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1 **Optimization of a method designed to extract and characterize microplastics in different packaged**  
2 **fish products**

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## **Highlights**

- Packaged seafood products were selected according to consumption habits.
- The method designed characterizes microplastics in the edible part of products.
- Additional steps were needed for non-filterable samples.
- Particles were characterized by stereomicroscopy and  $\mu$ -FTIR.
- Data on MP abundance are reported for the various seafood products.

## 24 **Abstract**

25 The study of the presence of microplastics (MPs) in seafood products is usually carried out by analyzing  
26 the gastrointestinal tract. However, this part is mostly removed during food processing. Moreover, few  
27 studies have produced results from processed fishery products. The primary objective of this study was  
28 to optimize methods for evaluating the presence of MPs in the edible portions of several products  
29 available in supermarkets. Seven seafood products, including smoked, canned, marinated, and those  
30 stored in polystyrene trays, were selected for analysis, with saithe fillets (*Pollachius virens*) and canned  
31 tuna (*Thunnus alalunga*) among them. To achieve optimal digestion of the samples, a 10% KOH  
32 solution at 40°C was used. For the fattiest samples, such as marinated anchovies, hydrophobic filters  
33 were employed. Additionally, bleach was utilized to lighten dark filters obtained, ensuring readability  
34 under the microscope. These parameters were defined to obtain suitable methods for each product. The  
35 average concentrations in different seafood matrices ranged from  $0.05 \pm 0.04$  to  $0.33 \pm 0.08$  MPs/g for  
36 saithe fillets and canned tuna, respectively. After extrapolation of these values to all the particles  
37 observed on each filter, the concentrations increased from  $0.06 \pm 0.05$  MPs/g to  $2.17 \pm 0.33$  MPs/g.  
38 Particles identification across all samples, using a microscope coupled with Fournier transform infrared  
39 spectroscopy ( $\mu$ -FTIR), confirmed the presence of MPs, including PET, PP, and EVA.

40  
41 **Keywords:** microplastic, seafood product, edible part,  $\mu$ -FTIR.

## 1. Introduction

For seven decades, plastics have been constantly increasing in the Earth's oceans. Nearly 32 million metric tons find their way into the oceans every year (Nelms et al, 2021). Microplastics, defined as less than 5 mm, can be primary-source, i.e. intentionally manufactured for the industry, or secondary-source, i.e. derived from the fragmentation of larger plastics (Arthur et al., 2009; El Hadri et al., 2020). These differently-sized MPs can be ingested by marine organisms, which have been shown to be incapable of differentiating food from MPs (Lusher, 2015). However, there is also evidence that fish are capable to spit out the microplastics (Ory et al., 2018). Fish are particularly impacted, studies having reported that this contamination affects 60% of fish worldwide (Sequeira et al., 2020). Moreover, the presence of MPs in food may be a threat to human health linked to their own composition (polymers, additives, metals, chemicals) and pollutants adsorbed on their surfaces. Particles are also a potential vector for pathogen transport (Kirstein et al., 2016). This subject is still infrequently documented, and there is a lack of available information. (Torres et al., 2021). Many studies have focused on contamination in the digestive tract of various organisms, but little information is available on fish tissues consumed by humans. It is important to evaluate the presence of MPs in edible parts in order to get a clearer idea of the risks to consumer health. So far, very few studies on processed seafood have been published in the literature (Akhbarizadeh et al., 2020; Hussien et al., 2021; Karami, et al., 2017a; Karami et al., 2018) and most of them have focused on canned products, especially tuna (Diaz-Basantos et al., 2022). Various tissue digestion protocols have been adopted in order to extract MPs from marine organisms in accordance with the methods applied to biological tissues and sediments. The most successful method remains potassium hydroxide (KOH) (Foekema et al. 2013, Dehaut et al. 2016 and Treilles et al. 2020). However, this methodology needs to be optimized because the composition of matrices varies widely among processed products (fat, impact of cooking, etc.), as do the ingredients included in these processes (salt, marinade, etc.). The aims of this study were (i) to test different methods to evaluate their ability to efficiently digest processed fish products corresponding to the most frequently purchased among French consumers (FranceAgrimer, 2020) and to propose an efficient protocol tailored to these products with as few steps as possible to reduce both the time needed for analysis and external contamination, and (ii) to apply the selected method(s) to different seafood products purchased in supermarkets with different packaging.

## 2. Materials and methods

### 2.1. Samples

Seven different seafood products were purchased from a local supermarket (Boulogne-sur-mer, France). The first two products were canned seafood and consisted of natural white tuna (*Thunnus alalunga*) and natural mackerel fillets (*Scomber scombrus*). The remaining products included a tray of salmon fillets

76 (*Salmo salar*) under a modified atmosphere, vacuum-packed smoked salmon, shrink-wrapped trays of  
77 cod fillets (*Gadus morhua*) and saithe fillets (*Pollachus virens*), and finally sealed trays of marinated  
78 anchovy fillets (*Engraulis encrasicolus*). Three samples of each product were analyzed (n=3). We also  
79 wished to target the maximum quantity of products to be as representative as possible.

## 80 **2.2. Development of MP extraction methods**

81 The products employed for the analyses were 10% (w/v) KOH solution from Chimieplus (Saint-Paul-  
82 de-Varax, France), analytical grade RPE water from Carlo Erba (Val de Reuil, France), 2.6% (v/v)  
83 sodium hypochlorite (NaOCl) from Orapi Europe (Saint-Vulbas, France), 17% (v/v) NaOCl from VWR  
84 (Fontenay-sous-Bois, France) and Carlo Erba absolute ethanol (Val de Reuil, France), which was  
85 subsequently diluted with bi-distilled water to obtain a 70% (v/v) ethanol solution. Two filters with  
86 different porosities were used during the analyses: Cytiva 1.6  $\mu\text{m}$  porosity GF/A glass fiber filter  
87 (Fribourg en Brisgau, France) and 4-10  $\mu\text{m}$  porosity Sartorius hydrophobic filter (3-602-090, Göttingen,  
88 Germany). All the filters had a diameter of 90 mm. Six methods were tested.

89 **Method 1** was adapted from Dehaut et al., 2016 and Treilles et al., 2020. This method was initially  
90 applied to canned tuna. Fish tissues were stirred at 200 rpm with 800 mL of 10% KOH solution then  
91 incubated at  $40 \pm 1^\circ\text{C}$ . Different time spans -16 and 24 hours -were also tested for this digestion step.  
92 The next day, samples were filtered with a GF/A filter, and conserved for an additional day at room  
93 temperature (from 18 to  $20^\circ\text{C}$ ) before observation/identification of the particles (Fig. 1).

94 **Method 1a** was developed and used only for oily seafood products. This trial included a preliminary  
95 step to filter the oily content present in the packaging before proceeding to digestion of the tissue with  
96 method 1 using hydrophobic filters (Fig. 1).

97 **Method 1b** is the same protocol as method 1 except for the addition of approximately 10 mL of 2.6%  
98 NaOCl onto the filters after filtration of the digestates (Fig. 1).

99 **Method 2** was adapted from Karami et al. (2017b) where fish tissues were stirred at 200 rpm with  
100 different volumes and concentrations of bleach solutions: 150 or 350 mL of 2.6% NaOCl and 250 or  
101 300 mL of 17% NaOCl. Samples were also incubated at  $40 \pm 1^\circ\text{C}$  for 24 h. (Fig. 1).

102 **Method 3** was adapted from Karami et al. (2017b). Fish tissues were stirred with two chemical products:  
103 300 mL of 2.6 % NaOCl for 2 h before adding 200 mL of 10% (w/v) KOH solution. Then the samples  
104 were incubated under agitation at 200 rpm at  $40 \pm 1^\circ\text{C}$  for 24 h. (Fig. 1).

