SEAFOOD TOMORROW

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Grant agreement no: 773400

Deliverable D2.4

Legislative proposal to harvest and detoxify PSP contaminated molluscs

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Dissemination Level	
PU Public	X
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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1. Summary

- An industrial protocol for PSP toxin reduction to safe levels, based on Decision 96/77/EC, was developed and applied for PSP mussel, clams and scallops detoxification.
- The procedure was applied to 6 batches of PSP-contaminated mussels, 2 batches of clams and 2 batches of scallops obtaining ± 85 % detoxification and a safe product.
- A significant reduction was obtained in all samples. Nevertheless, a batch of mussels with 9000 µg STX diHCl equiv./kg, reached a decrease of 90 % after applying the detoxification procedure, although it did not fall below the European limit.
- A viable industrial bivalve canning processing was developed guaranteeing the manufacture of a safe product.
- In order to apply this procedure, PSP level in the edible parts of molluscs should be higher than 800 µg equiv. STX diHCl/kg but lower than 5000 µg equiv. STX diHCl/kg
- A legislative proposal is included as a previous step to allow harvesting and detoxification of PSP contaminated shellfish.

2. Objective

The objective of this deliverable consists of information compilation regarding PSP shellfish detoxification in order to propose recommendations for a legislative proposal. Therefore, a draft text that defines the European legislative proposal in bivalves contaminated with PSP toxins is also included.

In this context, naturally PSP contaminated mussels, clams and scallops were specifically harvested to implement a thermal procedure described in the EU Decision. Slight modifications were applied, in order to obtain a better efficiency of detoxification and yield of bivalves.

3. Background

Paralytic shellfish poisoning (PSP) is caused by consumption of shellfish containing PSP toxins of the saxitoxins family (STX) (EFSA 2009). These toxins are produced by microalgae, mainly toxic marine dinoflagellates such as species of the genera *Alexandrium* and *Gymnodinium*, and also by certain freshwater cyanobacteria (Fabre et al. 2017; Gracia Villalobos et al. 2019; Pitois et al. 2018). These toxins are accumulated and sometimes metabolized into toxin derivatives in many species of filter-feeding bivalves, as mussels, clams and scallops, making them potentially toxic to humans. Harmful algal blooms (HABs) can also induce other ecological problems. In fact, some bivalves can be impaired during intense toxic episodes. For instance, a population of the surf clam *Mesodesma donacium* with high PSP toxic levels, died due to the desiccation caused by the incapability of the clams to burrow (Álvarez et al. 2019).

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To protect public health and ensure the quality of seafood, monitoring programs are implemented worldwide in order to detect and quantify these toxins, and eventually forbidding shellfish harvesting when levels of toxins exceed the legal limit laid down in current regulations. In Europe for example, harvesting and commercialization of bivalves is prohibited above the threshold of 800 µg STX diHCl equiv/kg of shellfish tissues (EC 2004). Closure of shellfish production areas has an important economic impact for producers and other associated industries. No solutions have been found to prevent these important episodes which are seldom predictable, and despite the influence of PSP events on human health and fisheries, studies on shellfish detoxification to mitigate this problem are still very scarce.

Natural detoxification occurs very slowly and it is conditioned by the presence of toxin producing microalgae in the water column. Lipophilic toxins are retained longer than the hydrophilic toxins, such as PSP toxins, although the detoxification rate depends on the species, concentration of toxins and environmental conditions (Lee et al. 2008). Several studies described that the concentration of some PSP toxin analogues in bivalves, but not all of them, can be reduced by exposing contaminated shellfish to a non-toxic diet (Reis Costa et al. 2018). Nevertheless, mitigating or modulating the presence of microalgae in the field is currently not possible, so this eventual solution should be applied by maintaining large stocks of shellfish in a closed space for several days, and the feasibility of this would be dubious.

Once harvested, toxin reduction or elimination from shellfish is mainly affected by the chemical properties of the toxins. In the particular case of PSP toxins, a regulation was published after performing scientific studies which proved that a suitable heat treatment decreased the levels of PSP toxins and guaranteed the safety of the cockle *Acanthocardia tuberculata* (Berenguer et al. 1993; EC 1996).

A detoxification procedure would result in an economically feasible solution for a shellfish canning industry in locations where PSP toxic episodes occur very often or are persistent, and large amounts of shellfish are affected. Besides, in view of the changing environmental conditions related to climate change, a rise in the incidence of these episodes could take place in the near future (Barbosa et al. 2019). Changes in the profiling and behavior of PSP toxic episodes, leading to lower toxicity values but longer toxic episodes have been proposed (Braga et al. 2018). It is important to mention that it would not be necessary to perform important modifications in factory installations to accomplish the PSP detoxification protocol. The required equipment is the same usually employed by the canning industry and factories applying this protocol do actually exist in the case of giant cockle. If a regulation for this detoxification protocol was finally approved, the importance of such modifications will depend on each individual factory and the decision to implement it or not would be due more to economic than technical reasons. Only the duration of the whole thermal process would be increased.

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4. Experimental design

4.1 Sampling of contaminated mussels, clams and scallops

Samples were obtained from different sampling points along the Spanish and Portuguese coasts from July 2018 to March 2019. Mussels (*Mytilus galloprovincialis*) were acquired from several mussel raft cultures in: a) Galicia, (samples coming from two different floating rafts in the Ría of Vigo, Pontevedra); b) Andalucía, (one batch of mussels from Benalmádena, Málaga), and c) Portugal, (one batch of mussels from Portinho da Costa, near Lisbon). In addition, other mussel batches were obtained in Catalonia, one sample, after exposure to a toxic bloom of the dinoflagellate *Alexandrium minutum*, inside a harbour, as explained below. All this information, as well as the phytoplanktonic species involved in the naturally contaminated batches of shellfish and results obtained are depicted in Table 1. Special permissions from the local authorities were obtained in order to harvest the toxic molluscs from the closed areas. Two batches of Japanese littleneck clams (*Ruditapes philippinarum*) were obtained from Pontevedra, Galicia (Spain) and both batches of scallops (*Pecten maximus*) were obtained from Málaga, Andalucia (Spain). Sampling zones where toxic mussels, clams and scallops were harvested are depicted in Figure 1.

