raw fresh mussels resulted in a 2-fold higher concentration of azaspiracids (AZAs) (AZA1, AZA2, and AZA3 expressed as AZA equivalents) in both whole flesh and digestive gland tissue compared to uncooked flesh. Also in this case the effect was attributed to the loss of water from the mussels into the cooking fluid. In addition, there was some evidence for conversion of a carboxylated AZA analogue (AZA17) into AZA3 during processing, resulting in a 3-fold increase in the level of the latter analogue.

For other lipophilic toxins such as yessotoxins (YTXs) and pectenotoxins (PTXs) there is no information on the influence of processing on their level in shellfish flesh, but there is no reason to assume that they will behave significantly differently to OA-group toxins and AZAs.

Based on the limited information available on the effect of processing on levels of lipophilic marine biotoxins in shellfish the CONTAM Panel concluded that processing of shellfish could lead to an approximate 2-fold increase in the concentration of lipohilic marine biotoxins in shellfish meat. Since limit values of marine biotoxins in shellfish meat are meant to protect the consumer, the effect of processing (cooking, steaming, autoclaving) should be considered when testing shellfish in official control.

Since cooking can lead to an increase in the levels of lipophilic marine biotoxins in shellfish meat, there is a need for harmonisation of sample pre-treatment practices (i.e. cooking versus non-cooking) before the actual analysis of lipophilic marine biotoxins is carried out.

Key words: processing, cooking, marine biotoxins, okadaic acid (OAs), azaspiracids (AZAs), lipophilic, shellfish, bivalve molluscs.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin[2] establishes maximum levels for marine biotoxins in live bivalve molluscs.

Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004[3] establishes the recognised testing methods for detecting marine biotoxins.

With the letter to Mr Koëter (PCA/khk/520587) of 4.7.2006 the Commission asked EFSA to assess the current limits and methods of analysis for marine biotoxins as established in the European Union (EU) legislation, in the light of the publication of the report of FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs (Oslo, September 26-30 2004). The opinion on Okadaic acid and its analogues was adopted on 27 November 2007 and published in January 2008.[4] This was the first in a series of nine opinions on the various biotoxins as requested by the European Commission.

The European Commission has been informed during the last workshop of Community and National Reference Laboratories on marine biotoxins held in Ljubljana from 22 to 24 October 2008, that the initial level of marine biotoxins belonging to the group of lipophilic toxins can be modified by the processing of molluscs in particular by steaming and other heat treatments. The levels of these toxins can increase after the processing.

Pearse McCarron, Jane Kilcoyne and Philipp Hess published in Toxicon[5] in 2008 an article on the effects of cooking and heat treatment on concentration and tissue distribution of okadaic acid and dinophysistoxin-2 in mussels (*Mytilus edulis*). Using high-performance liquid chromatography with mass spectrometry, the influence of conventional steaming and other heat treatment on the level of okadaic acid and dinophysistoxin-2 in mussels (*Mytilus edulis*) was investigated. It was found that concentrations increased correlated with water loss during steaming, and distribution of okadaic acid and dinophysistoxin-2 from the digestive glands to the remainder tissues was observed as a result of the processes examined.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to further elaborate and update its Opinion of the Scientific Panel on Contaminants in the Food Chain biological hazards related to okadaic acid (OA) and related toxins, that together form the group of OA-toxins, to verify if the initial levels of these marine

[2] Official Journal L 226, 25/06/2004 P. 0022 – 0082.
[3] Official Journal L338, 22/12/2005 P. 0027 – 0059.
[4] The EFSA Journal (2008) Journal number, 589, 1-62.
[5] Volume 51, Issue 6, May 2008, Pages 1081-1089.

biotoxins can be modified by the processing of molluscs in particular by steaming and other heat treatments.

Moreover, EFSA is asked to assess if the initial level of other marine biotoxins belonging to the group of lipophilic toxins can also be modified by the same processing.

