Analysis of small plastic particles in seafood

Evaluation and optimisation of sample preparation protocols

Julia Süssmann

Max Rubner-Institut

Federal Research Institute of Nutrition and Food

Plastic particles in seafood – How much do we eat?

environmental factors, but also…

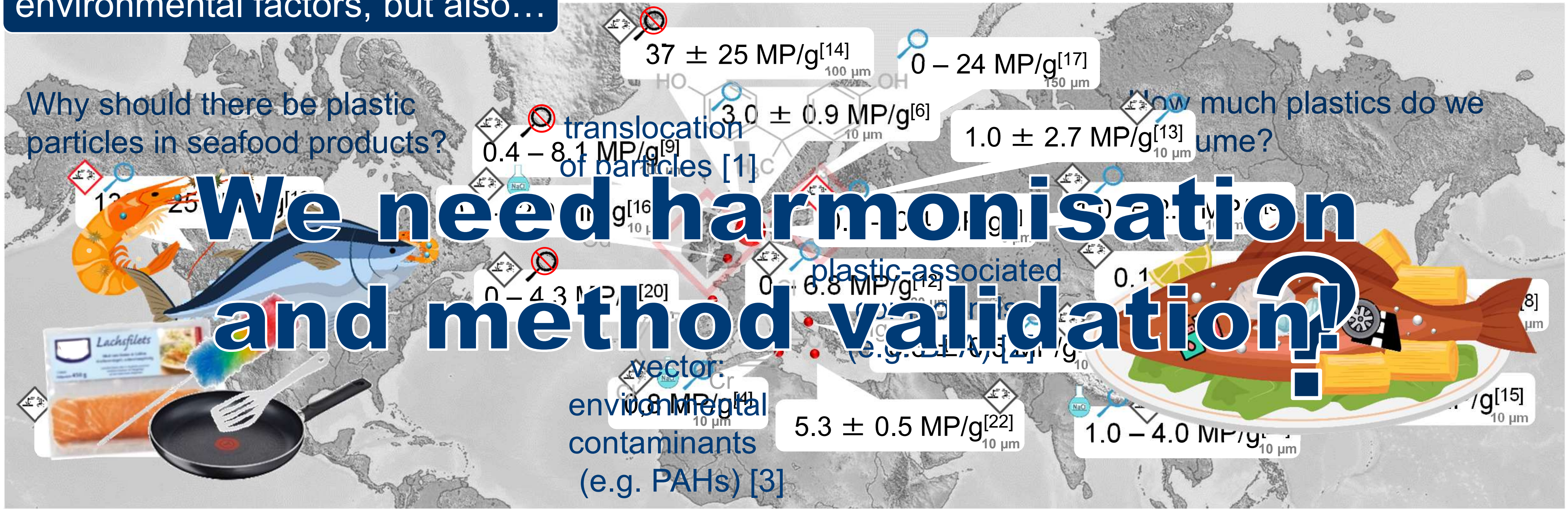


Figure 1: Microplastic content in mussels (*Mytilus* spp.) in particle number (MP) per gram (g) soft tissue (studies from 2014 – 2020).

No harmonised methods, limitation in comparison. Results are influenced by: **resolution of analytical technique**, possible polymer loss due to digestion method (◇, ◇), sub-optimal density separation ( ), incomplete ( ) or no identification ( ).

Sample preparation: What do we have to consider?