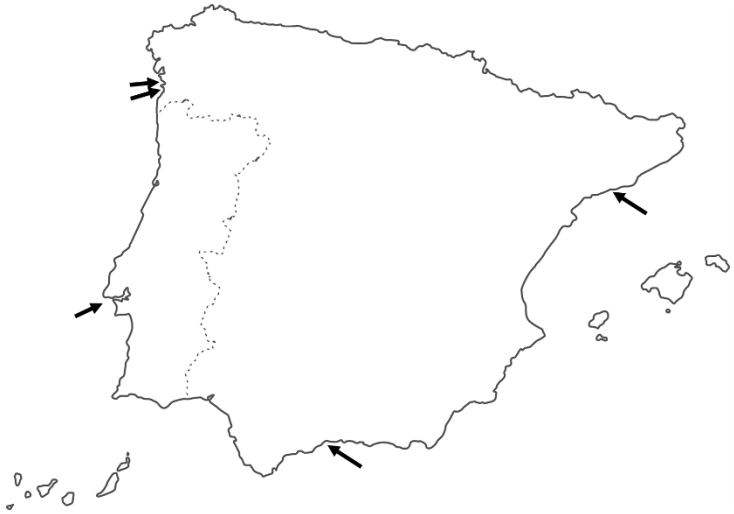

Fig 1: sampling points (marked by arrows) where PSP contaminated mussels and scallops were obtained during the study.

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Table 1. Origin and date of harvesting of live PSP contaminated mussels and scallops. Results of PSP toxins (mean values ± standard deviation, n=2) in raw bivalves analysed by HPLC-FLD, at both laboratories.

Species	Location	Harvesting date	Phytoplankton present	Average result (µg STX diHCl equiv/kg) (n=2)
Mussel (*Mytilus galloprovincialis*)	Ría of Vigo (Vigo A)	09/07/2018	*Alexandrium spp*	1072 ± 11
Mussel (*M. galloprovincialis*)	Ría of Vigo (Redondela C)	23/07/2018	*Alexandrium spp*	1604 ± 330
Mussel (*M. galloprovincialis*)	Ría of Vigo (Redondela C)	23/07/2018	*Alexandrium spp*	737 ± 134
Mussel (*M. galloprovincialis*)	Andalucía (Benalmádena)	02/08/2018	*Gymnodinium catenatum*	812 ± 270
Mussel (*M. galloprovincialis*)	Portinho da Costa (Lisbon)	22/10/2018	*Gymnodinium catenatum*	9001 ± 345
Mussel (*M. galloprovincialis*)	Catalonia	05/03/2019	*Alexandrium minutum*	4205 ± 43
Mussel (*M. galloprovincialis*), frozen	Catalonia	05/03/2019	*Alexandrium minutum*	2317 ± 261
Clam (*R. philippinarum*)	Ría of Pontevedra	27/07/2018	*Alexandrium spp*	1041 ± 23
Clam (*R. philippinarum*), frozen	Ría of Pontevedra	27/07/2018	*Alexandrium spp*	903 ± 204
Scallop (*Pecten maximus*)	Andalucía	22/08/2019	*Gymnodinium catenatum*	3232 ± 466
Scallop (*P. maximus*) eviscerated	Andalucía	22/08/2019	*Gymnodinium catenatum*	1976 ± 117
Scallop (*P. maximus*)	Andalucía	22/08/2019	*Gymnodinium catenatum*	3171 ± 30
Scallop (*P. maximus*), eviscerated	Andalucía	22/08/2019	*Gymnodinium catenatum*	1779 ± 126

Samples were refrigerated in thermally isolated boxes with cold accumulators after collection and shipped to the laboratory. Upon arrival, samples were processed as indicated in "Detoxification study" and analyzed as described below. Some subsamples of the different batches of mollusks were frozen at -20 °C and processing and analysis was performed after days or weeks until a maximum of 10 weeks.

4.2 Mussel exposure to a toxic bloom of the dinoflagellate Alexandrium minutum

A controlled field study was carried out in the Catalonian coast exposing 50 kg of mussel for 5 days to a toxic bloom of *Alexandrium minutum*. The objective was to allow high levels of PSP toxins to bioaccumulate in the mussels. Levels of *A. minutum* were always above 200000 cells/L and, as a result, the concentration of PSP toxins in mussels was higher than 4000 µg STX diHCl equiv/kg.

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4.3 Procedure for PSP mussels, clams and scallops detoxification

The regulated procedure (EC 1996), was applied to all different batches of PSP naturally contaminated mussels, clams and scallops with some modifications:

- Preliminary cleaning in running fresh water for two minutes.
- Pre-cooking in fresh water for three minutes at a temperature of 95 ± 5 °C.
- Separation of flesh and shells.
- Second cleaning in fresh water for 30 seconds.
- Cooking in fresh water for nine minutes at a temperature of 98 ± 5 °C.
- Cooling in running fresh water for approximately 90 seconds.
- Conditioning in containers closed hermetically in a non-acidified liquid medium.
- Sterilization in autoclave at 116 °C for 51 min (referred as "Canning") or Pasteurization at 90 °C for 10 min.

Separation of the edible parts (foot) from the non-edible parts (gills, viscera and mantle), in mussels and clams, was omitted in order to increase the yielding of the process. In the case of scallops, edible parts is the sum of adductor muscle and roe. During the different cleaning steps, mollusk flesh was submerged in fresh water, including a last rinse step. To facilitate toxin analysis, drinking water was employed as covering sauce, since the habitual covering medium used in processed *A. tuberculata* products (brine) can interfere with HPLC columns. Samples subjected to the detoxification method are identified along the text as "EC". Aliquots of the same batches of mussels, clams or scallops were sterilized or pasteurized without applying the detoxification procedure and are identified along the text as "normal". Table 2 shows the different treatments applied to mollusks along the work.

Table 2 keys used in figure legends along the text

Key word	Use of PSP detoxification procedure	Thermal processing
Raw	No	None.
Normal Canning	No	116 °C, 51 min
Normal Pasteurization	No	90 °C, 10 min
EC Canning	Yes	116 °C, 51 min
EC Pasteurization	Yes	90 °C, 10 min

4.4 Toxin extraction

Two laboratories, ANFACO and IRTA, were involved in the extraction and the analysis of PSP toxins in the samples, either processed or not. Both laboratories performed the same extraction and analysis protocol described below, only with variations related to the chromatographic columns and LC equipment used.

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4.5 Extraction, clean-up, hydrolysis and oxidation

The method was based on the HPLC-FLD Official Method (AOAC 2005; Lawrence et al. 2005), and refined as described by Turner et al. and Ben-Gigirey et al. (Turner et al. 2009; Ben-Gigirey et al. 2012). The method involves an acetic acid extraction through clean-up with SPE C18 cartridge extraction followed by periodate oxidation and analysis by HPLC-FLD. If the presence of any toxin is observed, peroxide oxidation and/or fractionation (F1, F2, F3) are then carried out by using COOH ion exchange SPE cartridges with periodate oxidation, injecting the obtained extracts in the HPLC-FLD.

