Session 1: Developments in analytical methods for micro- and nanoplastics

Definition of micro and nanoplastics & analytical challenges

Gilliland D [1]

[1] *European Commission, Joint Research Centre (JRC, IT)*

Abstract: Micro(nano)plastics are persistent pollutants whose increasing presence in land, water, food and even air may have the potential to produce harmful but poorly understood effects on the environment and human health. The sources of such materials are very varied but are generally classified into two main categories - primary microplastic which are polymeric particulates deliberately manufactured for use in products and secondary microplastics which are polymer fragments derived from the degradation of polymeric materials in use or by ageing and breakdown of bulk plastics in the environment. While the issue has been of environmental concern for many years there are continually increasing reports of micron and sub-micron sized (nanoplastics) polymer particulates directly in food products, in drinking water or released from food contact materials.

Irrespective of their source, small plastic particles are universal pollutants and research on the exposure and risks that they pose to man is ongoing but progress is highly dependent on the availability and quality of analytical methods. Micro and, above all, nanoplastic are difficult systems to analyse as their detection and quantification in real-world samples is very challenging. In fact, they combine the "classical" issues of accurate chemical detection and quantification with the need to properly measure the size (or better, the particle size distribution) of particles within a potentially very large size range from few millimetres to nanometres. In addition, they can be highly heterogeneous in terms of both chemical composition, size and even shape.

In the following presentation, consideration will firstly be given to the current understanding, both general and legislative, of what constitutes a microplastics after which an overview will be given of the current status of analytical methods for the detection and quantifications of this highly challenging class of materials.

Evaluation and optimization of extraction methods suitable for the analysis of microplastic particles occurring in the edible part of seafood

Süssmann J [1], Krause T [1], Martin D [1], Walz E [2], Greiner R [2], Rohn S [3,5], Fischer EK [4], Fritsche J [1]

[1] *Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish Products (DE),* [2]*Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Food Technology and Bioprocess Engineering (DE),* [3]*Universiity of Hamburg, Institute of Food Chemistry (DE),* [4] *University of Hamburg, Center for Earth System Research and Sustainability (CEN, DE),* [5] *Technische Universität Berlin, Institute of Food Technology and Food Chemistry, Department of Food Chemistry and Analysis (DE)*

Abstract: Findings of small plastic particles, called micro- (MP) or nanoplastics (NP), in the gastrointestinal tracts of aquatic organisms are reported frequently, including species used for human consumption (Hantoro et al., 2019). Due to a possible translocation of these particles from the gut into other tissues, MP might also be present in edible parts. Additionally, leaching and consecutive accumulation of additives or persistent organic pollutants adsorbed to the particle in the environment might occur. Therefore, an influence of MP on seafood and the human health might exist (Smith et al. 2018). However, for risk assessment besides assessment of the toxicity of MP, the occurrence of MP in the edible parts of seafood is an important prerequisite. Besides the low number of studies

analysing MP in the edible part of seafood no validated method for the assessment of MP in the edible parts of seafood currently exists. Therefore, this study was conducted to evaluate different approaches for extracting MP Ø ≥ 1 µm from the edible part of seafood considering for example digestion efficiency, sample preparation time, effort and costs in order to identify methods suitable for routine analysis. The preparation should be suitable for the most commonly used identification methods, including fluorescence microscopy, microspectroscopy (Infrared- and Raman-spectroscopy) and thermoanalytical methods (pyrolysis gas chromatography mass spectrometry (py-GC/MS)).

A literature research was conducted to determine the most promising extraction protocols for the isolation of MP from biota. The investigated protocols were selected depending on the overall time and effort of the method, the digestion efficiency and the corrosiveness towards plastics. Protocols using acidic, alkaline, oxidative, enzymatic as well as combinations thereof were taken into consideration. The protocols were applied with 10 g homogenized seafood matrix (fish fillet, the soft tissue of molluscs and crustaceans) to verify whether the filtration was possible with filters with a pore size of 1 µm. The polymer integrity of the most suitable and efficient protocol was tested by determining the plastic particle recovery (change in weight & size). In addition, the polymer identification was performed with py-GC/MS, FTIR- and Raman-spectroscopy before and after the digestion. Furthermore, the protocol was further optimized regarding digestion time and temperature, choice of filter material and post-digestive filter treatment. After optimization of the extraction protocol, precision and recovery were determined with seafood samples spiked with known amounts of fluorescent Nylon 12 (PA12)-particles (Ø approx. 20 µm). A qualitative detection of a mixture of polymers was tested with py-GC/MS.

The most suitable and efficient protocols were those based on the alkaline digestion with KOH according to Dehaut et al. (2016). By combining the alkaline digestion with a prior Pepsin digestion, the digestion temperature could be lowered to 37 °C, while the overall time for the complete extraction could be reduced to 6 h for most seafood sample matrices except for fish fillets with a fat content > 20 %. After reducing the digestion temperature for the alkaline treatment from 60 °C to 37 °C no adverse effects on all tested, commercially relevant polymers were examined with the exception of polyacrylonitrile. Particle integrity was analysed by FTIR- & Raman-spectroscopy as well as py-GC/MS and was not affected by the applied extraction procedure. In order to reduce analysis time with microscopic or spectroscopic methods, the application of smaller filter diameters was necessary. For the analysis of the complete filter with py-GC/MS, a smaller filter was required as well. Pre-filtration with polycarbonate (PC)-filters was the most applicable approach. However, contamination of the sample with PC from the filter occurred. A recovery of 88 % to 95 % of Ø 20 µm-PA12-particles was achieved for the lowest concentration level (15 – 20 particles per sample) with a reproducibility of 18 % to 29 %. As the particle number of the spiking solution itself could only be determined with a reproducibility of 17 % to 27 %, the reproducibility of the complete extraction procedure was deemed acceptable.