MRI Max Rubner-Institut

insufficient digestion

degradation of plastics

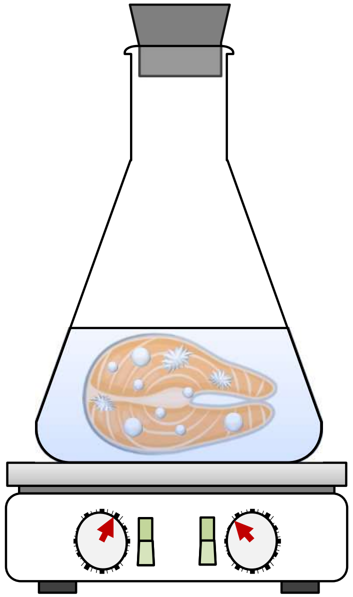
filter pore size

filter material

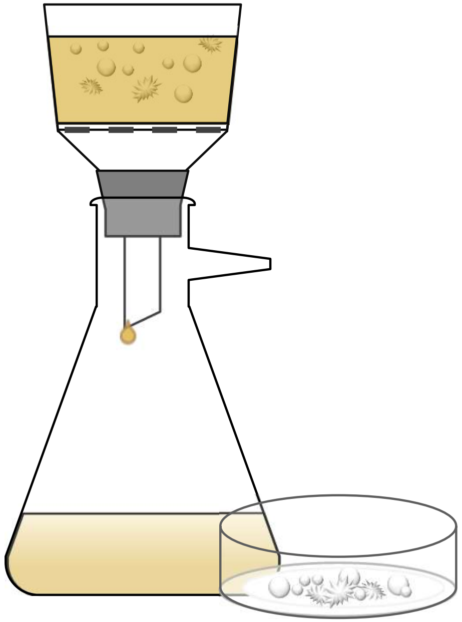
polymer identification

matrix interferences

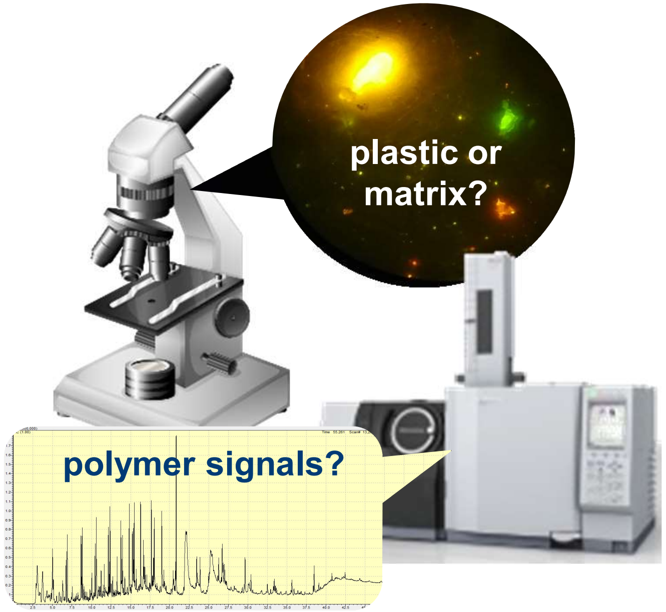
procedural contamination // loss due to adherence on labware surfaces



Digestion



Filtration



Analysis

Evaluation of sample preparation protocols

MRI Max Rubner-Institut

Literature research (n = 94, 2011-2018) digestion of aquatic organisms

Filtration with pore size 1 µm?

Impact on polymer integrity?

Suitable for routine analysis?

overall time, complexity, costs of reagents

Figure 2: Performance of digestion methods applied for isolating MP from fish fillet. Ideally, methods have high digestion efficiency and polymer integrity as well as low digestion time, number of preparation steps and low costs of reagents.

Optimisation: Minimising negative impacts on plastic particles

Max Rubner-Institut

Figure 3: Photographs of PAN before and after alkaline digestion.

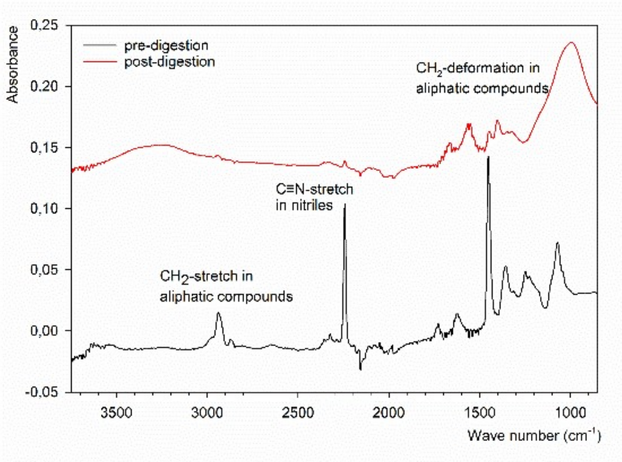


Figure 4: FTIR-Spectrum of PAN before (black) and after (red) digestion.

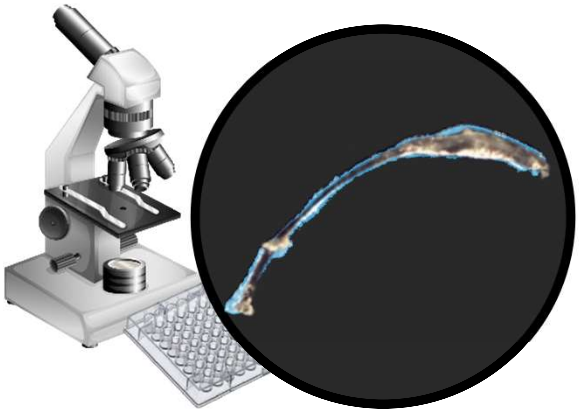


Figure 5: Photograph of a PET-particle before (blue) and after KOH-digestion at 60 °C.

weight recovery alone might miss small surface changes

possible loss of small particles → temperature reduction to 40 °C

Table 1: Polymer integrity after pepsin-KOH-digestion. Alkaline step conducted at 60 °C if not noted otherwise.

polymer	recovery	identification
	weight [%]	
PA6	96 ± 2	
PA12	98 ± 2	
PAN	-	
PC	96 ± 2	
PE	100 ± 2	
PET	91 ± <1	
40 °C	92 ± 2	
PP	98 ± <1	
PS	99 ± 2	
PSu	101 ± 1	
PTFE	100 ± 2	
PU	99 ± 2	
PVC	99 ± 1	