4.6 PSP toxin quantitation

The toxins dcGTX2,3, C1,2, dcSTX, GTX2,3, GTX5, STX were quantified in the C18 extract after peroxide oxidation; GTX1,4 and GTX6 in F2 fraction; NEO and dcNEO in F3 fraction and C3,4 in F1 hydrolizated fraction after periodate oxidation.
Total PSP toxin content, expressed as STX diHCl equivalents/kg, is calculated by summing individual toxin concentrations and applying toxicity equivalents factors (TEF) that are established for each toxin according to EFSA Scientific Opinion (EFSA 2009).
In the samples where no PSP toxins has been detected (below LODs), the histograms were left blank.

4.7 HPLC-FLD equipment and chromatographic conditions

PSP toxins analyses, at ANFACO, were carried out using an HPLC Alliance 2695 model and fluorescence detector 2474 model (Waters Corporation). A XSelect CSH C18 3.5 µm, 4.6 mm x 150 mm column and a XSelect CSH C18 3.5 µm, 3.9 mm x 5 mm precolumn from Waters were used. Chromatography conditions are described in the AOAC Method (Lawrence et al. 2005).

At IRTA, PSP toxins analyses were carried out using an UPLC Acquity H-Class model and FLR Acquity fluorescence detector (Waters Corporation). A Kinetex C18 4.5 µm, 4.6 x 150 mm column and a XSelect CSH C18 4.5 µm guard column from Phenomenex were used. Chromatography conditions used are those described in the rapid method by Hatfield et al. (Hatfield et al. 2016).

4.8 Method performance

The method acceptability criteria were selected to ensure the performance of the method, according to the International Organization for Standardization (ISO) 17025:2005 standards and the screening and semi-quantitation of PSP toxins EURLB-SOP quality requirements (EURLMB 2019). The minimum performance criteria were checked out throughout the study such as retention time deviation ± 0.2 min, peak area

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deviation (RSD ≤3.0%), linearity (R^2 ≥0.98), sensitivity (individual toxin LOD should be equal or lower than 1:20th of regulatory level), precision intra-batch ≤20% and inter-batch ≤25%.

5. Results and Discussion

The different batches of cultivated mussels (*M. galloprovincialis*), clams (*R. philippinarum*) and scallops (*P. maximus*), origin and sampling place, toxic phytoplankton involved, date of harvesting and analytical results initially obtained in the raw mollusks, are summarized in Table 1. In this table, mean values ± standard error of the mean (SEM) obtained for each sample analyzed by both laboratories are included. The different batches were split and samples were processed by the different thermal treatments as described above. A standard canning, a standard pasteurization, as usually performed in an industrial situation, and the detoxification procedure followed by canning or pasteurization were carried out. These treatments are referred, respectively, as Normal canning, Normal pasteurization, EC canning, EC Pasteurization. Normal pasteurization was not performed in all the batches, so in those cases, it is not depicted in the corresponding figure (Figs 3, 5, 6, and 9). The different keys used in figure's legend are summarized in Table 2. The HPLC results obtained by both laboratories showed good agreement, and good correlation was obtained, as shown in Figure 2.

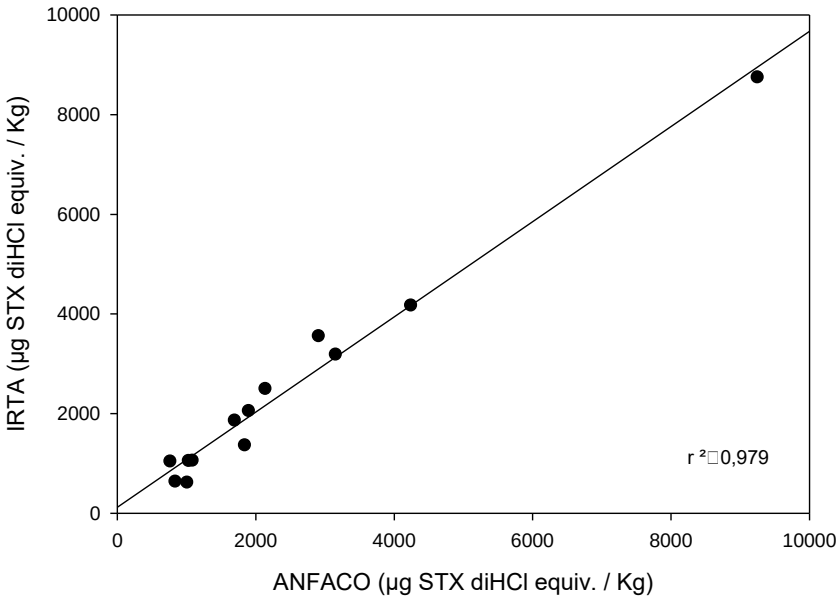

Fig 2: Correlation chart for the total content of PSP toxins present in raw bivalves set analyzed by HPLC-FLD (µg STX diHCl equiv/kg) (n=13) showing a good correlation between results obtained at ANFACO and IRTA laboratories.

Some of the most representative results, either as a total toxicity or as the different PSP analogues, are displayed next. Fig 3A, shows the levels of PSP toxins, expressed as µg STX diHCl equiv/kg (global toxicity), in raw and thermally processed mussels harvested during an Alexandrium bloom from Redondela, Galicia.

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Results show that the normal canning procedure, as well as the application of the detoxification process (EC) followed by sterilization or pasteurization, were able to decrease PSP levels below the limit of detection when applied to raw mussels containing around 1604 µg STX diHCl equiv/kg.

