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Ludovic Hermabessiere, Alexandre Dehaut, Anne-Laure Cassone, Laura Frère, Charlotte Himber, et al.. Identification of an optimized protocol for extraction and characterization of microplastics in seafood products. Journées des Doctorants et Post-Doctorants de l'ANSES, Oct 2016, Maisons-Alfort, France. anses-03889580

HAL Id: anses-03889580

https://anses.hal.science/anses-03889580

Submitted on 8 Dec 2022

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Identification of an optimized protocol for extraction and characterization of microplastics in seafood products

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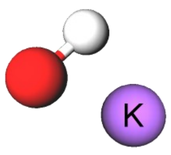
Introduction

Microplastics particles (<5 mm) are found in the Oceans worlwide and ingestion by marine species have been recently described. Microplastics can also represent a threat for human consumers through the consumption of seafood products. To date, a few studies have investigated the levels of contamination collected *in situ*. It is crucial for microplastics extraction to use solvent(s) or chemical(s) allowing efficient tissue dissolution without degrading plastic polymers. The purpose of this work was to benchmark six existing protocols on fifteen plastics polymers and assess their impact on polymers integrity coupled with an efficient digestion of biological matrices.

Material and methods

Experiment 1 : Testing 6 differents protocols on plastics integrity

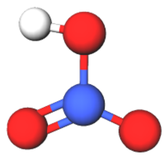




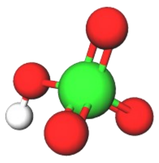
Protocol 1
KOH 10%, 60°C during 24h



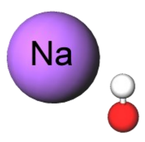
Protocol 2
Pepsin, 35°C during 2h



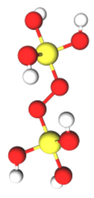
Protocol 3
HNO_3 65%, room temperature overnight then 60°C during 2h



Protocol 4
4:1 (v/v) HNO_3 65%:$HClO_4$ 65%, room temperature overnight then 100°C during 10min then dilution with 80°C water



Protocol 5
NaOH 10M, 60°C during 24h



Protocol 6
$K_2S_2O_8$ 0,27M et NaOH 0,24M, 65°C during 24h



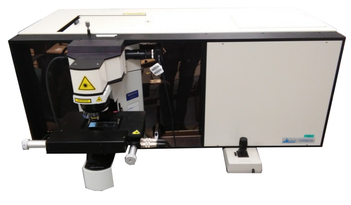
Microscopical observation



Weighting



Pyrolysis-GC/MS



Raman spectrometry

Fifteen tested families : high and low density polyethylene (HDPE & LDPE), polypropylene, (PP) expanded polystyrene (ePS), crosslinked polystyrene (PSXL), polystyrene (PS), poly(methyl-methacrylate) (PMMA), polylaurylactam (PA-12), polycarprolactam (PA-6), polyethylene terephtalate (PET), polyurethane (PUR), polytetrafluoroethylene (PFTE), unplasticized polyvinyl chloride (uPVC), polycarbonate (PC) and cellulose acetate (CA)

Experiment 2 : Application of selected protocols on seafood products



Black seabreams (digestive tract and filets)

and



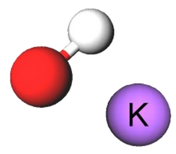
Mussel (whole content)

and



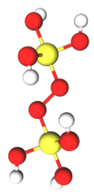
Velvet crab (whole content)

Protocol 1

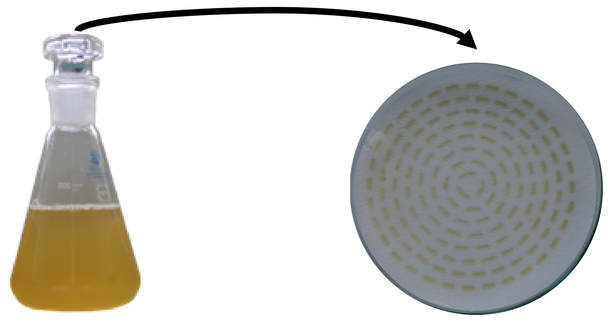


Or

Protocol 6



With 300 rpm of agitation



Calculation of digestion efficiency corresponding to the organic matter remaining after digestion

