Rapid screening: Enzymatic biosensor for fast screening of Xenobiotics in seafood





SUMMARY

Xenobiotics are environmental contaminants largely produced during industrial processing or synthetised for use in consumer goods. These compounds can accumulate in fish and seafood and pose a health risk to consumers and the environment. In order to protect human health and the marine environment, authorities have established acceptable concentrations for some of these compounds, and official reference methods to monitor them. At European Level, legal limits have been established for Polycyclic Aromatic Hydrocarbons (PAHs) (Commission Regulation (EU) No 1881/2006). However, only non-binding recommendations exist for several others, including Perfluorinated Compounds (PFCs) and Brominated Flame Retardants (BFRs). This knowledge output consists of a novel qualitative Enzyme Inhibition Screening Assay (extraction and assay protocols) for the detection of PAHs, PFCs and BRFs. The assays have been tested and validated for salmon and seabass samples.

KNOWLEDGE NEED

Current analytical methods for detecting and measuring xenobiotics are labour intensive, costly and require sophisticated laboratories, equipment and expertise. There is an urgent need for simple, specific, costeffective and fast screening methods that can be applied by seafood industry producers. Addressing this challenge, this output offers a novel solution based on enzyme inhibition technology, that can be applied by industrial endusers to detect PAH, BFRs and PFC in seafood.



RESULTS

In this study, enzyme inhibition assays and operating conditions have been optimised for application on seafood samples and sample treatment protocols for three target xenobiotics contaminant groups: PAHs, PFCs and BFRs. The two-step methodology includes a simple extraction protocol, followed by a novel fluorescence enzyme inhibition assay. Due to the different physico-chemical properties of target xenobiotics contaminants it was impossible to establish a common strategy for their extraction and analysis. Therefore, two different protocols have been optimised for polar (PFCs) and apolar (BFRs and PAHs) substances. Both protocols have been validated for application in two fish species: salmon (as a model for fatty fish) and sea bass (with less amount of fat). The sample extraction and assay can be performed with minimal professional training and laboratory equipment. The assay, which can be performed in 60 minutes, allows for the detection of multiple xenobiotics, to concentrations less than 10 μ g/kg of PAHs meeting current regulatory limits and between 90-500 μ g/kg of BFRs and PFCs, with proven precision and accuracy (less than 5% false negatives). If a sample tests positive, subsequent analysis by chromatography should be performed to identify the contaminant, however this method would reduce lag/response times significantly, compared to current methodologies. The technology has been evaluated and proven fit for purpose in salmon and seabass samples and will be commercialised as a kit for easy application by end-users. This method has the potential to help producers, and authorities, to control seafood safety hazards in products intended for consumption and thereby protect consumer health.

IMPACTS

This technology has significant potential to improve seafood safety in Europe. The method offers a quick solution to screen seafood for environmental contaminants and support producers in guaranteeing product safety in a low-cost manner. The method would have significant impact on several levels: increase the availability of safer seafood on the market, improving consumer trust and seafood consumption habits, and contributing to an improved health profile of the European population. The method could support an overall growth in the seafood sector, sustainability and profitability.

Contributes to the UN Sustainable Development Goal 12: Responsible consumption and production

END-USERS & APPLICATIONS

Research and Development community: scientists, researchers and academics working in the field of seafood safety can use the new knowledge to support further research on xenobiotics, including their regulation under new monitoring programme and supporting legislation.

• Industry – seafood producers/managers: further development at industrial level is needed to develop a commercial kit (work is currently underway) which would allow producers to apply the method and to ensure the production of safer seafood. This knowledge could also be applied by production site managers to manage contamination hazards and risks more effectively.

O Regulators/policy makers - EU food and national food safety authorities: new knowledge generated by this research can be used to support near-future EU regulation on xenobiotics, including the development of monitoring programmes to ensure the production and sale of safer seafood.

DISSEMINATION AND EXPLOITATION

Exploitation activities for seafood industry:

- Polycyclic Aromatic Hydrocarbon Screening Assay Protocol - video and flyer (developed by Biorex Food Diagnostics): seafoodtomorrow.eu/media
- Demonstration of the strategy at SEAFOOD^{томовкоw} demonstration workshops, in France, and Spain (February – March 2021) to support uptake and use of the method.
- Horizon Results Platform: please see seafoodtomorrow. eu/horizon-results-platform

Exploitation activities for policy makers:

• Managers, regulators and policy makers will be reached through the final **SEAFOOD^{TOMORROW}** event, info session, and a dedicated EU policy event.

Dissemination activities for all users:

- Project newsletter and news articles
- Promotion on Twitter and LinkedIn







INNOVATION STATUS

Technology Readiness Level 7 - system has been demonstrated in an operational environment **Patents and IPR:** This technology has been patented. Patent number: EP20383173 | Owner: AZTI



FUTURE RESEARCH

There is significant interest in this technology for the detection of PAHs, as they are currently regulated. Commercialising this solution in the form of a kit would provide end-users with the necessary reagents to perform the analysis. The stability of reagents (i.e. the enzymes) should be improved in order to increase shelf life and avoid the use of ultra-freezers. Nevertheless, the protocols are well-defined and can be used by anyone with the necessary equipment.

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