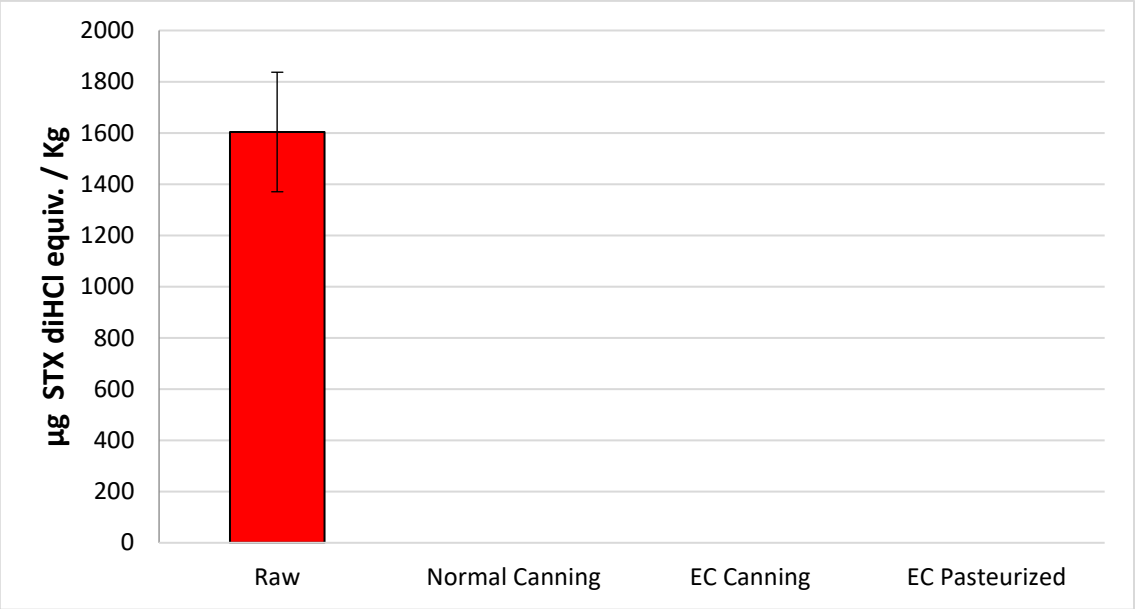

Fig 3A. PSP toxins (global toxicity) in naturally contaminated mussels from Redondela, NW Spain analyzed by HPLC. Mean values (n=2) are represented, and vertical bars indicate the SEM.

Fig 3B shows the quantification of the different PSP analogues of samples from Fig 3A, expressed as STX diHCl equiv/kg. It is worth to mention that in the sample, GTX1,4 was the dominant toxin. Very low concentration of other analogues, such as dcSTX, GTX2,3 and C3,4 toxins were detected as well. Similar results were obtained in another batch of mussels from the same location and bloom with a PSP concentration near the legal limit, 800 µg STX diHCl equiv/kg due to GTX1,4; C3,4 and GTX2,3, where no PSP toxins were detected after processing (not shown).

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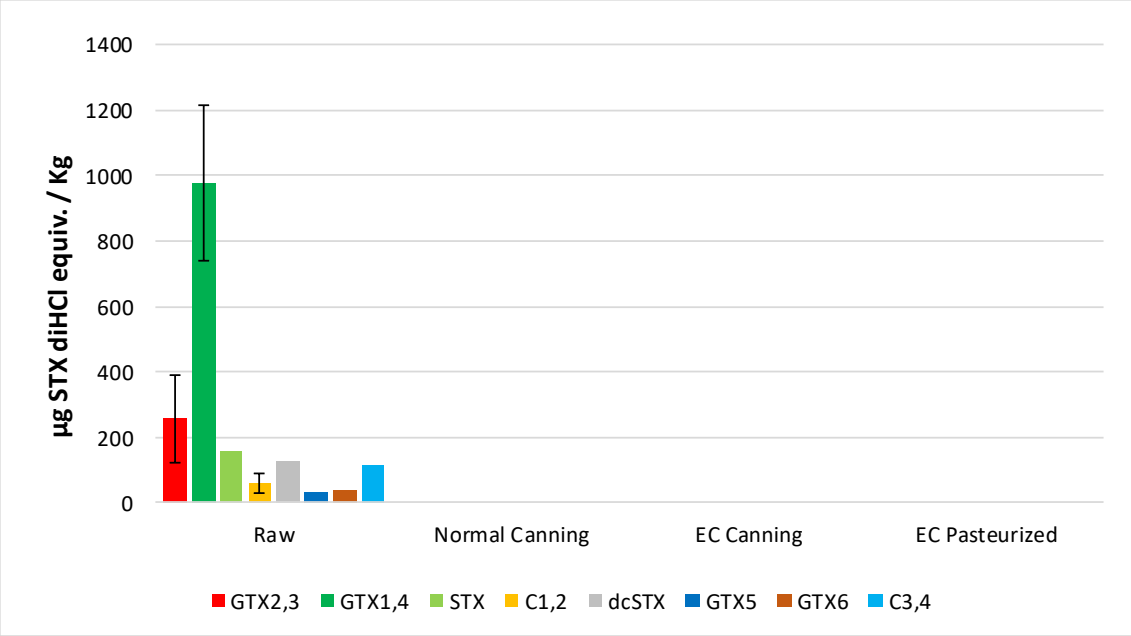

Fig 3B. PSP toxins in naturally contaminated mussels from Redondela, NW Spain. Figure shows all the PSP analogues detected in each sample, raw or processed under different conditions. Mean values (n=2) are represented, and vertical bars indicate the SEM.

Similar results were obtained in the batch of mussels from Catalonia containing a final concentration of 4206 µg STX diHCl equiv./kg (Fig 4A). It is worth mentioning that, the same sample, after frozen storage at -20ºC for 3 weeks, showed a decrease in toxicity to 2318 µg STX diHCl equiv/kg (data not shown). Both treatments, the normal sterilization and the detoxification protocol, followed by sterilization or pasteurization, produced a significant decrease in PSP levels, below the legal limit (t-Student, $p < 0.05$).

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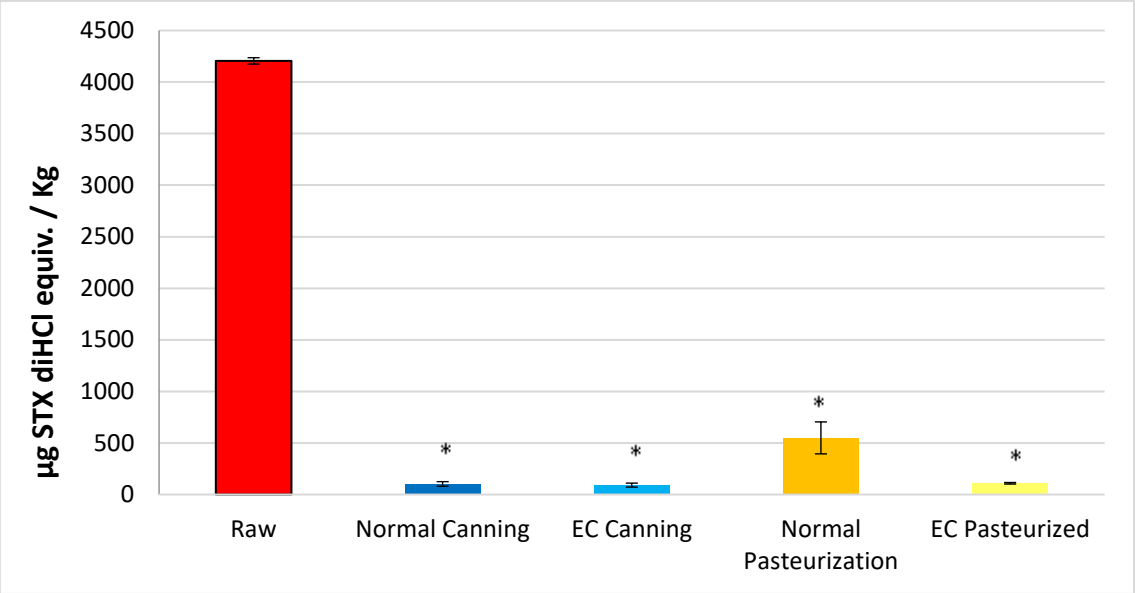