105 **Method 4** was adapted from Karami et al., (2017b). Fish tissues were stirred at 200 rpm with two  
106 chemical products: 250 mL of 17% NaOCl for 2 h before adding 450 mL of 20% (w/v) KOH solution.  
107 Then the samples were incubated at  $40 \pm 1^\circ\text{C}$  for 24 h. (Fig. 1).

108 A method was considered to be suitable when the filtration was successful, with no clogging and using  
109 a light-colored filter, observable under a stereomicroscope. These efficient methods were applied to a  
110 triplicate series of the seven seafood end products in order to estimate the presence of MP particles and  
111 their shape, size and type.

### 112 ***2.3. Prevention of contamination and QA/QC***

113 In order to avoid any contamination, all glassware (except measuring cylinders), filters in Petri dishes  
114 and stainless-steel tools (which had been washed beforehand) were transferred to the Nabertherm L  
115 40/11 muffle furnace (Lilienthal, Germany) and kept at  $450^\circ\text{C}$  for 6 h in order to destroy any particles  
116 present. Each solution employed during the analyses was filtered using a  $0.7\ \mu\text{m}$  pore size 90mm GF/F  
117 glass fiber filter. Due to stability issues, 2.6% bleach was extemporaneously filtered with the same type  
118 of filter. All manipulations were carried out under a Thermo Scientific Herasafe 2030i laminar flow  
119 cabinet (Saint-Herblain, France) in order to prevent atmospheric contamination of the samples. Before  
120 starting the analyses, the bench of the laminar flow cabinet as well as the measuring cylinder were  
121 cleaned with bi-distilled water, 70% (v/v) ethanol solution and bi-distilled water again. Each item and  
122 all the packaging for the seafood products were previously dusted with compressed dry gas (Argenteuil,  
123 France). Additionally, a set of different controls described below was used for each batch of analyses.  
124 Atmospheric controls were carried out during each manipulation—one for the preparation of digestion  
125 and one for the filtration—in order to monitor the level of contamination inside the cabinet. To ensure  
126 consistency, the negative controls were executed concurrently with the analysis sessions, as all sessions  
127 adhered to identical parameters. In our specific case, an analysis session entails initiating a digestion  
128 process involving both a negative and positive control, along with three replicates of a distinct matrix.  
129 Depending on equipment availability, one or two matrixes were processed during an analysis session.  
130 Four negative samples (, i.e., Erlenmeyer flask containing the same volume of 10% KOH without a  
131 sample,) were carried out during the four analysis sessions. A positive control was also added in order  
132 to monitor recovery efficiency (Dehaut et al., 2023). This control was composed of a gelatin capsule,  
133 containing three particles of five different MPs (n=15) including PE (polyethylene), PP (polypropylene)  
134 and PS (polystyrene) fragments, a section of PA-6 (nylon) monofilament and PES (polyester)  
135 microfibers, which is placed in KOH. This positive control was employed during the analysis sessions  
136 in order to assess any potential systematic errors. The purpose was to determine if all the MP digestion  
137 products would be collected at the end of the filtration process, as it has been observed that after  
138 filtration, approximately 90% of the plastics are recovered, while the remaining portion persists within  
139 the Erlenmeyer flask, even after the conducted washes. This positive control served as a reference point

140 for each analysis session, enabling verification of the aforementioned systematic error. Four positive  
141 controls were carried out.

#### 142 ***2.4. Observation and identification of microparticles***

143 Before the observation stage, filters were conserved in a Petri dish for 24 h at room temperature (from  
144 18 to 20°C). Observations were carried out with an Olympus SZX-16 stereomicroscope (Rungis, France)  
145 equipped with a UC90 camera and an SDFPLAPO PF 1x/0.15 objective. All the filters were observed  
146 at 4x magnification in order to save an image of each observed particle. Once acquired, images were  
147 then processed with Olympus Cellsens 4.2 and OlyVia in order to measure the size of each particle. Two  
148 categories were defined according to their shape: fiber or fragment, respectively measured using  
149 freehand polyline and freehand polygon tools. Fibers were characterized by length and color, while  
150 fragments were characterized by mean Feret diameter and color. Size categories were defined for both  
151 fibers and fragments [0 – 50 µm], [50 – 100 µm], [100 – 500 µm] and [500 – 5000 µm]. Prior to the use  
152 of micro-Fourier transform infrared spectroscopy (µ-FTIR), a strategy was established in order to  
153 rationalize the time spent on identification and to cover the widest diversity of microparticles  
154 (Supplementary data 1). Briefly, particles with different shapes and colors were systematically analyzed.  
155 The remaining particles on the filter were processed in either of two ways depending on their number:  
156 If the total number of identical particles was higher than 50, a subsample of 10% was analyzed; if this  
157 number was below 50, five particles were systematically analyzed. A label listing all the particles  
158 selected for identification was placed on the lid of the glass Petri dish to make it easier to find them with  
159 µ-FTIR afterwards. Before identification, to ensure good traceability, all the tagged particles were  
160 visually observed under the microscope to ascertain that the particle was one of the ones that we were  
161 looking for. Particles were identified using a Perkin Elmer Spectrum-3-Spotlight™ 400 µ-FTIR  
162 (Villebon-sur-Yvette, France) equipped with an MCT detector and an automatic ATR module. After  
163 that, all the pre-selected particles were processed. The area surrounding the particle to be targeted was  
164 mapped to ascertain the presence and characteristics of this particle. After acquiring the background,  
165 spectra were recorded with 25 acquisitions from 4000 to 600 cm<sup>-1</sup> with a resolution of 4. Afterwards,  
166 each spectrum was compared with different databases, including a custom Perkin Elmer database,  
167 Flopp/Flopp-e (De Frond et al., 2021) used with Openspecy (Cowger et al., 2021). Identification was  
168 validated when the score was higher than 0.7.

#### 169 ***2.5. Data processing***

170 All the particles present on each filter were counted (Supplementary data 1). Particles observed on the  
171 controls were deducted from the sample filters. These subtractions were performed according to particle  
172 shape, color, size and identification. An initial dataset was compiled based on the concentration of  
173 particles for each sample. A second dataset was determined using the results of polymer identification,

174 i.e., the number of microplastics found based on the particle sub-samples analyzed for each filter  
175 (Supplementary data 1). A third dataset was compiled based on the extrapolated MP count, which  
176 corresponds to the estimated number of MPs based on the previous two datasets. Briefly, based on  
177 particle observations, each particle that was not analyzed with  $\mu$ -FTIR but that had the same shape, color  
178 and size class attributes as an identified particle was estimated to be an MP with the same polymeric  
179 composition as its reference. The percentages reported in this paper correspond to the third dataset, i.e.,  
180 extrapolated number of MPs for each type of sample, based on the sum of particles recovered from the  
181 three replicates. The symbol " $\pm$ " is used in this paper to denote the standard deviation of the mean value.

### 3. Results and Discussion

#### 3.1. Optimization of the extraction method

184 The aim of this work was to develop an efficient method of extracting MPs from seafood products with  
185 as few steps as possible from sample to result. The can content was fractionated to test different ratios  
186 between the mass of tissue and the volume of digestion solution (**Table 1**). Regardless of the ratio, the  
187 results always showed incomplete degradation of tissue with an incubation time below 24 hours.  
188 However, the digestion of canned tuna was successful after 24 hours with an optimal ratio of 1:4.5.  
189 Method 1 was then tested on the six remaining seafood products: saithe fillets, cod fillets, salmon fillets,  
190 smoked salmon, marinated anchovies, and canned mackerel, applying different ratios ranging from 1:  
191 2.6 to 1: 15.8 between the mass of tissue and the volume of 10% KOH (**Table 1**). In order to homogenize  
192 the analysis process, the 24-hour incubation was then tested as a priority on these matrices. The results  
193 for digestion showed that method 1 led to complete tissue digestion for all but marinated anchovies (**Fig.**  
194 **2**). The results for filtration revealed that the filters can be observed, except in the case of canned  
195 mackerel, where the contrast between the coloring of filters and particles was insufficient to correctly  
196 detect all the microplastics under the stereomicroscope (**Fig. 2**). In order to make use of these canned  
197 mackerel filters, a supplementary step was added to method 1 immediately after the digestate filtration  
198 (method 1b). This entailed adding 10 mL of 2.6% NaOCl directly onto the GF/A filter during filtration  
199 so its color faded, making it easier to observe (**Fig.2**). Method 1b was only applied to canned mackerel  
200 and the addition of 2.6% NaOCl had no visual impact on the polymers of the positive controls; indeed,  
201 this method had previously been used for MP research (Karami et al., 2017b).