Optimisation: The importance of filter choice

MRI Max Rubner-Institut

compatibility with analytical method

- chemical stability
- thermal stability

optical / spectroscopic
e.g. Al_2O_3, PTFE, metal(-coated)

solvent extraction
e.g. glass/quartz fiber, PTFE

thermoanalytical
e.g. glass fiber, quartz fiber, Al_2O_3

avoiding filter clogging

- pore size
- filter material (adsorption)

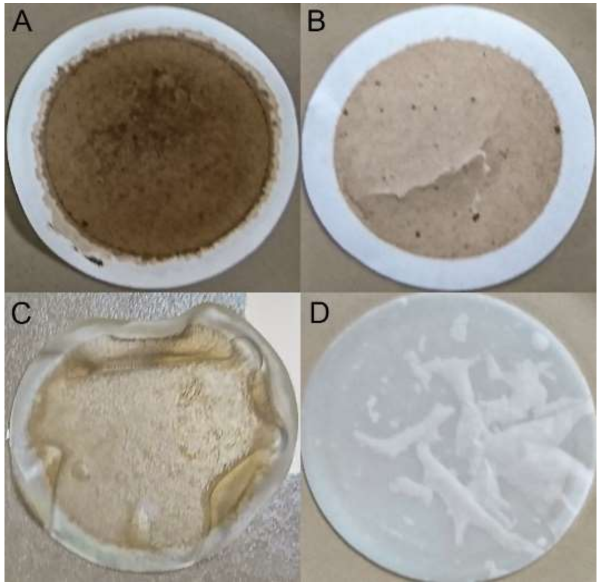


Figure 6: Photograph of filters (pore size ~ 1 µm) after filtering 10 g digested herring fillet.
A) Cellulose nitrate; B) Glass fiber;
C) Cellulose acetate; D) Polycarbonate

influence of structure on particle retention[23]

- hidden between layers
- passing pores lengthwise

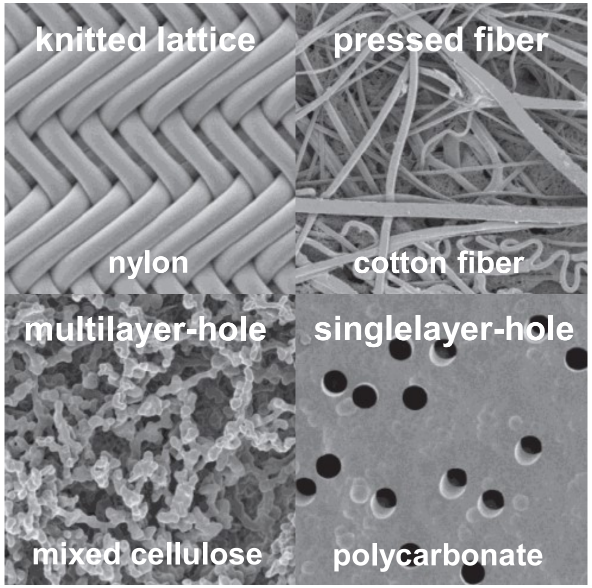


Figure 7: SEM-image of surface morphology-types of membrane filters; Cai et al. (2020).

Optimisation: Post-filtration treatment

MRI Max Rubner-Institut

solvent extraction (^{1}H-NMR, Py-GC/MS)

remove fatty residues

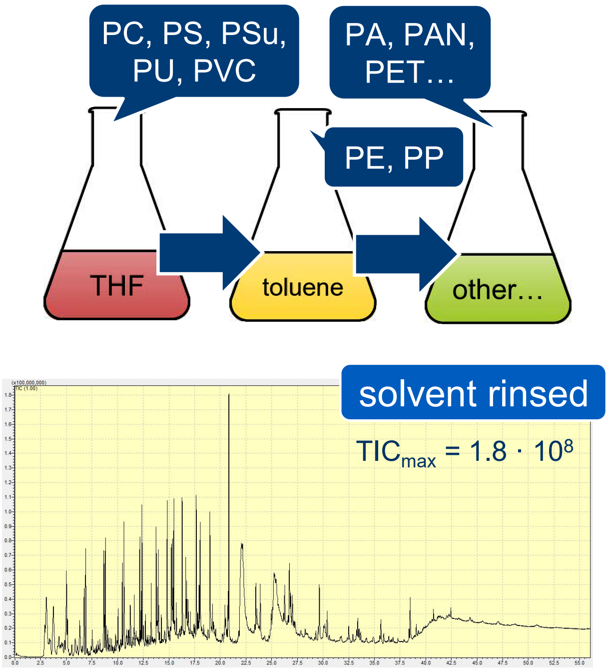


Figure 8: Pyrogram of herring fillet on glass fiber filter spiked with commercially relevant polymers after rinsing with ethanol.