Fig 4A. Naturally contaminated mussels after controlled immersion into an area with *A minutum* in Catalonia, Spain analyzed by HPLC (global toxicity). Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the global toxicity between raw and the four thermal treatments (t-Student, $p < 0.05$).

The raw sample of immersed mussels coming from Catalonia contained several toxins of the group, mainly GTX1,4 and GTX2,3. STX was the dominant analogue in all four processed samples, and was not present in the raw sample. This fact suggests that a transformation to STX takes place due to the thermal process (Figure 4B).

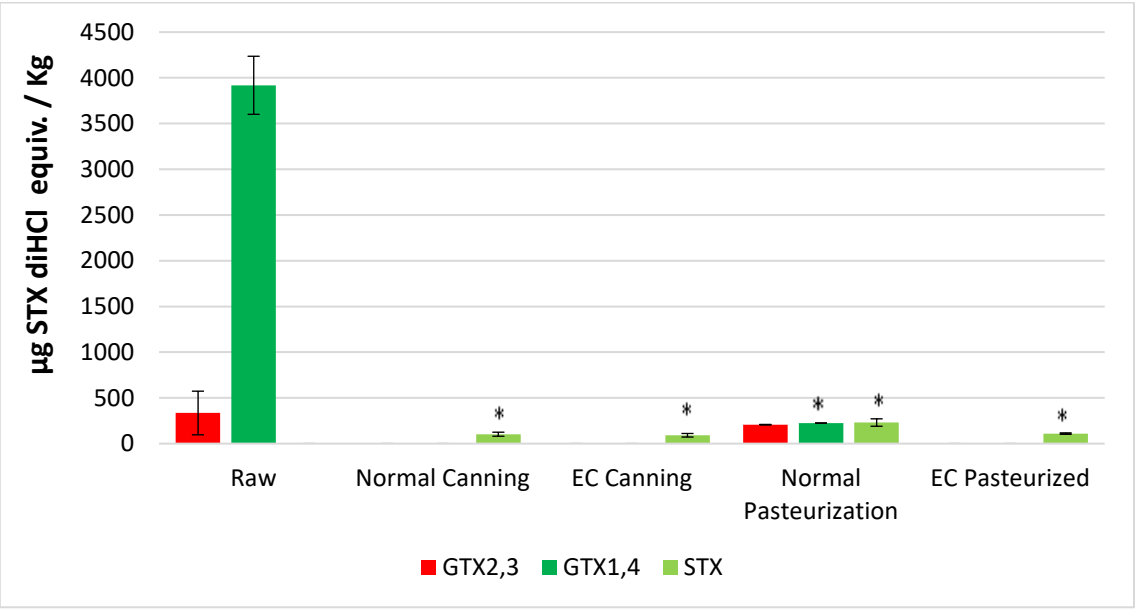

Fig 4B. PSP toxins in naturally contaminated mussels after controlled immersion into an area with *A minutum* from Catalonia, Spain, fresh sample. Figure shows all the PSP analogues detected in each sample, raw or processed under different conditions. Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the toxins profile between raw and the four thermal treatments (t-Student, $p < 0.05$).

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A new batch of mussels with an extremely high concentration of PSP toxins was harvested from Portugal during a *Gymnodinium catenatum* bloom, as shows Fig 5A. In this case, mussels exposed to the toxic episode for a long time, presented a huge toxin concentration (9000 µg STX diHCl equiv/kg), exceeding more than 10 times the legal limit. Although after application of the detoxification procedure, PSP toxins concentration decreased in a significant way (t-Student, $p < 0.05$), reaching 90 % of detoxification, no safe products were attained in this case. PSP concentration in mussels elaborated with the detoxification protocol and then canned was 1054±33 µg STX equiv/kg, higher than the legal limit, whereas those samples pasteurized after the detoxification procedure showed lower PSP concentrations (783±183 µg STX diHCl equiv/kg), which was a surprising finding.

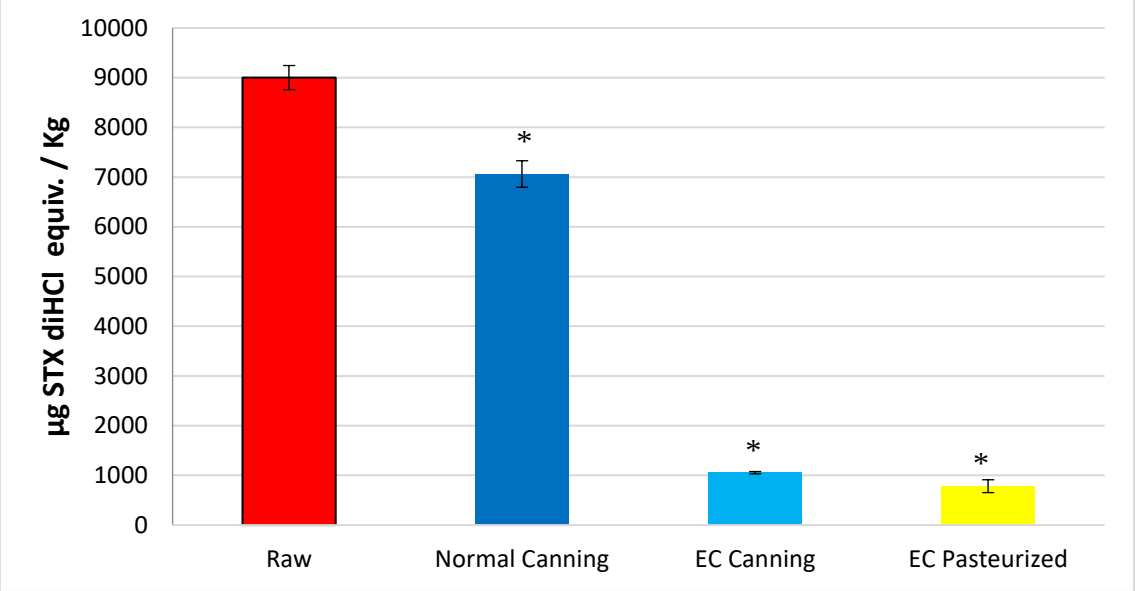

Fig 5A. Naturally contaminated mussels from Lisbon, Portugal, analyzed by HPLC (global toxicity). Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the global toxicity between raw and the three thermal treatments (t-Student, $p < 0.05$).