202 Other extraction methods were tested on the marinated anchovies. The digestion of the entire product  
203 with method 1 led to the obtention of two distinct phases in the digestate due to the presence of fatty  
204 content in the marinade. Other chemical products were used in order to digest the whole product (**Table**  
205 **1**). Many analyses were carried out using methods 2, 3, and 4 with different digestion ratios (**Table 1**),  
206 but none of the tested conditions led to efficient digestion. After all these attempts, a modification of  
207 method 1 was considered, which involved a preliminary step before including digestion with 10% KOH

208 (method 1a). This method involved separating the marinade from the anchovies using a specific filter to  
209 isolate the fattiest part of the sample. The anchovy tray was therefore opened under the laminar flow  
210 cabinet in order to directly filter the marinade using a hydrophobic filter and then process all the anchovy  
211 tissues with a classical digestion scheme (method 1). Method 1a improved digestion of the marinated  
212 anchovies and allowed us to observe these hydrophobic filters (**Fig.2**) to check for the presence of  
213 particles in the oily marinade.

214 All the methods developed were carried out until effective digestion was achieved in order to determine  
215 an optimal digestion ratio, verified three times, in order to reproduce the method on other products of  
216 the same nature. Indeed, although methods 1, 1a and 1b enabled us to extract particles and observe the  
217 filters, the optimal proportion between the tissue mass and the KOH volume ranged from 1:4 to 1:8  
218 (w/v) for cod and saithe fillets, respectively. The results tend to show that a higher volume of KOH was  
219 required when the lipid level was higher, but this is not the only parameter influencing digestibility.  
220 Indeed, the values were 1:6.9 for canned mackerel and 1:5.8 for smoked salmon, which contained 17%  
221 and 13% of lipids, respectively, while saithe fillets required a ratio of 1:8 despite it being one of the low-  
222 fat white fish species, with a lipid content of 1% (Anses, 2020). These different proportions were  
223 difficult to compare with other studies using KOH to digest tissue. Indeed, the mass of the tissues or the  
224 precise volume of KOH used in previous studies has usually remained unspecified (Da Silva et al., 2022;  
225 Daniel et al., 2021; Fernández Severini et al., 2020; Karami et al., 2017b) Additionally, the aim of this  
226 study was to analyze the entire product, which differs from other studies where only a fraction of the  
227 sample was collected to extract the particles (Akhbarizadeh et al., 2020; Süßmann et al., 2021). Splitting  
228 up the sample remains of questionable utility as no test has yet demonstrated the perfect distribution of  
229 particles at the end of the homogenization step. This additional step might even increase the risk of  
230 external contamination during the analysis of these samples. It is with the goal of limiting contamination  
231 that the present study focused on the entire sample. The three methods—i.e., methods 1, 1a and 1b—are  
232 based on the use of 10% KOH at 40°C for 24 hours in order to digest fish matrices. Since its first use in  
233 2013 (Foekema et al., 2013), potassium hydroxide has proved to be advantageous in the extraction of  
234 MPs from seafood tissues compared with other methods (Dehaut et al., 2016). The incubation  
235 temperature of 40°C had already been applied in several commercial seafood products such as dried fish  
236 (Karami et al., 2017a), canned sardines and sprats (Karami et al., 2018), longtail or canned yellowfin  
237 tuna, or even canned mackerel (Akhbarizadeh et al., 2020). Moreover, alkaline hydrolysis at 40°C avoids  
238 the loss of integrity in plastic particles (Süßmann et al., 2021) whereas digestion at 60°C can partly  
239 deteriorate polyethylene terephthalate fibers (Treilles et al., 2020). The 24-hour incubation time with a  
240 chemical treatment was also found to be beneficial by several authors, although this time can be extended  
241 to 72 h for different food products (Akhbarizadeh et al., 2020; Daniel et al., 2021; Sridhar et al., 2022).  
242 In this study, the digestates were vacuum filtered through a 90mm diameter GF/A filter, which is the  
243 most commonly used format. This diameter was smaller than the 125 mm filters used for dried fish and

244 canned sardines (Karami et al., 2018; Karami, Golieskardi, Ho, et al., 2017) but larger than the 70 mm  
1 245 filters tested for edible tissues of shellfish (Daniel et al., 2021). It enabled us to obtain a single filter per  
2 246 digestate while avoiding clogging. These filters retained a maximum of particles that  $\mu$ -FTIR could  
3 247 identify. A higher porosity, such as a 149  $\mu$ m filter membrane, was used in previous studies for oily  
4 248 products (Karami et al., 2018) but probably induced an underestimation of the microplastic  
5 249 concentration. This is particularly true for the smallest particles (Toussaint et al., 2019), which are  
6 250 actually known to be the biggest class of particles. Our choice of using entire samples increases the  
7 251 representativeness of results for the consumer, who most often ingests the whole product.

### 14 252 ***3.2. Applications on miscellaneous seafood products***

17 253 Based on the optimization results, samples were processed according to different methods: method 1a  
18 254 (marinated anchovies), method 1b (canned mackerel) and method 1 for the remaining products. All the  
19 255 samples, analyzed in triplicate, were successfully digested and filtered. However, this choice to perform  
20 256 triplicate, in order to provide reliable results on different matrices, induce limits. This study found that  
21 257 the optimal proportion between tissue mass and KOH volume may vary for different seafood products,  
22 258 depending on their composition. This last can also be linked to the origin and the different brands on the  
23 259 market. Therefore, it might be necessary to adapt the digestion ratios for specific products to achieve  
24 260 efficient results. Moreover, marinated products may present challenges due to the presence of fatty  
25 261 content in the marinade. Method 1a, which improved the digestion of marinated anchovies, may not be  
26 262 applicable to all types of marinated seafood products. However, this study emphasizes the importance  
27 263 of analyzing the entire sample to maintain representativeness for consumers. These few points should  
28 264 be taken into account before applying these methods to other types of products available on the market.

#### 38 265 ***3.2.1. Blank and negative controls***

41 266 Among the 12 procedural blank filters obtained, the mean value of  $0.87 \pm 0.99$  particles per filter was  
42 267 observed in atmospheric controls, whereas  $10.5 \pm 8.4$  particles per filter were counted in negative  
43 268 controls. In these negative controls, the number of fibers and fragments per filter ranged from 0 to 17  
44 269 and 0 to 20, respectively. The fibers and fragments identified in the blanks that were associated with  
45 270 sample processing were systematically subtracted from the respective sample, taking the color and size  
46 271 class of these particles into account. The plastic recovery level, which had a mean value of  $93.3 \pm 9.43\%$ ,  
47 272 was calculated using the positive controls.