oxidative treatment (all identification techniques)

reduce matrix residues

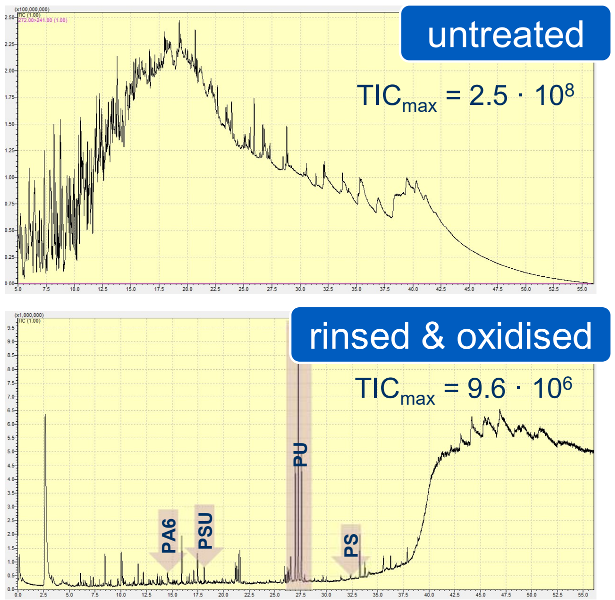


Figure 9: Pyrogram of herring fillet on glass fiber filter spiked with commercially relevant polymers before (above) and after (below) H_2O_2-treatment and ethanol rinsing. Interfering matrix signals are reduced significantly after treatment.

particle staining (fluorescence microscopy)

increase visibility

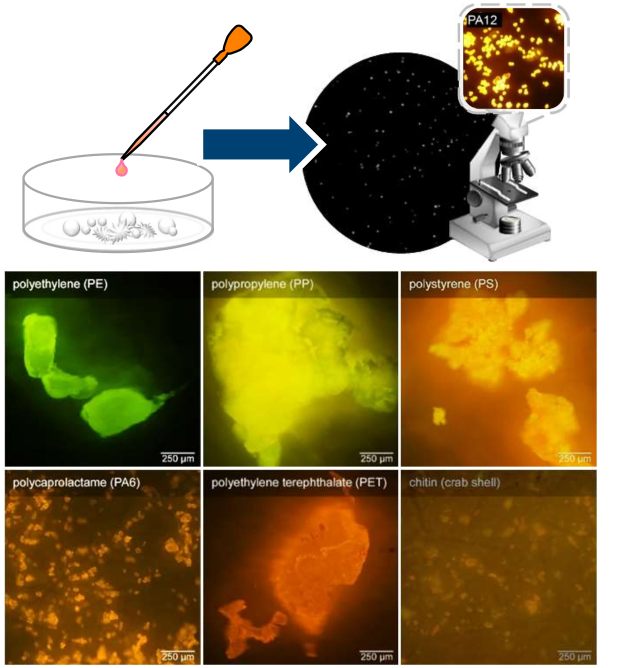


Figure 10: Photographs of Nile Red stained particles (fluorescence: FITC-filter). Red shift of emitted fluorescence with increasing polarity.

Optimisation: Preventing procedural contamination

MRI Max Rubner-Institut

reagents & solutions	glass-/labware & filters	atmospheric deposition
pre-filtration	rinsing & thermal treatment	

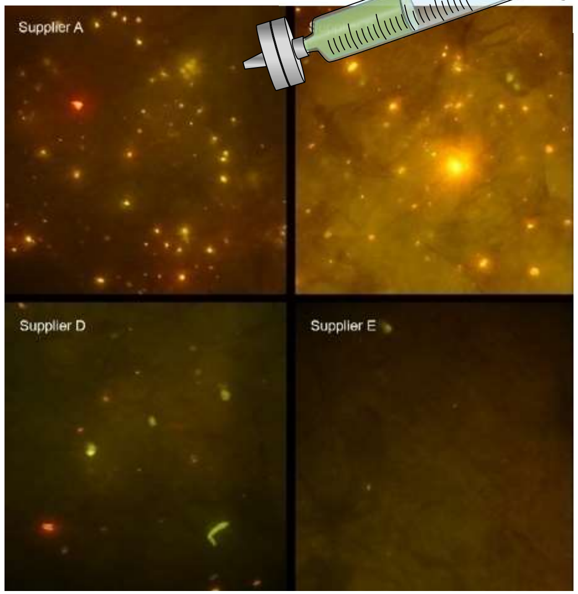


Figure 11: Photographs of Nile red-stained filters after filtration of pepsin from different suppliers. Particles with green, yellow or orange fluorescence are MP-suspect.