Results and discussion

Experiment 1

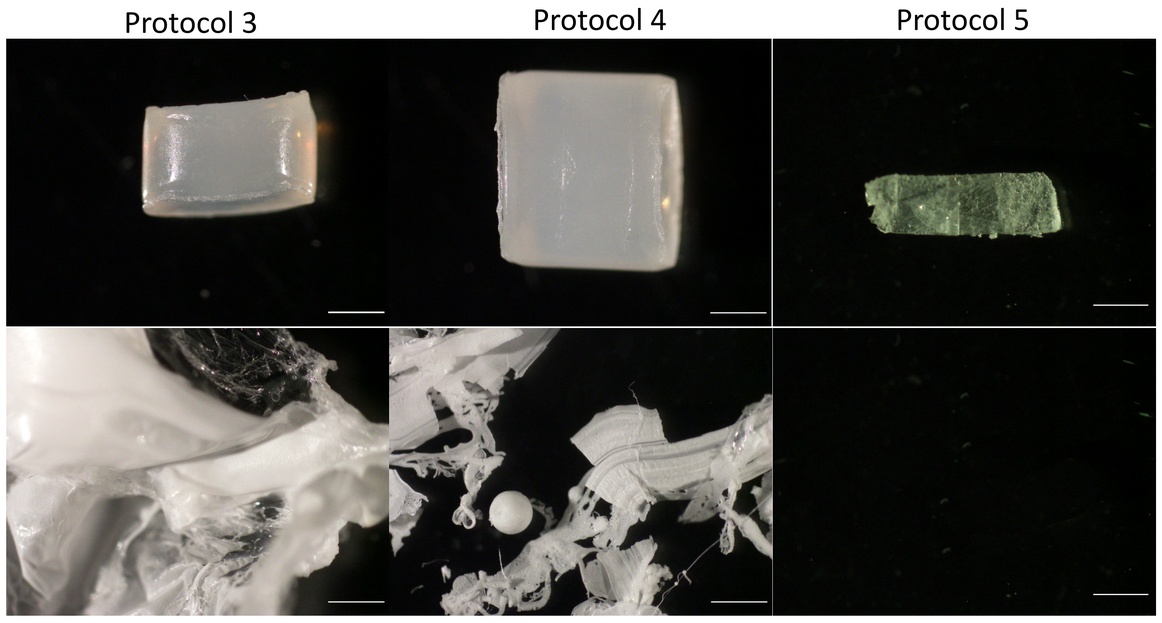


Figure 1 : Pictures of plastics degraded using protocol 3, protocol 4 and protocol 5. White bars represents 1mm

During experiment 1, with protocol 3 and 4, PA-12 plastic are partially degraded and with protocol 5 PC is totally degraded (Figure 1). Moreover, protocol 2 using pepsin is too expensive to be kept to be applied in field survey. Finally none of these protocols (2, 3, 4 and 5) were kept as promising protocols for the following experiment 2. Protocols 1 and 6 were used for experience 2 as they have no impact on plastic integrity and identification using Pyrolysis-GC/MS and Raman spectrometry.

Experiment 2

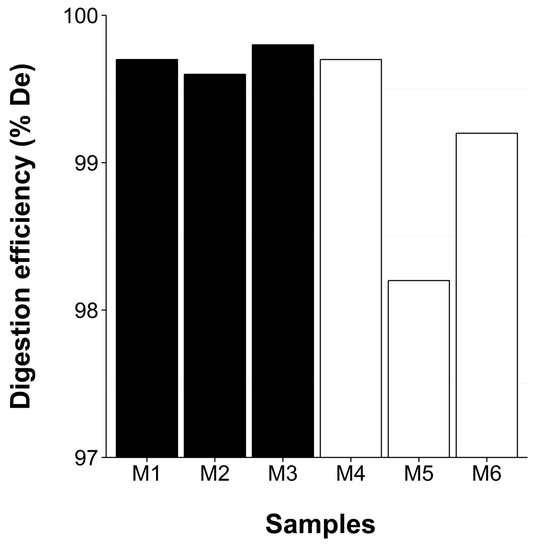


Figure 2 : Comparison of digestion efficiency for three musells treated with protocol 1 (M1 to M3) and with protocol 6 (M4 to M6)

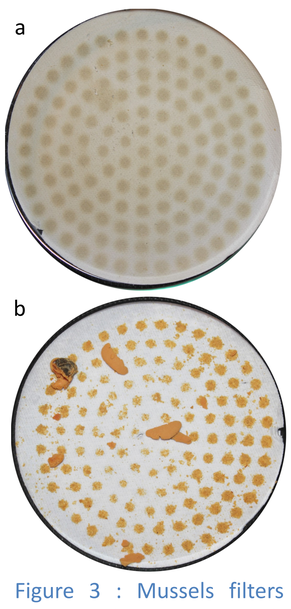


Figure 3 : Mussels filters after digestion with protocol 1 (a) and protocol 6 (b)

During experiment 2, protocol 6 did not efficently digest whole mussels (Figure 2) as pieces of mussels were still visible (Figure 3b). Protocol 1 allowed a good filtration and revealed negligible debris (Figure 3a) on the filter after digestion of mussels. Digestion of velvet crabs and black seabreams using protocol 1 led to easy filtration and observable filters.

Conclusion

Protocol 1 using KOH 10% at 60°C during 24h at 300rpm is proposed to be a suitable protocol for extraction and characterization of microplastics in seafood products. This protocol should be recommended by internationals bodies. The selected protocol is now apply in a field study to determine the prevalence of microplastics in the major seafood products consumed in France.

Acknowledgments

Ludovic Hermabessiere is thankful to Région Hauts de France—Nord Pas de Calais Picardie and Anses for the financial support of his PhD grant.