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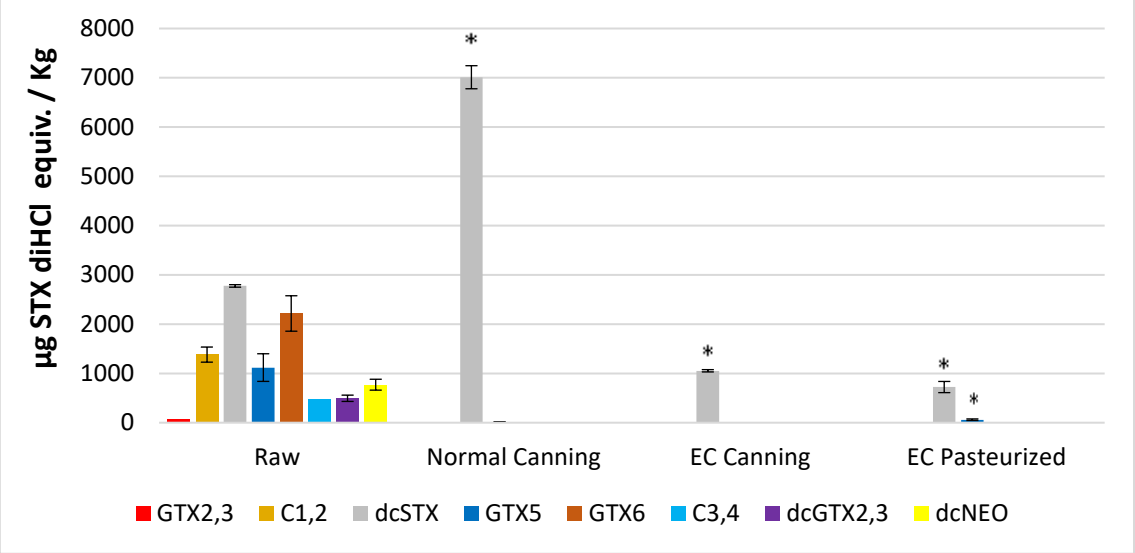

Fig 5B. Naturally contaminated mussels from Lisbon, Portugal, analyzed by HPLC. Figure shows all the PSP analogues detected in each sample, raw or processed under different conditions. Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the toxins profile between raw and the three thermal treatments (t-Student, $p < 0.05$).

Fig 5B shows all PSP analogues identified in the samples represented in Fig 5A. The raw sample contained several toxins of the group, mainly dcSTX; GTX6; GTX5, C1,2 and dcGTX2,3. Again, dcSTX was the dominant analogue in the processed samples, even at higher levels than in the raw sample after "Normal Canning". This fact suggests that a transformation of other toxins to dcSTX takes place due to the thermal process.

The analysis of contaminated clams and the products obtained after processing offered similar results to those obtained in mussels. Fresh raw clams obtained from Pontevedra (NW of Spain) during an *Alexandrium* spp bloom showed a PSP concentration of 1041 ± 22 µg STX diHCl equiv/kg (Fig 6A). The same sample after frozen storage at -20ºC for 7 weeks, contained 903 ± 204 µg STX diHCl equiv/kg (data not shown). Application of both treatments: the normal sterilization procedure and the detoxification protocol followed by sterilization or pasteurization, produced a decrease in PSP levels to not detectable levels (Fig 6B).

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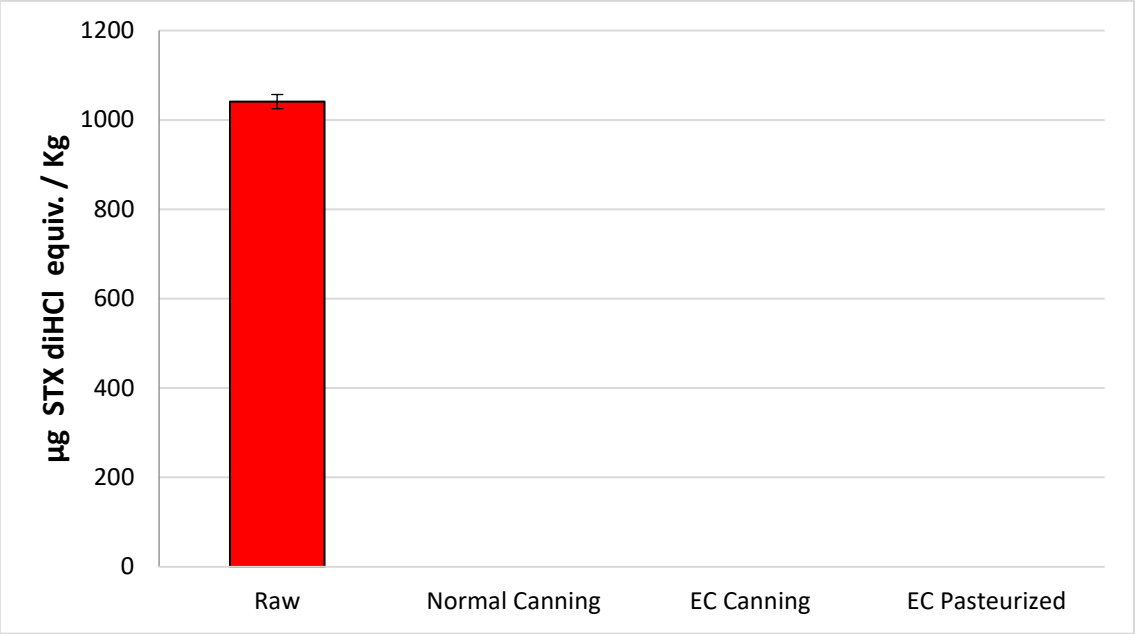

Fig 6A. PSP toxins in naturally contaminated clams from Pontevedra, NW Spain analyzed by HPLC (global toxicity). Mean values (n=2) are represented, and vertical bars indicate the SEM.

