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DIFFERENTIAL ACCUMULATION OF DOMOIC ACID IN EUROPEAN OYSTERS, QUEEN SCALLOPS AND ASCIDIANS *Microcosmus spp.*

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INTRODUCTION

Being filter feeders, shellfish and ascidians can accumulate contaminants present in environment becoming their vectors to higher food chain. Consumption of bivalves contaminated with potent neurotoxin - domoic acid (DA) **(Figure 1)** can cause Amnesic shellfish poisoning syndrome (1). The aim of this study was to determine differences in occurrence and accumulation of this hydrophilic marine biotoxin in European oysters (*Ostrea edulis Linnaeus,* 1758) (n = 46) **(Figure 2)**, Queen scallops (*Aequipecten opercularis Linnaeus,* 1758) (n = 53) **(Figure 3)** and edible ascidians of the Microcosmus spp. (n = 107) **(Figure 4)** originating from the same harvesting area in the Istrian peninsula waters located in the northern part of the Adriatic Sea.

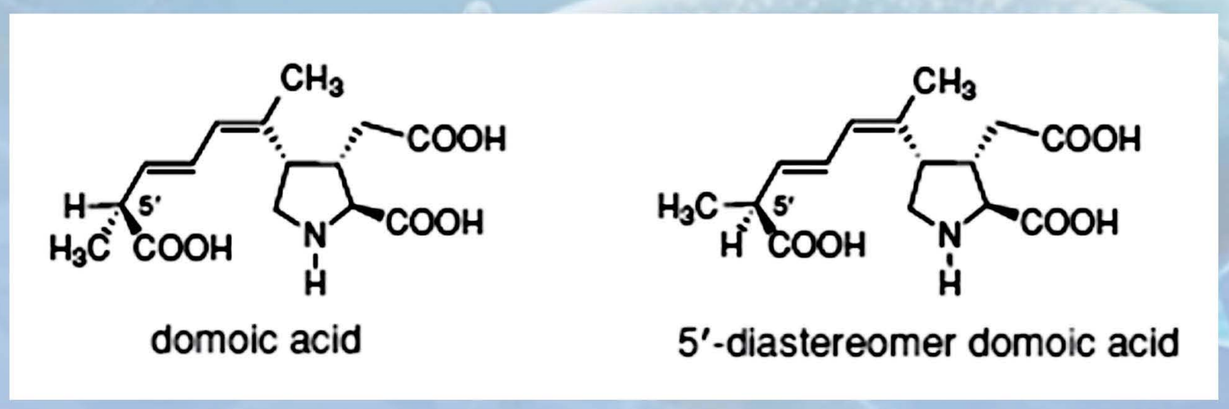

Fig. 1 Structural formula of domoic acid and epi-domoic acid

Fig. 2 European oysters

Fig. 3 Queen scallops

Fig. 4 Ascidians

MATERIALS AND METHOD

Sample extraction and determination was carried out using dansyl chloride derivatization and liquid chromatography-tandem mass spectrometry (2). Determination of DA was performed using a 1290 Infinity UPLC (Agilent Technologies, Singapore) coupled with G6460 Electrospray Ionisation Triple Quad Mass Spectrometer (Agilent Technologies, Waldbronn, Germany) **(Figure 5).** Chromatographic separation was done on a Zorbax SB-C18RRHT 2.1 × 50 mm, 1.8 µm column (Agilent Technologies, Santa Clara, USA), Method was validated in line with Commission Decision 2002/657/EC (3) applying InterVAL Plus Software (quo data, Gesellschaft für Qualitätsmanagement und Statistik GmbH, Dresden, Germany).

Fig. 5 UPLC-MS/MS Agilent Technologies

EXTRACTION MeOH:H 2 O (1:1) →

SAX CLEAN-UP →

CONCENTRATION →

DERIVATIZATION →

LC-MS/MS DETECTION

RESULTS

Validation results are compliant with the requirements of Commission Decision 2002/657/EC, showing the suitability of this method to determine DA in the investigated species. In order to minimize matrix effect for quantification, matrix-matched calibration for every investigated species indivdualy was used. The obtained results showed the presence of DA in all species **(Table 1).** Statistical analysis revealed a difference for DA accumulation between species. Ascidians and European oysters showed no significant difference ($p = 0.210$), whilst between oysters and Queen scallops and Ascidians and scallops significant difference was determined ($p < 0.001$). Queen scalops showed the greatest affinity for DA accumulation with a mean of DA concentration 2.3 fold higher than in European oysters. Compared to oysters, Ascidians showed almost twice a greater affinity for DA accumulation, but significantly lower mean and maximum concentrations detected.

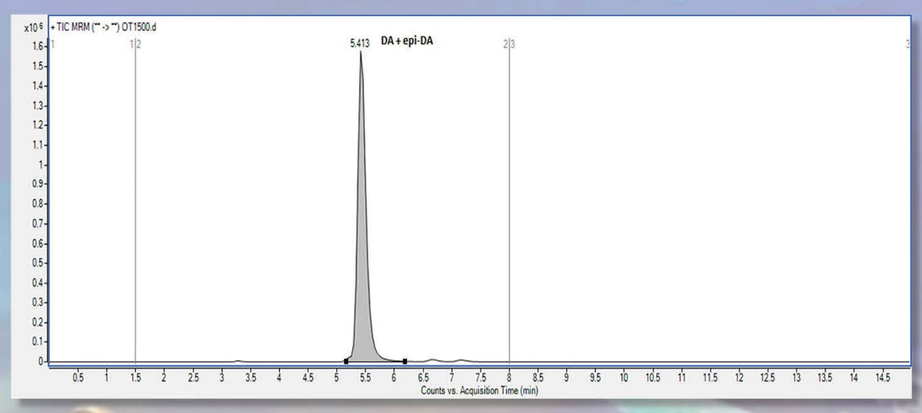

Fig. 6 Total ion chromatogram of domoic acid

Table 1. Domoic acid in shellfish and ascidians.

Species	n	% of positives	Mean ± SD of positives (µg/kg)	Median (µg/kg)	Maximum (µg/kg)
European oysters	46	17.4	65.64 ± 69.79	0	212.37
Queen scallops	53	56.6	152.65 ± 233.91	2.96	809.54
Ascidians	107	32.7	5.50 ± 6.36	0	24.28

CONCLUSIONS

This study reveals differences in occurrence and accumulation of DA among investigated species. Though it was detected in all of them, Queen scallops have a greater preference for this phycotoxin accumulation as compared to other two, making them suitable sentinel species for monitoring the level of DA in seafood. Even though DA was found only in trace levels, it`s occurence in one third of samples indicates the presence of DA producing species in waters of Istrian peninsula and presents a potential threath for consumers safety due to sublethal, rather than acute exposure.

REFERENCES

1. Nicolas et al. (2017) *Glob. Food Secur.* 15:11–21.
2. Beach et al. (2014) *Anal. Methods* 7: 1000-1007.
3. Commission Decision (EC) 2002 *Official J Eur Union* L 221/8-L 221/36.