#### 54 273 ***3.2.2. Shape, size, polymer /chemical composition of MPs***

56 274 A total of 2137 particles were counted onto filters after filtration, and reported as fragments or fibers  
57 275 (Supplementary data 2). Fragments between 0 and 100  $\mu$ m were dominant (>50%), while the majority  
58 276 of fibers (>70%) measured between 100 and 5000  $\mu$ m (Fig. 3). The sizes of fibers ranged from 15 to

277 4238  $\mu\text{m}$  and fragments from 10 to 1632  $\mu\text{m}$ . Overall, 911 particles (40.7%) were analyzed by  $\mu\text{-FTIR}$ .  
1 278 The identification strategy applied to each filter led to the characterization of a percentage of analyzed  
2  
3 279 particles ranging from a minimum of 16% to a maximum of 90%. There were more fibers in the fillets  
4  
5 280 of saithe, cod, and salmon whereas fragments exceeded 50% in other products (Supplementary data 3).  
6  
7 281 The  $\mu\text{-FTIR}$  results for all the species showed that cellulose was commonly identified in the majority of  
8  
9 282 fibers found in filters, ranging from 11% to 44% per seafood product (**Fig. 4**). This result is corroborated  
10  
11 283 by another study showing that cellulose was the most frequently found fiber in the digestive tract of 24  
12  
13 284 fish species (Wu et al., 2020). The second most frequently identified microparticle was usually  
14  
15 285 polyethylene terephthalate (PET). This polymer was distributed differently depending on the seafood  
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17 286 product. A high proportion of PET was found, for example, among the fragments in canned tuna. PET  
18  
19 287 is still one of the most common polymers used in our daily life, especially in the food industry where it  
20  
21 288 is very widely used for items ranging from water bottles to textiles or transparent film, etc. (Soong et  
22  
23 289 al., 2022). Moreover, the present results are in line with (Diaz-Basantes et al., 2022), whose study on  
24  
25 290 commercial canned tuna also showed a majority of PET fragments. The other targeted particles were  
26  
27 291 grouped in different categories, such as polypropylene (PP) and ethylene vinyl acetate (EVA). This  
28  
29 292 copolymer of ethylene and vinyl acetate (VA) is not commonly found in the environment, but its  
30  
31 293 presence in the seafood products varied from 10 to 40%. EVA is used for different purposes depending  
32  
33 294 on the proportions of ethylene and VA. The higher the VA content, the more EVA will be characterized  
34  
35 295 as rubber. It is mainly used in hot melt formulations for coatings and bags used to package frozen foods  
36  
37 296 (Buonocore et al., 2014). These identification results underline the difficulty in defining the origin of  
38  
39 297 contamination of these seafood products.

### 3.2.3. *Particles in canned samples*

39 299 Canned tuna had the highest concentration of particles, with  $2.28 \pm 0.38$  particles/g net weight (**Fig. 2**).  
40  
41 300 The abundance in mackerel was seven times lower, with a concentration of  $0.30 \pm 0.12$  particles/g net  
42  
43 301 weight. To our knowledge, no canned white tuna MP concentrations have previously been described in  
44  
45 302 the literature to compare with these results. Based on the identification of selected particles, the MP  
46  
47 303 concentration observed in white tuna was  $0.33 \pm 0.08$  MPs/g net weight. The extrapolation of these  
48  
49 304 values to all the particles observed on the filter increases this value to  $2.17 \pm 0.33$  MPs/g net weight.  
50  
51 305 The large discrepancy between these two values is explained by the fact that 968 out of 1078 particles  
52  
53 306 were identical in color and shape. Another study carried out in a similar canned product containing  
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55 307 longtail tuna fish in brine showed an abundance 1.5 times lower ( $0.22 \pm 0.02$  MPs/g muscle)  
56  
57 308 (Akhbarizadeh et al., 2020) or 318 times lower than other values observed in lyophilized water-soaked  
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59 309 samples (Diaz-Basantes et al., 2022). These comparisons between MP concentrations are nevertheless  
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61 310 to be taken with caution as the MP extraction procedure and the tuna species of the brand products were  
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63 311 different. Another study of canned products highlighted the presence of MPs in two brands of canned  
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312 sardines or sprats with a concentration of 1 MP/can (Karami et al., 2018). These authors concluded that  
1 313 there was contamination during the canning process linked to the morphology of the observed MPs.  
2 314 However, MPs could be present from the beginning of the process in the raw materials. The ingestion  
3 315 of particles by the two species has already been highlighted by several authors, who found particles in  
4 316 the gut but not in edible parts. The presence of four plastic fragments was observed for the first time in  
5 317 the stomach of 31 *Thunnus alalunga* specimens in 2015 (Romeo et al., 2015). Microplastics have also  
6 318 been detected in the digestive tract of other tuna species. For example, six specimens of skipjack tuna  
7 319 (*Euthynnus affinis*) from Indonesia had a total of 19 MPs (Andreas et al., 2021), and  $3 \pm 2.65$   
8 320 MPs/individual ( $0.03 \pm 0.03$  MPs/g) were detected in the digestive tract of *Thunnus tonggol*  
9 321 (Hosseinpour et al., 2021). In the case of *Scomber scombrus*, MPs have already been detected in their  
10 322 stomach content at a concentration of  $0.46 \pm 0.78$  MPs/individual (Neves et al., 2015) or  $0.37 \pm 1.03$   
11 323 MPs/individual (López-Martínez et al., 2022). However, the present study on commercial seafood  
12 324 products did not investigate such organs. Contamination of white tuna muscle or canned mackerel fillets  
13 325 by stomach MPs during the canning process could result from two different origins: Major  
14 326 contamination of the stomach by MPs could lead to their dispersion in the flesh. Contamination could  
15 327 also occur via the ingredients used in the manufacturing process. As has already been observed in canned  
16 328 yellowfin and longtail tuna, there may be a positive correlation between MP concentration and the salt  
17 329 content of samples (Akhbarizadeh et al., 2020). The content of MPs in salt has indeed been proven by  
18 330 some authors, with concentrations ranging from  $0.085 \pm 0.063$  per g to 0.212 particles/g according to  
19 331 brands and geographic origins (Kosuth et al., 2018; Kuttykattil et al., 2022). However, the highest  
20 332 abundance of particles observed in canned tuna and mackerel products cannot only depend on the salt,  
21 333 as water in the brine could also be a source of MP contamination. Moreover, the precise composition of  
22 334 the brine was not indicated on these commercial products.

#### 3.2.4. Particles in samples conserved in marinade

335  
336 The method described above was used to observe the concentrations of particles in both the fillets of  
337 anchovies (Figure 2) and in the oil in which they were packaged (Table 2). The total number of particles  
338 was  $0.28 \pm 0.09$  and  $0.48 \pm 0.25$  items/g for fillets and oil marinade, respectively. Based on the  
339 identification of a selection of particles, the abundance of MPs in the fillets was  $0.10 \pm 0.05$  MPs/g,  
340 while that measured in the oil was  $0.17 \pm 0.07$  MPs/g. Finally, regarding the extrapolated data, values  
341 ranged from  $0.13 \pm 0.05$  MPs/g for fillets to  $0.24 \pm 0.16$  MPs/g for the marinade. Various authors have  
342 studied MP contamination in European anchovies. Their contamination level was found to be  $0.15 \pm$   
343  $0.04$  MPs in the gastrointestinal tract (GIT) of fish from the southern Black Sea coast (Eryaşar et al.,  
344 2022), for example, or  $0.46 \pm 0.25$  microparticles and 1.25 MPs in the stomach content of anchovies  
345 from the Adriatic and Ligurian Seas, respectively (Misic et al., 2022; Renzi et al., 2019). All these  
346 studies evaluated the MP level per individual. Although European anchovies are often consumed after

347 being gutted and beheaded, the present results highlight contamination of the fish muscle. In the  
1 348 marinated products, the level of particles seemed to be equivalent to these values per individual.  
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3 349 However, in this case, the anchovy fillets were macerated in an oily marinade whose measured MP  
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5 350 concentration indicates a possible source of contamination.  
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### 7 351 **3.2.5. Particles in tray-packed fillets**