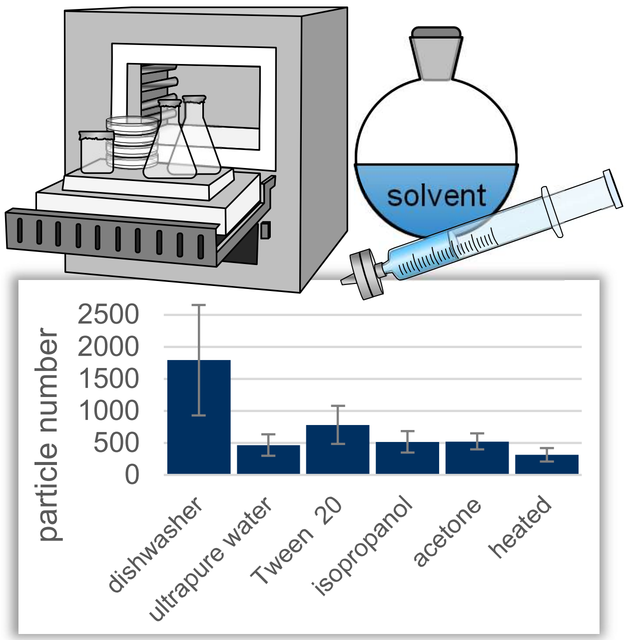


Figure 12: Number of MP-suspect particles rinsed off glass flasks after application of different cleaning procedures.

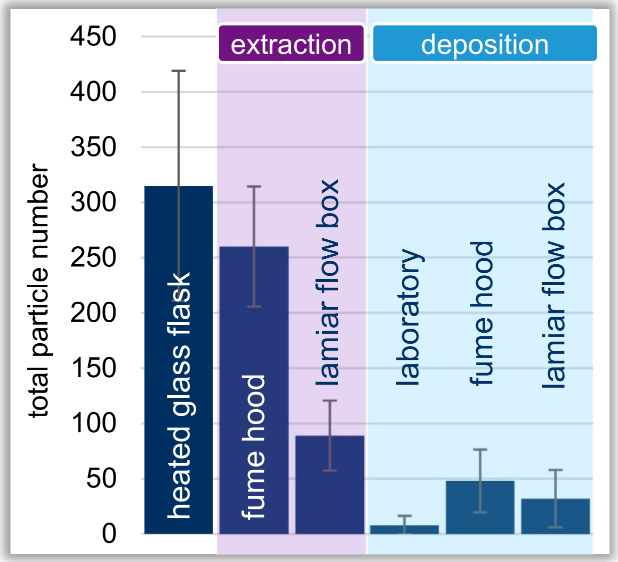
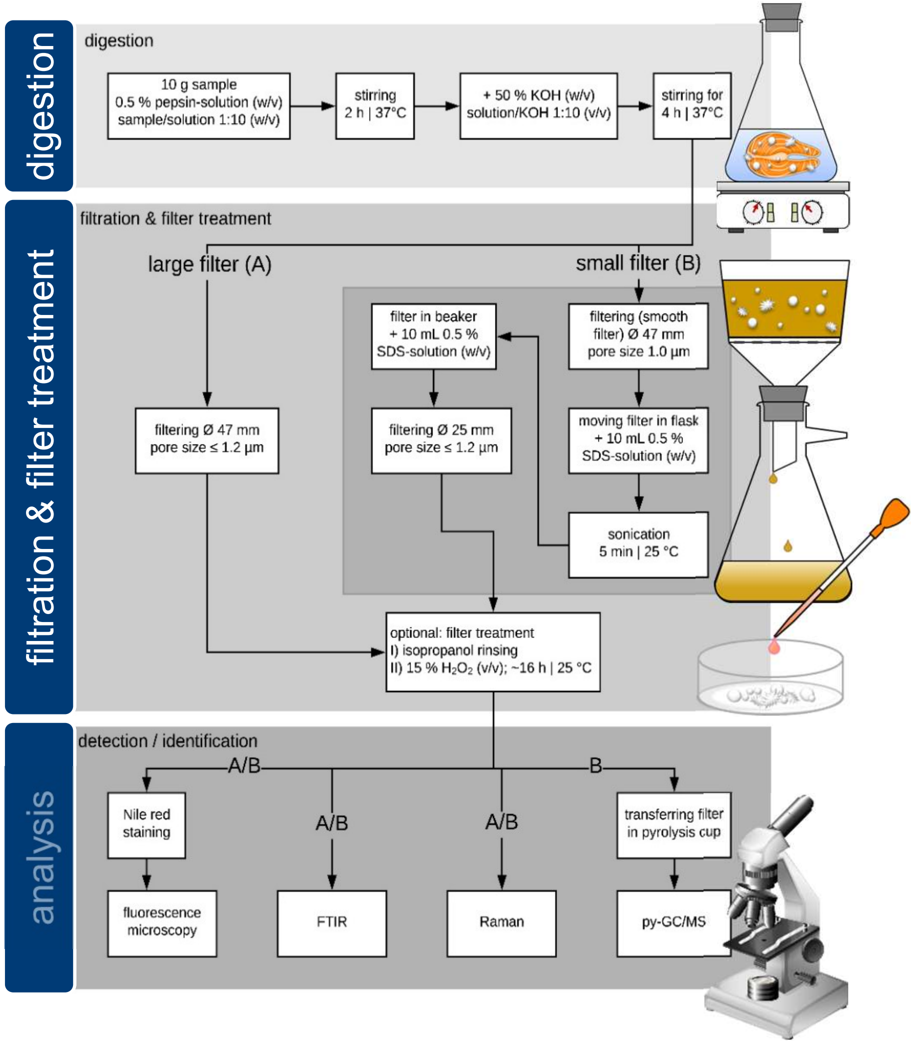


Figure 13: Number of MP-suspect particles rinsed off from heated glassware (c.f. Figure 12) in comparison to blank samples of a simulated digestion procedure (purple) and particles deposited from air within one hour (blue). The biggest entry path for particles seemed to be insufficiently cleaned glassware.