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ASSESSMENT

1. Introduction

The European Food Safety Authority (EFSA) opinions on okadaic acid (OA) and its analogues and on azaspiracids (AZAs) did not specifically assess the effect of processing (cooking, steaming, autoclaving) of shellfish due to limited information available at the time of preparing these opinions. Some information on the effects of processing on the levels of marine biotoxins in shellfish was included in the report of Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxin in Bivalve Molluscs (FAO/IOC/WWHO, 2004). However, it did not address the issue of processing in detail due to a lack of information on the effects of processing on toxin levels, interconversion of analogues, redistribution of the toxins in different parts of the shellfish, and the effect of water loss.

After publication of the EFSA opinion on okadaic acid and its analogues adopted in November 2007, McCarron *et al*. (2008) published a study on the effect of processing on the levels of okadaic acid in shellfish. This information was discussed at the workshop of the Community and National Reference Laboratories on marine biotoxins in Ljubljana in October 2008, and therefore the European Commission has asked EFSA to further elaborate on the effect of processing on levels of lipophilic marine biotoxins in shellfish.

2. Lipophilic toxins

2.1. Okadaic acid (OA) and related toxins

OA-group toxins, comprising okadaic acid (OA) and its analogues, the dinophysistoxins DTX1, DTX2 and DTX3, cause Diarrhoeic Shellfish Poisoning. In its opinion on OA, the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) established the following toxicity equivalency factors (TEFs): OA=1; DTX1=1; DTX2=0.6. For DTX3 the TEF value equals that of the corresponding unesterified toxins.

The current European Union (EU) regulatory limit is 160 µg OA equivalents/kg shellfish meat. The CONTAM Panel identified 400 g of shellfish meat as the high portion size to be used in the acute risk assessment of marine biotoxins. It concluded that in order for a 60 kg adult not to exceed the acute reference dose (ARfD) of 0.3 µg OA equivalents/kg b.w., a 400 g portion of shellfish should not contain more than 18 µg toxin, corresponding to 45 µg OA equivalent/kg shellfish meat.

The literature on the influence of cooking on OA-group toxins is sparse. The EFSA opinion referred to Hess and Jorgensen (2007) reporting that loss of fluid during cooking could result in a 25-80 % increase in the concentration of the lipophilic toxins in cooked shellfish compared to uncooked shellfish, and mentioned that during heat treatment of shellfish contaminated with OA-group toxins OA appears to be somewhat more heat stable than DTX2, as OA degrades significantly at a temperature of 120°C and higher, whereas DTX2 starts to degrade at about 100°C.

In a recent paper of McCarron *et al*. (2008) the influence of conventional steaming (over boiling water for 10 minutes) and autoclaving (121°C for 15 minutes) on the level of OA and DTX2 in mussels was investigated. The authors studied the effect of processing on whole flesh, the digestive glands and the remainder tissues (remaining after careful dissection of the digestive glands). After steaming a 30 % and 70 % increase in the level of OA-group toxins

(expressed as mg OA equivalents/kg) in the whole flesh was found for the two samples studied. After autoclaving, the increase in the level of OA equivalents was of 70 % and 84 % for the two samples. Measurements of the moisture content indicated that these increases were caused by water loss during processing. Although not consistent for both samples, the results of the study suggested that redistribution from the digestive gland to the remainder tissues might occur during processing. The authors concluded that this suggested that analysis of whole shellfish flesh, as opposed to the digestive gland, was more appropriate for regulatory purposes, particularly when processed shellfish is analysed.

Earlier information on degradation of OA and DTX2 during processing was confirmed by this study of McCarron *et al*. (2008). After exposure to temperatures of 130°C and higher for 10 minutes the level of OA decreased significantly. At 150°C the degradation was of about 40 % compared to the control situation. Degradation of DTX2 appeared to start at 100°C, and was of about 60 % at 150°C.