10 352 The average number of particles for salmon samples was  $0.16 \pm 0.50$  for salmon fillets and  $0.58 \pm 0.03$   
11 353 items/g for smoked salmon. Based on the identification of a selection of particles, the abundance of MPs  
12 354 observed in the fillets ranged from  $0.06 \pm 0.05$  MPs/g (salmon fillets) to  $0.23 \pm 0.03$  MPs/g (smoked  
13 355 salmon). Regarding the extrapolated data, these values rise to  $0.08 \pm 0.06$  and  $0.40 \pm 0.08$ , respectively.  
14 356 Few investigations concerning the contamination of Atlantic salmon by plastics are available. The first  
15 357 data produced indicated that no particles bigger than 1 mm were found to have been ingested according  
16 358 to GIT content of Atlantic salmon from Canada (Liboiron et al., 2019). MPs have since been detected  
17 359 in the liver and muscle tissue of these species with a concentration of  $0.15 \pm 0.10$  MPs/g and  $0.10 \pm 0.04$   
18 360 MPs/g, respectively (Gomiero et al., 2020). The latter values, obtained from dissected wild salmon, were  
19 361 very different from the packaged fillets studied here. To our knowledge, only one study has investigated  
20 362 MP contamination in a transformed salmon end product, but the species was not specified (Hussien et  
21 363 al., 2021). It highlighted the absence of MPs in canned salmon. Each manufacturing step or ingredient,  
22 364 such as salt, included in the process could represent an external source of contamination recovered *in*  
23 365 *fine* in these end products.  
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34 366 For the latter two products, the particle count was  $0.15 \pm 0.08$  and  $0.17 \pm 0.02$  items/g for saithe and cod  
35 367 fillets, respectively, which is of the same order of magnitude as salmon fillets. Based on the  
36 368 identification of selected particles, the number of MPs ranged from  $0.05 \pm 0.06$  to  $0.08 \pm 0.01$  MPs/g  
37 369 for the same products. Finally, when extrapolated data were taken into account, the abundance of MPs  
38 370 ranges from  $0.06 \pm 0.05$  to  $0.08 \pm 0.01$  MPs/g. The MP contamination in these types of fillets has never  
39 371 previously been investigated since the few studies available focused on the ingestion of plastics. For  
40 372 example, an ingestion prevalence rate from 3% to 29%, with 1 to 7 plastic particles/individual, was  
41 373 observed in four studies focusing on the GIT of cod (Bråte et al., 2016; Liboiron et al., 2016, 2019;  
42 374 Walls et al., 2022). A study performed on the GIT of cod and saithe highlighted an average of 0.23 MPs  
43 375 and 0.28 MPs per individual, respectively (de Vries et al., 2020). Although these values cannot be related  
44 376 to the edible part, they suggest that the fillet contamination in our study may not only come from the  
45 377 raw material.  
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## 56 378 **4. Conclusion**

58 379 This work aimed to design a method to evaluate microplastic contamination in various seafood end  
59 380 products. An incubation of tissue in 10% KOH for 24 h at 40°C allowed us to extract MPs but a pre-  
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381 digestion step of oil filtration was necessary in oil-conserved products and a post-step of clarification  
1 382 needed when the filter color was too dark. These methods were applied to evaluate for the first time the  
2 383 abundance of microplastics and their type in different edible parts of some seafood products.  
3 384 Microplastics were observed in all the studied samples, but the exact origin of the polymers detected  
4 385 was not possible to define due to the fact that only end products were studied. Further studies on the raw  
5 386 material and the manufacturing processes leading to these end products could help to define the possible  
6 387 origin of these microplastics and to develop some solutions to reduce this contamination.  
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## 15 389 **5. CRediT roles**

18 390 **Simge Duman:** Methodology, Investigation, Formal analysis, Data curation, Writing - original draft;  
19 391 **Périne Doyen:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Funding  
20 392 acquisition; **Pauline Hogede:** Investigation; **Nicolina Andersson:** Investigation; **Romane Bayeuil:**  
21 393 Investigation; **Thierry Grard:** Funding acquisition, Writing - review & editing; **Alexandre Dehaut:**  
22 394 Conceptualization, Methodology, Supervision, Funding acquisition, Visualization, Writing - review &  
23 395 editing; **Guillaume Duflos:** Methodology, Supervision, Funding acquisition, Writing - review &  
24 396 editing.  
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## 43 402 **7. Abbreviations**

46 403 ABS: Acrylonitrile butadiene styrene; EVA: Ethylene-vinyl acetate; NA: Non-analyzed; NI: Non-  
47 404 identified; PBMA: Poly butyl methacrylate; PE: Polyethylene; PEA: Polyethylene adipate; PET:  
48 405 Polyethylene terephthalate; PES: Polyester; PLA: Polylactic acid; PP: Polypropylene; PTFE:  
49 406 Polytetrafluoroethylene; PU: Polyurethane; PVC: Polyvinyl chloride.  
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## 54 407 **8. References**

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## 11 12 13 578 **9. Figure captions**

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16 579 **Table 1:** Methods tested on different seafood matrices in order to define an optimal method with a  
17 580 tailored volume of digestion solution and ratio designed to explore the microplastic concentration in a  
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19 581 whole end product.

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22 582 **Table 2:** Concentrations of particles, identified microplastics and extrapolated microplastics observed  
23 583 in the seven seafood products studied.

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26 584 **Figure 1:** Presentation of the tested method

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28  
29 585 **Figure 2:** Examples of filters obtained by the end of filtration for each matrix

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32 586 **Figure 3:** Distribution size of particles: A for the fragments (n = 1604) and B for the fibers (n = 446)

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34 587 **Figure 4:** Identification of polymers observed in the seven seafood products studied: NI corresponds to  
35 non-identified particles. Polymer acronyms are as follows: ABS: Acrylonitrile butadiene styrene; EVA:  
36 588 Ethylene-vinyl acetate; PBMA: Poly butyl methacrylate; PE: Polyethylene; PEA: Polyethylene adipate;  
37 589 PET: Polyethylene terephthalate; PLA: Polylactic acid; PP: Polypropylene; PTFE:  
38 590 Polytetrafluoroethylene; PU: Polyurethane; PVC: Polyvinyl chloride. The figures displayed hereinafter  
39 591 correspond to the total number of particles (n) collected for the three replicates of each sample, together  
40  
41 592 with the total weight of the replicates (w). Saithe fillets (n = 114 - w = 748.1 g), cod fillets (n = 103 - w  
42 = 597.6 g), salmon fillets (n = 94 - w = 581.2 g), smoked salmon (n = 240 - w = 412.4 g), marinated  
43 593 anchovies (n = 130 - w = 465.1 g), marinade from anchovies (n = 87 - w = 180 g), canned mackerel (n  
44 594 = 160 - w = 524.6 g), canned tuna (n = 1209 - w = 529.6 g).  
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1 2 3 <i>Matrix</i> 4 5 6 7	Methods (Figure 1)	Time / T°C	Range of tissue weight (g)	Nature of digestion solutions	Volume of digestion solution (mL)	Range of digestion ratios Mass of tissue (g) / digestion volume (mL)	Optimal volume digestion (mL)	Optimal digestion ratio Mass of tissue (g) / volume of 10 % (w/v) KOH	Direct observation of digestate filters
8 9 <b>Canned tuna</b> 10 11	Method 1	16H / 40°C	132 - 190	10 % (w :v) KOH	500 - 800	1 : 2.6 to 1 : 6	800	-	-
12 13 <b>Canned mackerel</b> 14	Method 1	24H / 40°C	132 – 190	10 % (w :v) KOH	500 - 800	1 : 2.6 to 1 : 6	800	1 : 4.5	ok
15 16 <b>Saithe fillets</b> 17	Method 1	24H / 40°C	100	10 % (w :v) KOH	800	1 : 8	800	1 : 8	ok
18 19 <b>Cod fillets</b> 20	Method 1	24H / 40°C	61 - 199	10 % (w :v) KOH	300 - 800	1 : 4 to 1 : 4.9	800	1 : 4	ok
21 22 <b>Salmon fillets</b> 23	Method 1	24H / 40°C	117 - 208	10 % (w :v) KOH	800	1 : 3.8 to 1 : 6.8	800	1 : 5.8	ok
24 25 <b>Smoked Salmon</b>	Method 1	24H / 40°C	137.5	10 % (w:v) KOH	800	1 : 3.8 to 1 : 4.3	800	1: 5.8	ok
26 27 28 29 30 31 32 33 34 <b>Marinated Anchovies</b> 35 36 37 38 39 40 41 42 43	Method 1	24H / 40°C	45 -145	10 % (w :v) KOH	300 - 800	1 : 5.5 to 1 : 6.7	800	-	-
	Method 2	24H / 40°C	50	NaOCl 2.6% + NaOCl 17 %	350mL of 2.6% + 300mL of 17%	1 : 13	800	-	-
		24H / 40°C	81	NaOCl 2.6% + NaOCl 17 %	150mL of 2.6% + 250mL of 17%	1 : 4.9	800	-	-
	Method 3	24H / 40°C	56 – 134	NaOCl 2.6% + 10 % (w/v) KOH	300mL (2.6%) + 200mL (10%)	1 : 3.7 to 1 : 8.9	800	-	-
	Method 4	24H / 40°C	134	NaOCl (17%) + 20% (w :v) KOH	250mL of 17% + 450mL of 20%	1 : 5 .2	800	-	-
	Method 1a	24H / 40°C	70 - 155	10 % (w :v) KOH	800	1 : 5.2 to 1 : 11.4	800	1 : 5.2	-