Preliminary validation of the optimised protocol[24]



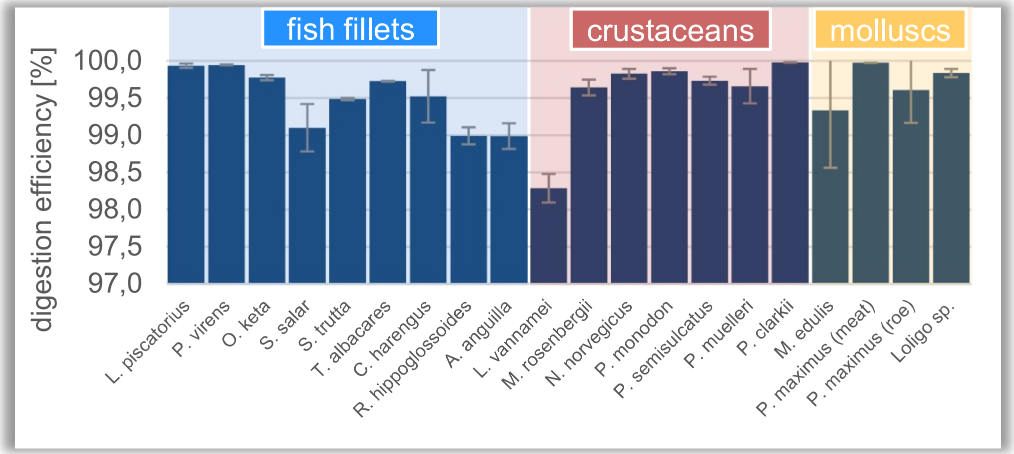
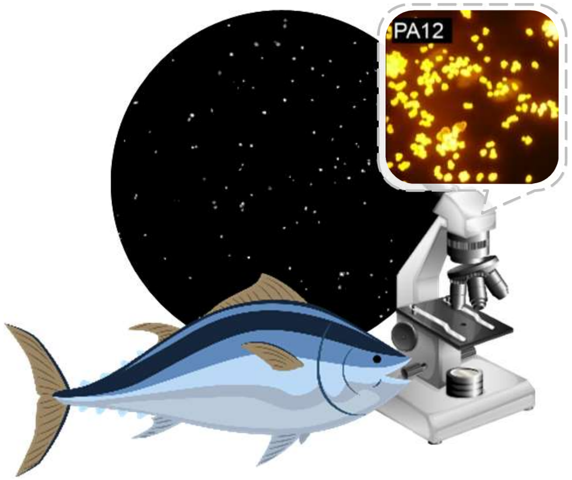


Figure 14: Digestion efficiency of edible parts from different seafood species. Fishes are sorted according to their fat content (increasing).



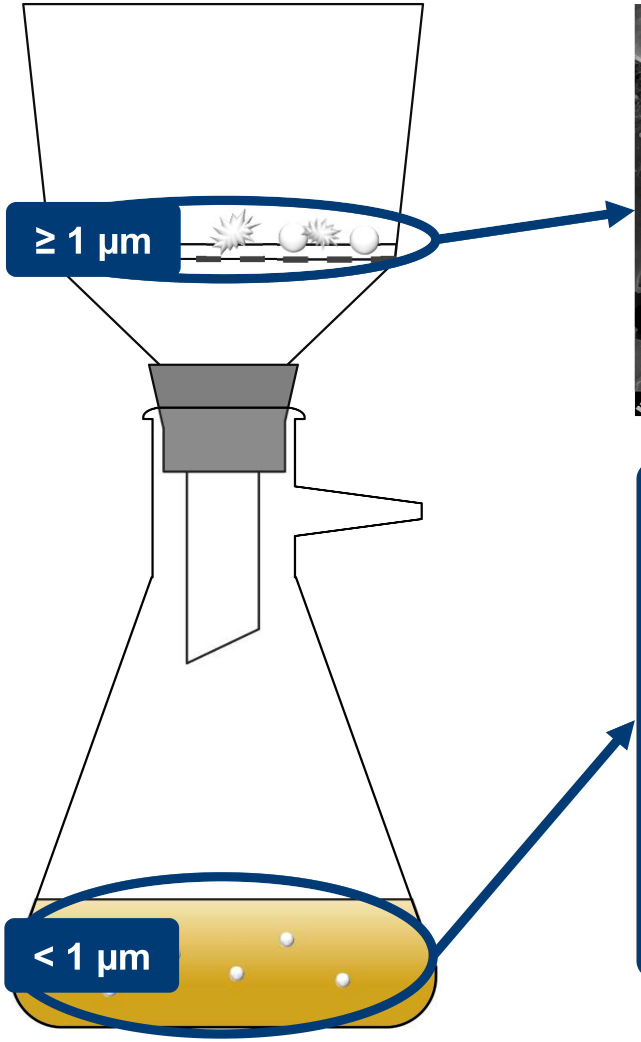
recovery rates
Ø 10 – 50 µm
pre-stained PA12