2.2. Azaspiracids (AZAs)

Azaspiracids (AZAs) are a group of shellfish toxins causing AZA poisoning. Approximately 20 different analogues have been identified, of which AZA1, AZA2 and AZA3 are the most important ones based on occurrence and toxicity.

In the EFSA opinion on the AZAs adopted in June 2008, the CONTAM Panel adopted the following TEFs applied in some countries for AZAs: AZA1=1; AZA2=1.8; AZA3=1.4. The current EU regulatory limit is of 160 µg AZA1 equivalents/kg shellfish meat. The CONTAM Panel concluded that in order for a 60 kg adult not to exceed the ARfD of 0.2 µg AZA1 equivalents/kg b.w., a 400 g portion of shellfish should not contain more than 12 µg toxin, or 30 µg AZA1 equivalent/kg shellfish meat.

It was mentioned in the opinion that AZAs in shellfish are not decomposed at temperatures relevant for cooking. In addition it was mentioned that information from Hess *et al*. (2005) indicated that steaming of raw fresh mussels resulted in a 2-fold higher level of AZAs (AZA1, AZA2, and AZA3 expressed as AZA equivalents/kg) in both whole flesh and digestive gland tissue compared to the uncooked flesh. This effect was attributed to the loss of water from the mussels into the cooking fluid.

In a recent study McCarron *et al*. (2009) investigated the effect of heating on AZAs in the absence of water loss. For that purpose aliquots of mussel tissue homogenates were put into capped centrifuge tubes which were heated for 10 minutes at 90°C. No differences in the concentration of AZA1 and AZA2 were observed, but the concentration of AZA3 increased 3-fold. It was shown by the authors that a carboxylated AZA analogue, AZA17, was converted under these conditions into AZA3.

2.3. Yessotoxins (YTXs) and pectenotoxins (PTXs)

There is no information on the effects of processing (e.g. cooking, steaming, autoclaving) on the levels of yessotoxins (YTXs) or pectenotoxins (PTXs) in shellfish. However, it can be assumed that, as for other lipophilic marine biotoxins, OA-group toxins and AZAs, cooking may lead to an increase in concentration of YTXs and PTXs in shellfish flesh due to the loss of water loss during processing.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Processing (cooking, steaming, autoclaving) of shellfish can lead to an approximate 2-fold increase in the level of lipophilic marine biotoxins e.g. okadaic acid (OA) and its analogues and azaspiracids (AZAs) in shellfish meat due to water loss.
- At high temperatures (>100°C) degradation of OA and dinophysistoxin-2 (DTX2) may occur.
- There is no information on the effect of processing on the levels of yessotoxins (YTXs) and pectenotoxins (PTXs) in shellfish meat, but there is no reason to assume that they will behave significantly differently to OA-group toxins and AZAs.
- The Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) noted that there is no information available on other forms of processing such as frying or grilling.

RECOMMENDATIONS (INCL. KNOWLEDGE/DATA GAPS)

- The effect of processing (cooking, steaming, autoclaving) should be considered when testing shellfish in official control.
- Since cooking leads to an increase in the level of lipophilic marine biotoxins in shellfish meat, there is a need for harmonisation of sample pre-treatment practices (i.e. cooking versus non-cooking) before the actual analysis of lipophilic marine biotoxins is carried out.
- The database on the effects of different types of processing on levels of lipophilic marine biotoxins in shellfish should be extended, comprising information on all lipophilic biotoxins and all the individual shellfish species.

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ABBREVIATIONS

ARfD	Acute reference dose
AZA	Azaspiracid
CONTAM Panel	Panel on Contaminants in the Food Chain
DTX	Dinophysistoxin
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
IOC	Intergovernmental Oceanographic Committee of the United Nations
OA	Okadaic acid
PTX	Pectenotoxin
TEF	Toxicity equivalency factor
WHO	World Health Organization
YTX	Yessotoxin