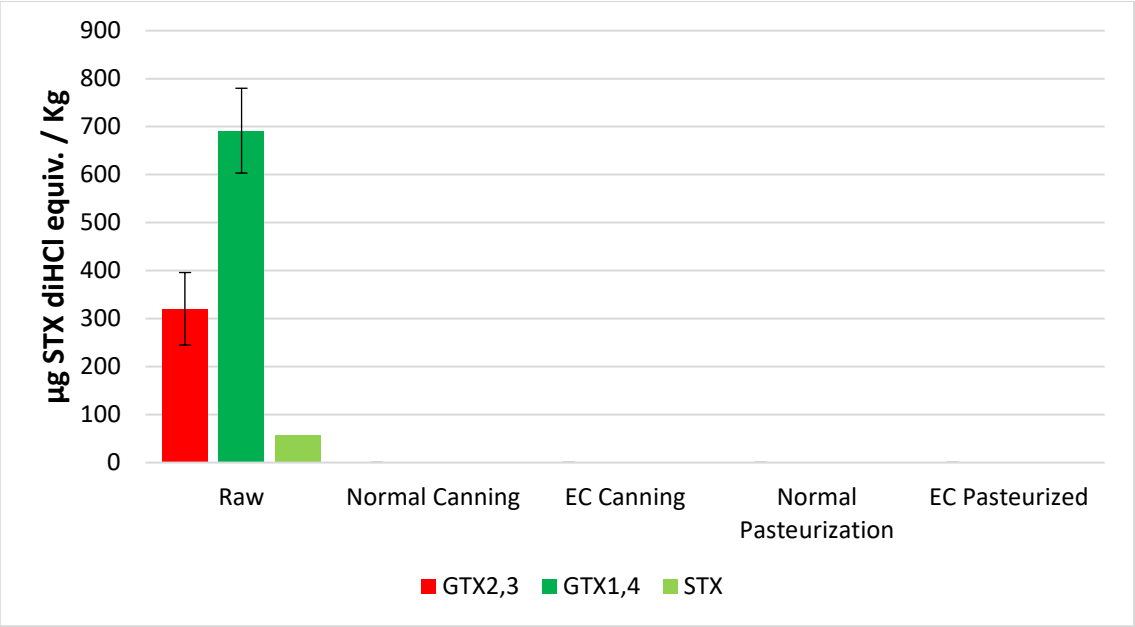

Fig 6B. Naturally contaminated clams from Pontevedra, Galicia, Spain, analyzed by HPLC. Figure shows all the PSP analogues detected in each sample, raw, eviscerated or processed under different conditions. Mean values (n=2) are represented, and vertical bars indicate the SEM.

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In addition to mussels and clams, scallops contaminated with PSP were harvested during a *Gymnodinium catenatum* bloom. In this species, evisceration of raw scallops reduced significantly PSP concentration, as expected (t-Student, $p < 0.05$) (Fig 7A). Evisceration was performed previously to all the thermal processes applied and reduced drastically all the congeners to non-detectable levels, in canned samples (with or without detoxification protocol). Also, it reduced to levels well below the legal limit in pasteurized samples. The different analogues detected in the raw mollusk, whole body or eviscerated were: dcSTX, GTX5, GTX2,3; STX; C1,2; and dcGTX2,3). Mostly dcSTX (327±69 µg STX diHCl equiv/kg) and, in a much lower level, GTX5 (28±9 µg STX diHCl equiv/kg) were detectable when pasteurization was carried out after a conventional pre-cooking step. In addition, only dcSTX was detectable to a lower level (144±19 µg STX diHCl equiv/kg) when pasteurization was carried out after the detoxification protocol (Fig 7B).

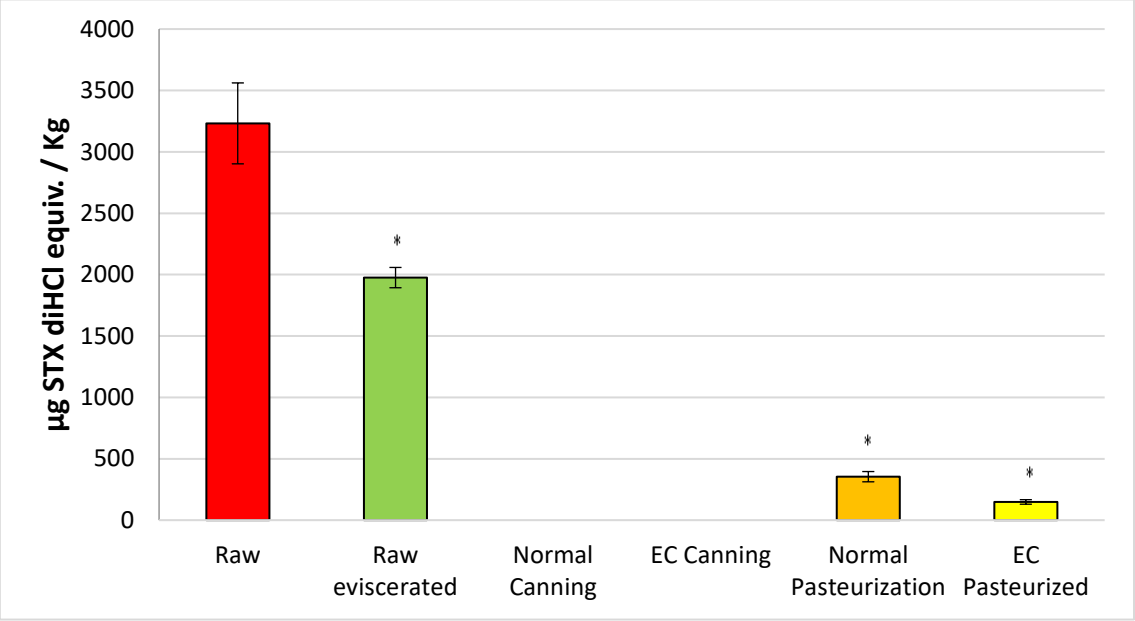

Fig 7A. Naturally contaminated scallops (*Pecten maximus*) from Marbella, Andalucía, Spain (global toxicity). Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the global toxicity between raw, raw eviscerated and the four thermal treatments (t-Student, $p < 0.05$).