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46 599 **Table 1:** Methods tested on different seafood matrices in order to define an optimal method with a

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48 600 tailored volume of digestion solution and ratio designed to explore the microplastic concentration in a

49 601 whole end product.

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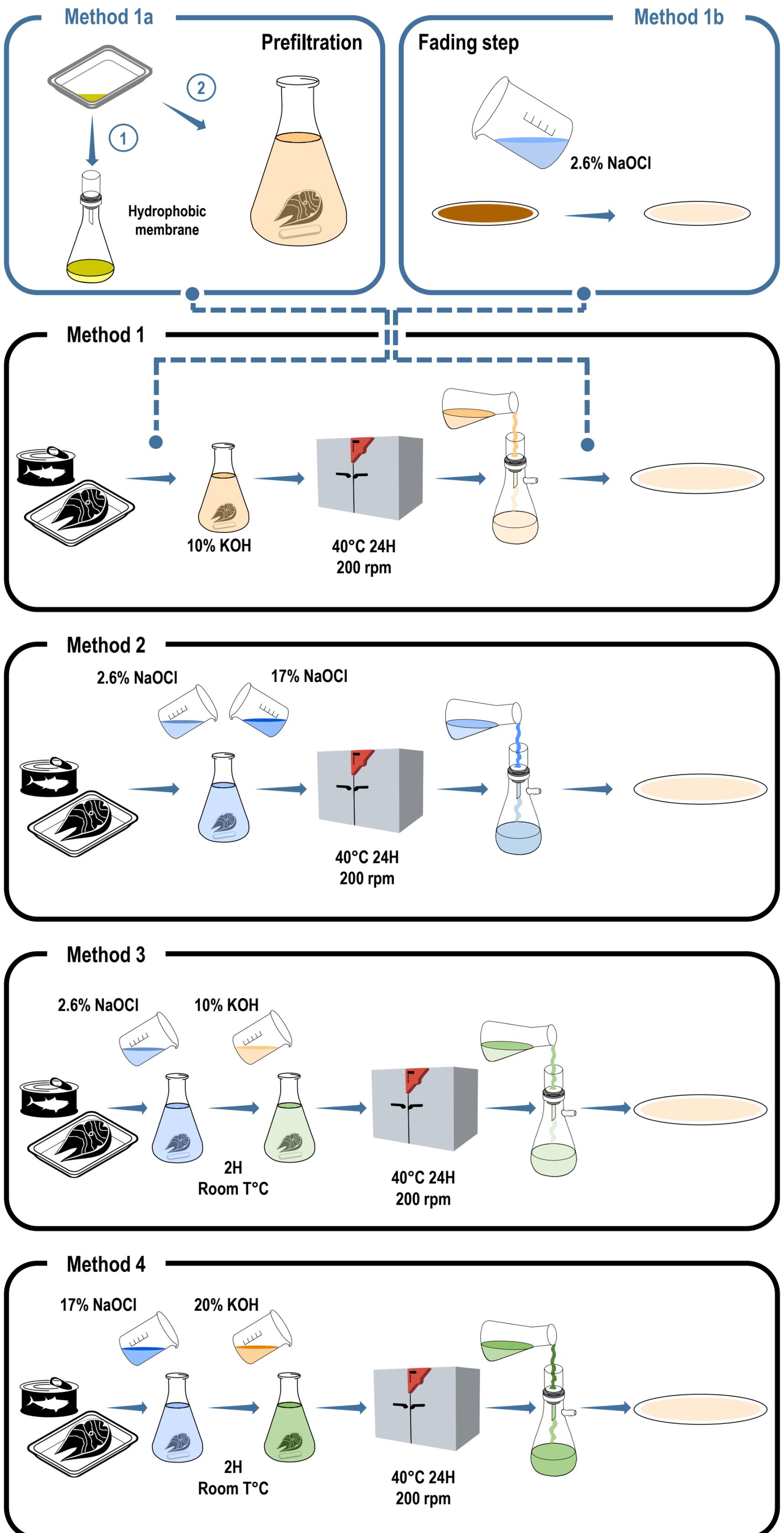
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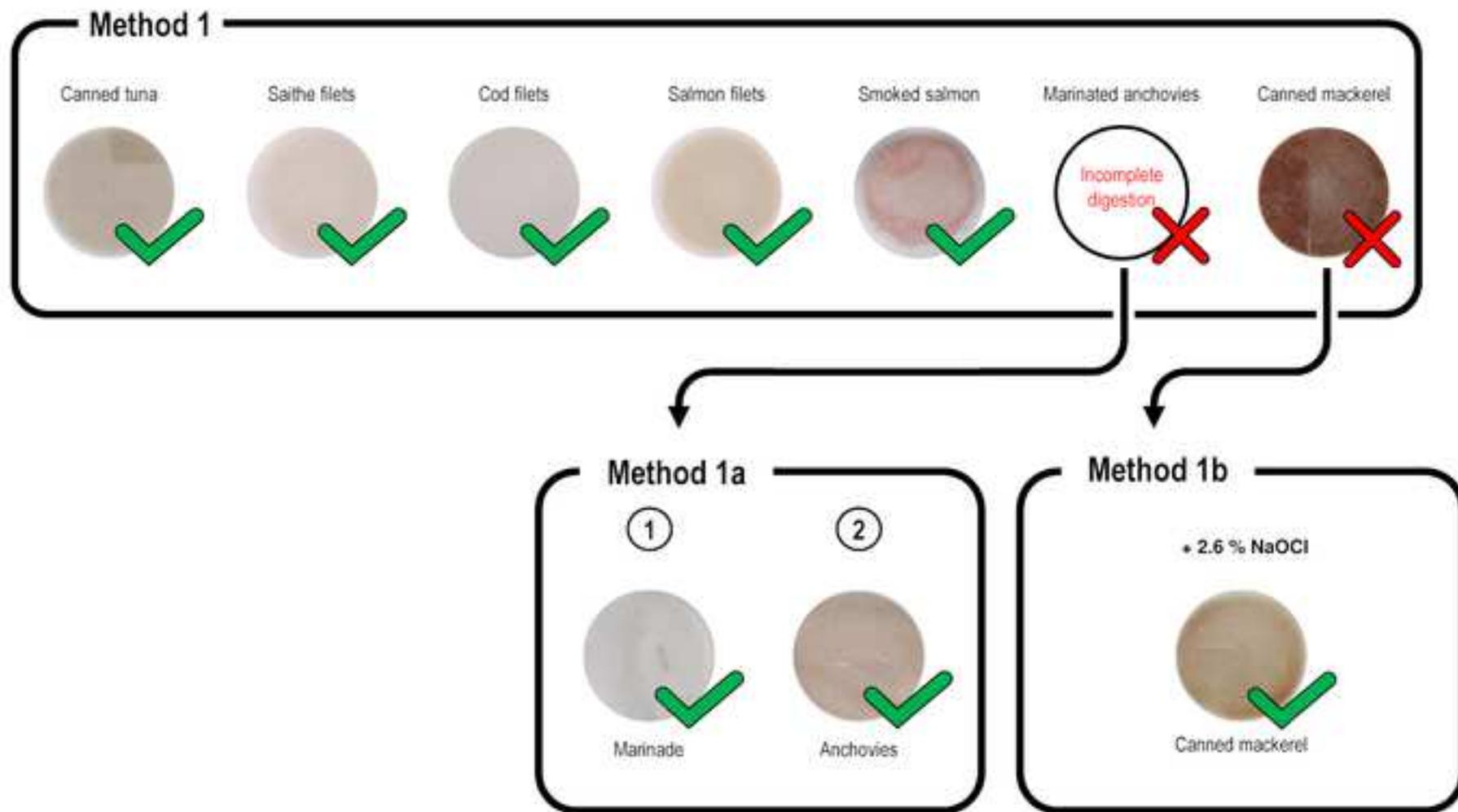