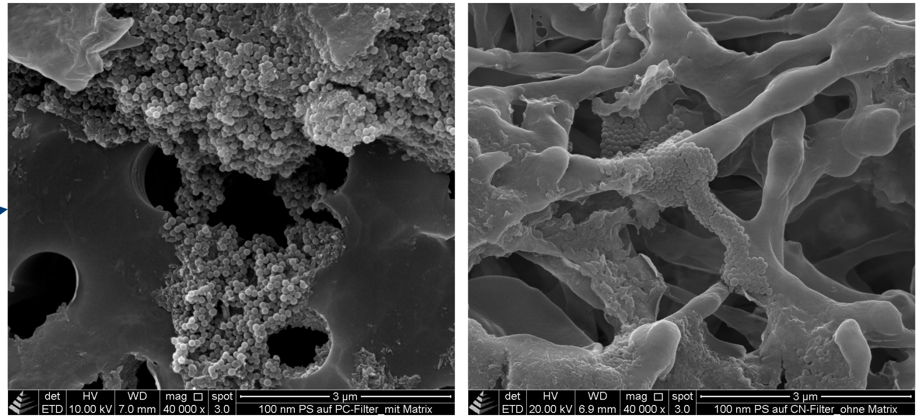
n = 10	88 ± 16 %
n = 100	89 ± 12 %
n = 1000	103 ± 13 %

qualitative with common analytical techniques

Preliminary results: Challenges of nanoplastics analysis

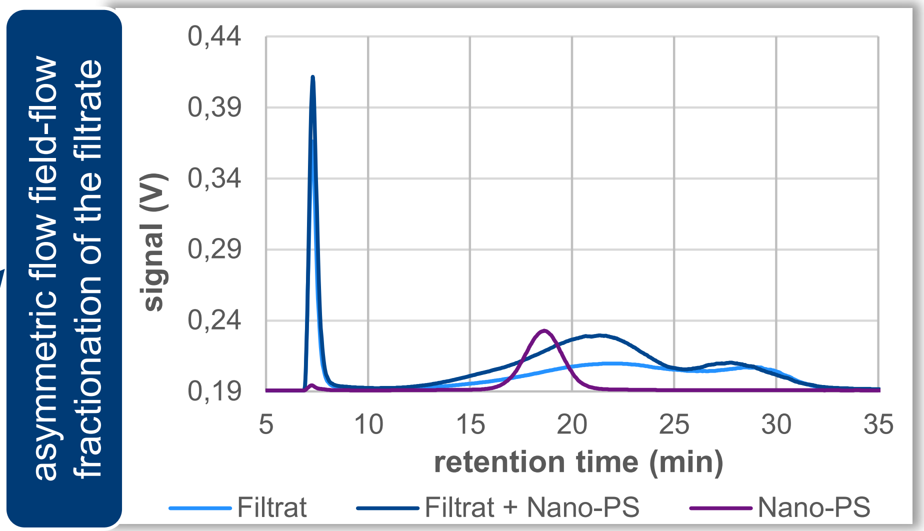
Max Rubner-Institut





„Lost“ by filtration?

Figure 15: SEM-images of polystyrene nanospheres (Ø 100 nm) and residues of digested herring filet adhering to the surface of polycarbonate (left) and cellulose nitrate (right) filters.



Hidden by residual matrix?

Figure 16: MALS-signals of solutions with and without 1 ppm polystyrene-nanobeads (Ø 150 nm) after separation with AF4. Purple – pure nanobead-solution; light blue – filtrate of digested herring fillet; dark blue – filtrate of digested herring fillet spiked with nanobeads.

Thank you for your support…

Max Rubner-Institut

Federal Research Institute of Nutrition and Food

Safety and Quality of Milk and Fish

Jan Fritsche
Julia Süssmann
Torsten Krause
Dierk Martin
Björn Neumann
Longina Reimann
Karen Zurheide
Martina Gerull
Kevin Pohl

- Microscopy
- Py-GC/MS
- ATR-FTIR

Food Technology and Bioprocess Engineering

Ralf Greiner
Elke Walz
Birgit Hetzer
Andrea Tauer
Christian Geuter

- DSC
- AF4-MALS
- REM(-EDX)

cen

Universität Hamburg
DER FORSCHUNG | DER LEHRE | DER BILDUNG

University of Hamburg

Center for Earth System Research and Sustainability

- Fluorescence Microscopy
- µ-Raman

Jürgen Böhner
Elke Fischer
Matthias Tamminga

Organic Chemistry

- ^{1}H-NMR

Thomas Hackl
Claudia Wontorra

Technische Universität Berlin

Technical University Berlin

Sascha Rohn

Thank you for your attention!