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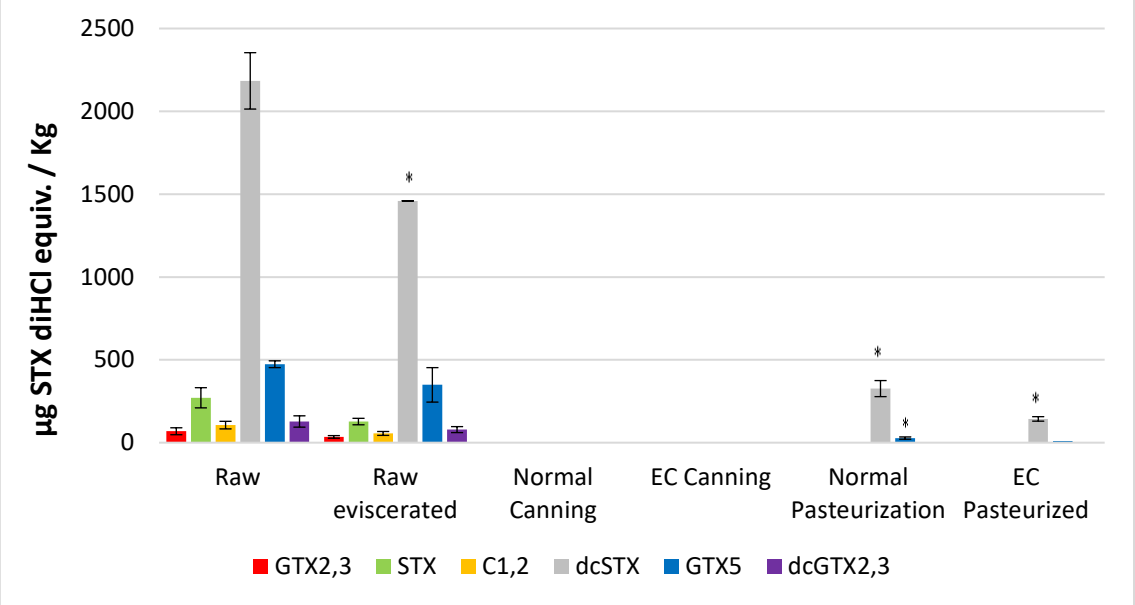

Fig 7B. Naturally contaminated scallops from Marbella, Andalucía, Spain, analyzed by HPLC. Figure shows all the PSP analogues detected in each sample, raw, eviscerated or processed under different conditions. Mean values (n=2) are represented, and vertical bars indicate the SEM. Asterisk (*) means significant differences for the toxins profile between raw, raw eviscerated and the four thermal treatments (t-Student, $p < 0.05$).

6. Conclusions

In conclusion, an efficient and inexpensive "detoxification procedure" can be applied in PSP contaminated mussels, clams and scallops to decrease PSP toxins below the legal limit (800 µg STX diHCl equiv/kg). However, a maximum threshold level in raw material should be previously established to define if the processing will efficiently reduce PSP toxins below the legal limit. Based on our data, a theoretical value of 5300 µg STX diHCl equiv/kg, based on the percentage of detoxification, would be the highest level. Although it is still necessary that the industry should proceed with quality controls of the final product to ensure that it responds to the legal requirements and levels of PSP toxins are safe, in the same way as stated in the reference legislation for *Acanthocardia tuberculatum*.

These data have been published in Food and Chemical toxicology as *Ana G. Cabado, Jorge Lago, Virginia González, Lucía Blanco, Beatriz Paz, Jorge Diogène, Laura Ferreres, Maria Rambla-Alegre, Detoxification of paralytic shellfish poisoning toxins in naturally contaminated mussels, clams and scallops by an industrial procedure, Food and Chemical Toxicology, Volume 141, 2020, 111386,* https://doi.org/10.1016/j.fct.2020.111386

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7. Legislative proposal

…….: Commission Decision of ……. establishing the conditions for the harvesting and processing of certain bivalve molluscs coming from areas where the paralytic shellfish poison level exceeds the limit laid down by REGULATION (EC) No 853/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs

Article 1

This Decision applies only bivalve molluscs: mussels, clams and scallops

Article 2

1. Member States can authorize the harvesting of the bivalve molluscs referred to in Article 1 in production areas where the PSP level in the edible parts of these molluscs is higher than 800 µg equiv. STX diHCl/kg but lower than 5000 µg equiv. STX diHCl/kg.

The harvested molluscs must:

- be transported in containers or vehicles sealed by the competent authority, directly to an approved establishment, especially authorized to carry out their treatment,

- be accompanied by a document issued by the competent authority which authorizes the transport, attesting to the nature and quantity of the product, area of origin and plant of destination,

- be subjected to the heat treatment defined in the Annex to this Decision.

2. The products resulting from the molluscs referred to in paragraph 1 must be analysed in order to confirm that they do not contain a PSP level over 600 µg equiv. STX diHCl/kg. Analysis must be performed by the methodologies allowed by COMMISSION REGULATION (EC) No 2074/2005 after the application of the heat treatment. Each batch shall be tested. Analysis must be performed by laboratories accredited under ISO 17025.

Article 3

The competent authority shall check that the HACCP records drawn up and implemented by the person responsible for the establishment authorized for this thermal treatment defined in the Annex to this Decision.

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Heat treatment applicable to bivalve molluscs with the objective of reducing the PSP toxin to a level lower than 800 µg equiv. STX diHCl/kg.

Molluscs have to undergo the following operations sequentially:

1. Preliminary cleaning in fresh water for a minimum of two minutes at a temperature of 20 °C, plus or minus 2 °C.

2. Pre-cooking in fresh water for a minimum of three minutes at a temperature of 95 °C, plus or minus 5 °C.

3. The separation of flesh and shells.

4. Second cleaning in running fresh water for a minimum of 30 seconds at a temperature of 20 °C, plus or minus 2 °C.

5. Cooking in fresh water for a minimum of nine minutes at a temperature of 98 °C, plus or minus 3 °C.

6. Cooling in running cold fresh water for approximately 90 seconds.

7. The separation of the edible parts (foot) from the non-edible parts (gills, viscera and mantle) mechanically with water pressure. This step is not mandatory for mussels and clams.

8. Conditioning in containers closed hermetically.

9. Sterilization in autoclave at a minimum temperature of 116 °C for a time calculated according to the dimension of the containers used but which cannot be lower than 51 minutes. Alternatively, a pasteurization treatment at a minimum temperature of 90 °C for a time calculated according to the dimension of the containers used but which cannot be lower than 10 minutes.

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