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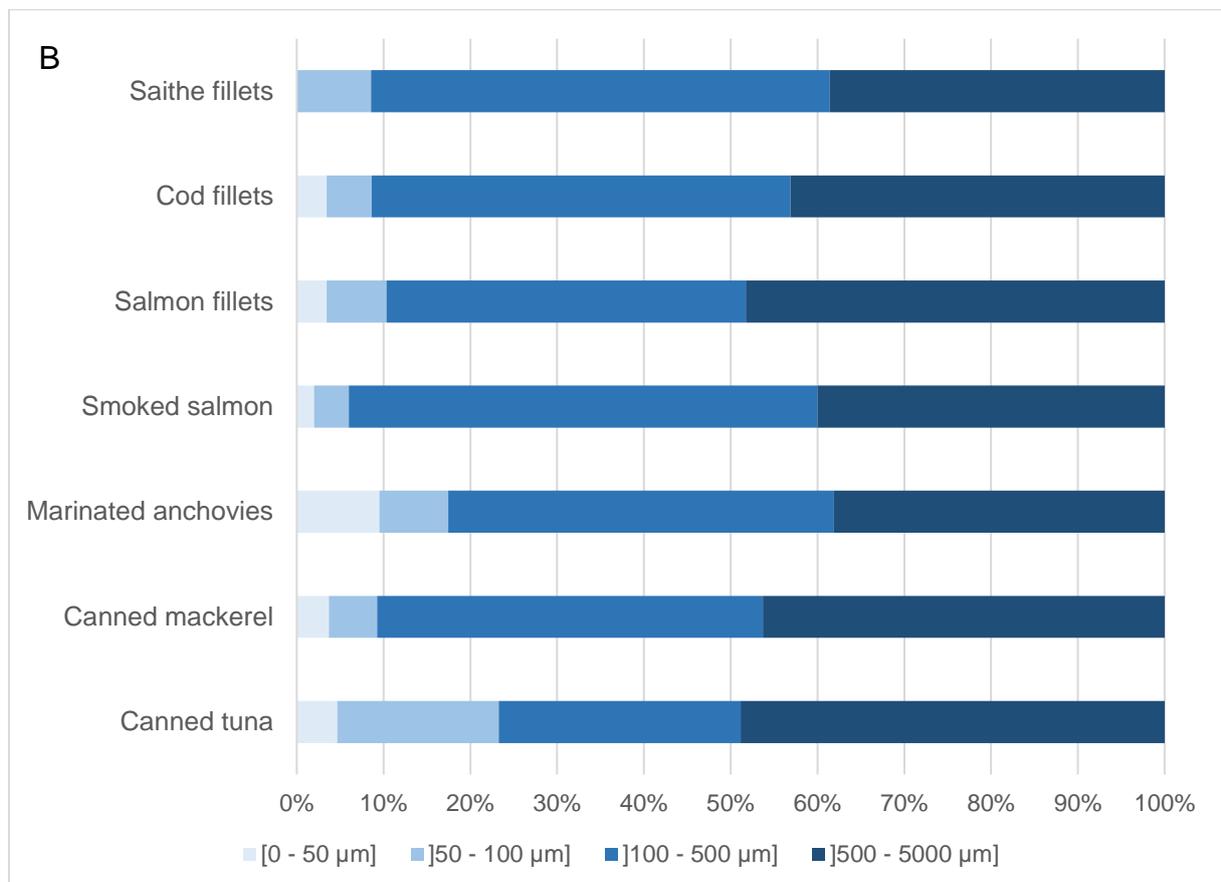
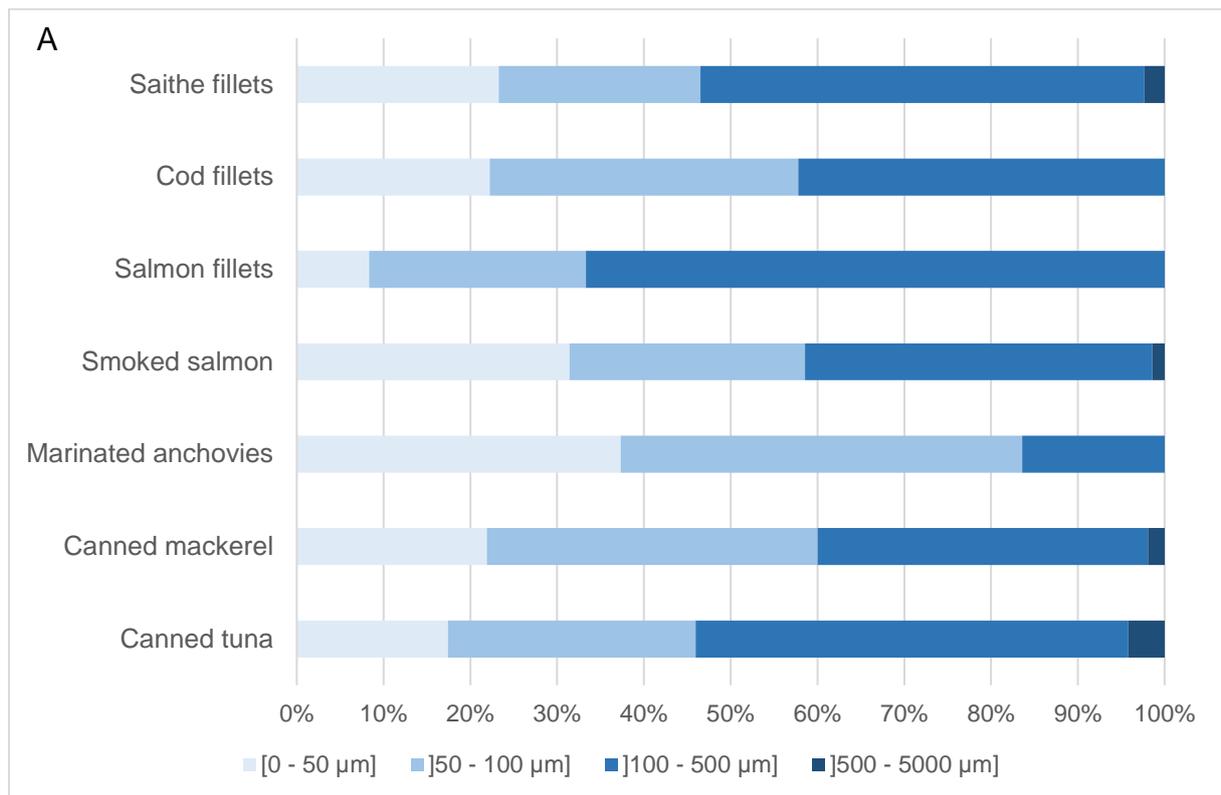
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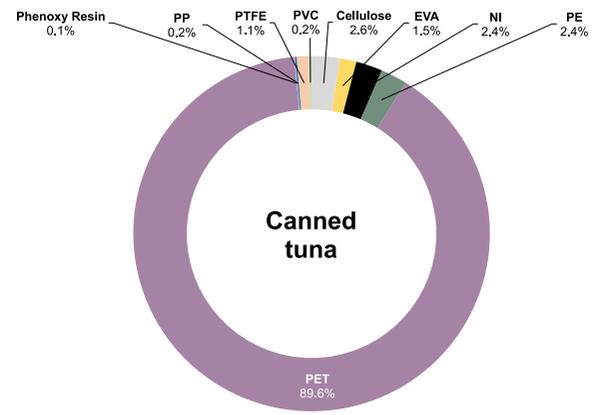
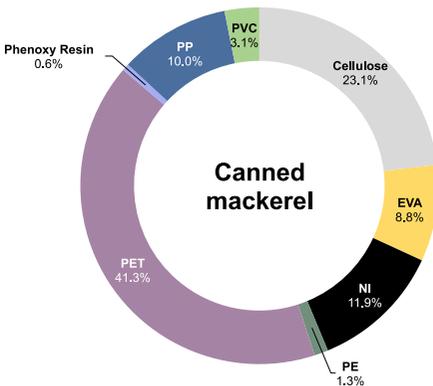
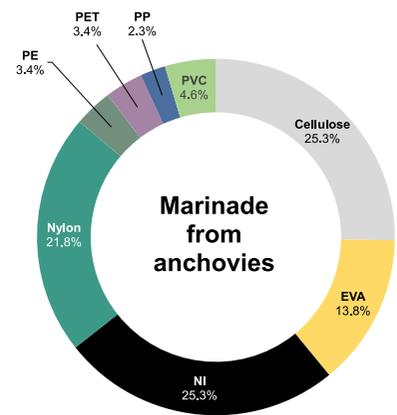
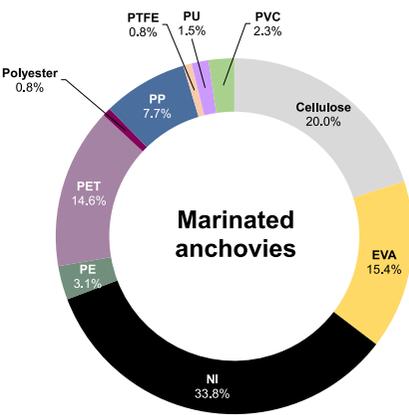
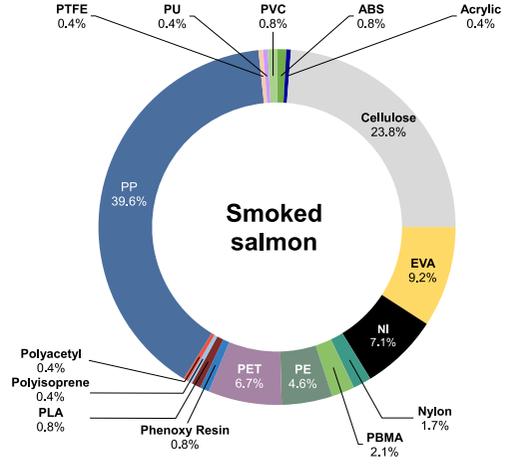
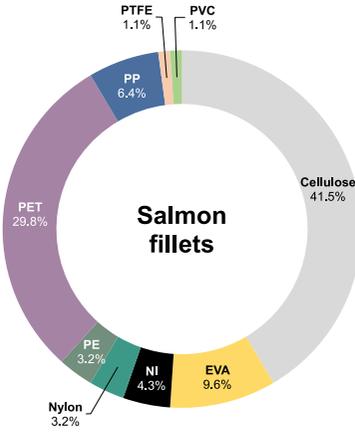
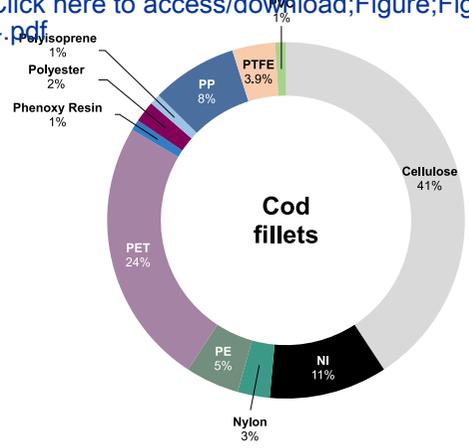
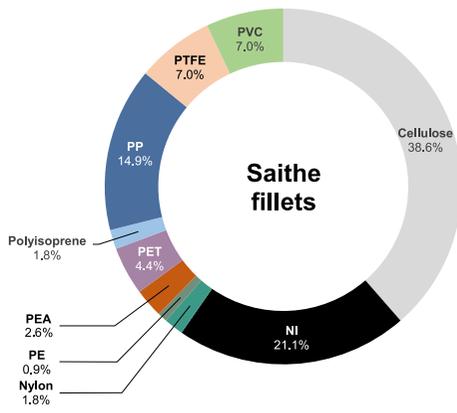
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Figure