References

[1] McIlwraith, H. K., Kim, J., Helm, P., Bhavsar, S. P., Metzger, J. S., & Rochman, C. M. (2021). Evidence of Microplastic Translocation in Wild-Caught Fish and Implications for Microplastic Accumulation Dynamics in Food Webs. Environmental Science & Technology, 55(18), 12372-12382.

[2] Barboza, L. G. A., Cunha, S. C., Monteiro, C., Fernandes, J. O., & Guilhermino, L. (2020). Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. Journal of hazardous materials, 393, 122419.

[3] Tien, C. J., Wang, Z. X., & Chen, C. S. (2020). Microplastics in water, sediment and fish from the Fengshan River system: Relationship to aquatic factors and accumulation of polycyclic aromatic hydrocarbons by fish. Environmental Pollution, 265, 114962.

[4] Abidli, Sami, Youssef Lahbib, and Najoua Trigui El Menif. "Microplastics in commercial molluscs from the lagoon of Bizerte (Northern Tunisia)." Marine pollution bulletin 142 (2019): 243-252.

[5] Bråte, Inger Lise N., et al. "Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study." Environmental Pollution 243 (2018): 383-393.

[6] Catarino, Ana I., et al. "Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal." Environmental pollution 237 (2018): 675-684.

[7] Van Cauwenberghe, Lisbeth, and Colin R. Janssen. "Microplastics in bivalves cultured for human consumption." Environmental pollution 193 (2014): 65-70.

[8] Cho, Youna, et al. "Abundance and characteristics of microplastics in market bivalves from South Korea." Environmental pollution 245 (2019): 1107-1116.

[9] De Witte, B., et al. "Quality assessment of the blue mussel (Mytilus edulis): Comparison between commercial and wild types." Marine pollution bulletin 85.1 (2014): 146-155. mussels collected from coastal and offshore areas of the northern and central Adriatic Sea." Environmental Science and Pollution Research 26.24 (2019): 24407-24416.

References

[10] Digka, Nikoletta, et al. "Microplastics in mussels and fish from the Northern Ionian Sea." Marine pollution bulletin 135 (2018): 30-40.

[11] Ding, Jinfeng, et al. "Detection of microplastics in local marine organisms using a multi-technology system." Analytical Methods 11.1 (2019): 78-87.

[12] Fischer, Elke. "Distribution of microplastics in marine species of the Wadden Sea along the coastline of Schleswig-Holstein, Germany." Final Report University Hamburg (2019).

[13] Iversen, Karine Bue. Microplastics in blue mussels (Mytilus edulis) from the marine environment of coastal Norway. MS thesis. Norwegian University of Life Sciences, Ås, 2018.

[14] Karlsson, Therese M., et al. "Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation." Marine pollution bulletin 122.1-2 (2017): 403-408.

[15] Li, Jiana, et al. "Microplastics in mussels along the coastal waters of China." Environmental pollution 214 (2016): 177-184.

[16] Li, Jiana, et al. "Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom." Environmental pollution 241 (2018): 35-44.

[17] Lusher, A. L., et al. "Sampling, isolating and identifying microplastics ingested by fish and invertebrates." Analytical methods 9.9 (2017): 1346-1360.

[18] Mankin, Chloe, and Andrea Huvard. "Microfibers in Mytilus species (Mollusca, Bivalvia) from Southern California Harbors, Beaches, and Supermarkets.“

[19] Murphy, Fionn, et al. "The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland." Marine pollution bulletin 122.1-2 (2017): 353-359.

References

[20] Reguera, Pablo, Lucía Viñas, and Jesús Gago. "Microplastics in wild mussels (Mytilus spp.) from the north coast of Spain." Scientia Marina 83.4 (2019): 337-347.

[21] Li, Jiana, et al. "Microplastics in commercial bivalves from China." Environmental pollution 207 (2015): 190-195.

[22] Gomiero, Alessio, et al. "First occurrence and composition assessment of microplastics in native mussels collected from coastal and offshore areas of the northern and central Adriatic Sea." Environmental Science and Pollution Research 26.24 (2019): 24407-24416.

[23] Cai, Huiwen, et al. "Microplastic quantification affected by structure and pore size of filters." Chemosphere 257 (2020): 127198.

[24] Süssmann, J., Krause, T., Martin, D., Walz, E., Greiner, R., Rohn, S., ... & Fritsche, J. (2021). Evaluation and optimisation of sample preparation protocols suitable for the analysis of plastic particles present in seafood. Food Control, 125, 107969.