The evaluation of extraction protocols suitable for microplastic analysis in the edible part of seafood confirmed the applicability of proposals reported by Bessa et al. (2019) dedicated for the extraction of MP from biota. Introducing Pepsin into the digestion procedure lead to a quick breakup of the overall seafood sample structure. To avoid expensive enzymatic treatment costs fatty tissues were destroyed with KOH instead of a broad range of specific enzymes. Consequently, in contrast to the sole alkaline digestion described by Karami et al. (2017), the optimized seafood protocol developed in this study was able to digest 10 g to 100 g of a broad range of seafood species within 6 h instead of 72 h at a temperature of 37°C to 40°C while not affecting the integrity of most polymers. However, the seafood protocol is only of limited suitability for analysing polyacrylonitrile, which mostly derives from textile fibres. That polymer is significantly altered even at room temperature within the first 10 min of the alkaline treatment.

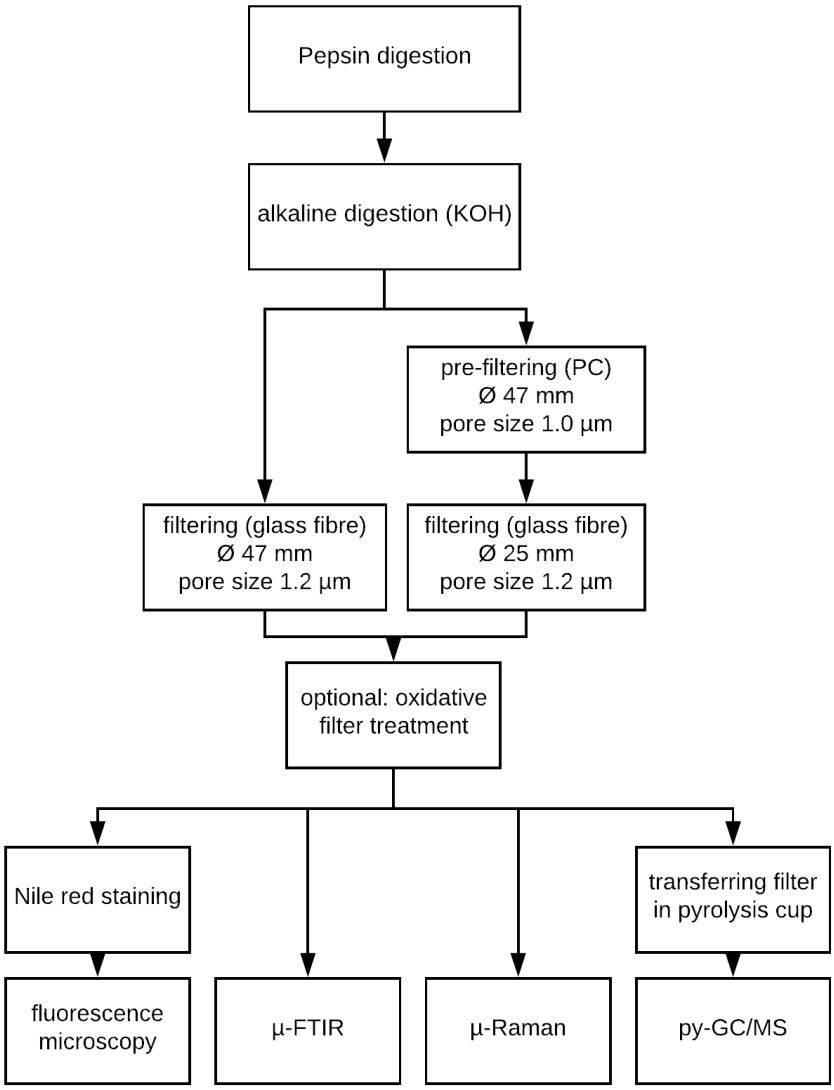

Figure 1: Overview of sample preparation for the analysis of MP in seafood with different analytical procedures.

Bibliography

Bessa et al. 2019. Harmonized protocol for monitoring microplastics in biota. JPI-Oceans BASEMAN project.

Dehaut A, Cassone AL, Frère L, Hermabessiere L, Himber C, Rinnert E, Rivière G, Lambert C, Soudant P, Huvet A, Duflos G, Paul-Pont I. 2016, Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ Pollut. 215:223-233.

Hantoro I, Löhr AJ, Van Belleghem FGAJ, Widianarko B, Ragas AMJ. 2019. Microplastics in coastal areas and seafood: implications for food safety. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 36(5):674-711.

Karami A, Golieskardi A, Choo CK, Romano N, Ho YB, Salamatinia B. 2017. A high-performance protocol for extraction of microplastics in fish. Sci Total Environ. 578:485-494.

Smith M, Love DC, Rochman CM, Neff RA. 2018. Microplastics in Seafood and the Implications for Human Health. Curr Environ Health Rep. 5(3):375-386.