<i>Matrix</i>	<b>Methods</b> (Figure 1)	<b>Time / T°C</b>	<b>Range of tissue weight (g)</b>	<b>Nature of digestion solutions</b>	<b>Volume of digestion solution (mL)</b>	<b>Range of digestion ratios</b> Mass of tissue (g) / digestion volume (mL)	<b>Optimal volume digestion (mL)</b>	<b>Optimal digestion ratio</b> Mass of tissue (g) / volume of 10 % (w/v) KOH	<b>Direct observation of digestate filters</b>
<b>Canned tuna</b>	Method 1	16H / 40°C	132 - 190	10 % (w :v) KOH	500 - 800	1 : 2.6 to 1 : 6	800	-	-
	Method 1	24H / 40°C	132 - 190	10 % (w :v) KOH	500 - 800	1 : 2.6 to 1 : 6	800	1 : 4.5	ok
<b>Canned mackerel</b>	Method 1	24H / 40°C	38 - 97	10 % (w:v) KOH	300 - 800	1 : 3.1 to 1 : 15.8	800	1 : 6.9	If too dark <a href="#">method 1b (Figure 1)</a>
<b>Saithe fillets</b>	Method 1	24H / 40°C	100	10 % (w :v) KOH	800	1 : 8	800	1 : 8	ok
<b>Cod fillets</b>	Method 1	24H / 40°C	61 - 199	10 % (w :v) KOH	300 - 800	1 : 4 to 1 : 4.9	800	1 : 4	ok
<b>Salmon fillets</b>	Method 1	24H / 40°C	117 - 208	10 % (w :v) KOH	800	1 : 3.8 to 1 : 6.8	800	1 : 5.8	ok
<b>Smoked Salmon</b>	Method 1	24H / 40°C	137.5	10 % (w:v) KOH	800	1 : 3.8 to 1 : 4.3	800	1 : 5.8	ok
<b>Marinated Anchovies</b>	Method 1	24H / 40°C	45 -145	10 % (w :v) KOH	300 - 800	1 : 5.5 to 1 : 6.7	800	-	-
	Method 2	24H / 40°C	50	NaOCl 2.6% + NaOCl 17 %	350mL of 2.6% + 300mL of 17%	1 : 13	800	-	-
		24H / 40°C	81	NaOCl 2.6% + NaOCl 17 %	150mL of 2.6% + 250mL of 17%	1 : 4.9	800	-	-
	Method 3	24H / 40°C	56 - 134	NaOCl 2.6% + 10 % (w/v) KOH	300mL (2.6%) + 200mL (10%)	1 : 3.7 to 1 : 8.9	800	-	-
	Method 4	24H / 40°C	134	NaOCl (17%) + 20% (w :v) KOH	250mL of 17% + 450mL of 20%	1 : 5.2	800	-	-
	<a href="#">Method 1a</a>	24H / 40°C	70 - 155	10 % (w :v) KOH	800	1 : 5.2 to 1 : 11.4	800	1 : 5.2	-



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**Supplementary Interactive Plot Data (CSV)**  
Supplementary datas.docx

