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A high-performance protocol for extraction of microplastics in fish

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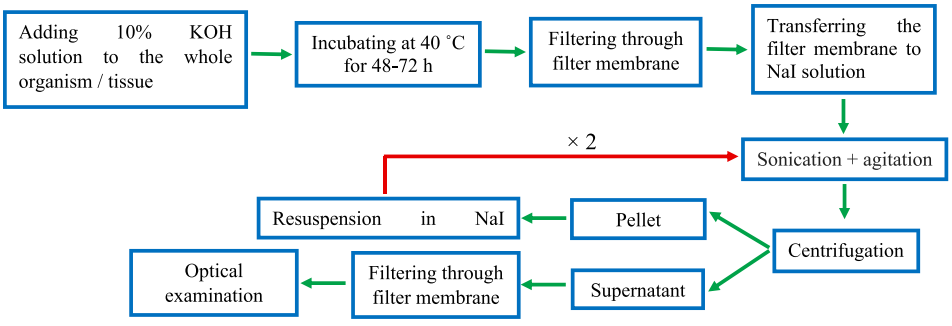
HIGHLIGHTS

- Acids, oxidative agents, and NaOH solution were not suitable candidates.
- Incubating the tissues in KOH solution at 40 °C for 48 h was the best treatment.
- Extracting the digestate with NaI solution was efficient in separating bone fragments.

GRAPHICAL ABSTRACT



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ABSTRACT

So far, several classes of digesting solutions have been employed to extract microplastics (MPs) from biological matrices. However, the performance of digesting solutions across different temperatures has never been systematically investigated. In the first phase of the present study, we measured the efficiency of different oxidative agents (NaClO or H_2O_2), bases (NaOH or KOH), and acids [HCl or HNO_3; concentrated and diluted (5%)] in digesting fish tissues at room temperature (RT, 25 °C), 40, 50, or 60 °C. In the second phase, the treatments that were efficient in digesting the biological materials (>95%) were evaluated for their compatibility with eight major plastic polymers (assessed through recovery rate, Raman spectroscopy analysis, and morphological changes). Among the tested solutions, NaClO, NaOH, and diluted acids did not result in a satisfactory digestion efficiency at any of the temperatures. The H_2O_2 treatment at 50 °C efficiently digested the biological materials, although it decreased the recovery rate of nylon-6 (NY6) and nylon-66 (NY66) and altered the colour of polyethylene terephthalate (PET) fragments. Similarly, concentrated HCl and HNO_3 treatments at RT fully digested the fish tissues, but also fully dissolved NY6 and NY66, and reduced the recovery rate of most or all of the polymers, respectively. Potassium hydroxide solution fully eliminated the biological matrices at all temperatures. However, at 50 and 60 °C, it degraded PET, reduced the recovery rate of PET and polyvinyl chloride (PVC), and changed the colour of NY66. According to our results, treating biological materials with a 10% KOH solution and incubating at 40 °C was both time and cost-effective, efficient in digesting biological materials, and had no impact on the integrity of the plastic polymers. Furthermore, coupling this treatment with NaI extraction created a promising protocol to isolate MPs from whole fish samples.

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1. Introduction

There is an increasing trend in the production and usage of plastics worldwide (PlasticsEurope, 2012, 2014) and constitute between 5.8 and 10% of the solid waste mass in different countries (Barlaz, 2006; Sokka et al., 2007). It is estimated that around 5.25 trillion plastic particles weighing about 270,000 tons are floating on the ocean's surface (Eriksen et al., 2014). Microplastics (MPs) are defined as particles ranging from 1 to 1000 μm (Karami et al., 2016b) and may be found abundantly in aquatic environments with reported concentrations of up to 100,000 particles/m^3 seawater (Norén and Naustvoll, 2010). Due to their small size and resemblance to prey, MPs may be ingested by a broad spectrum of organisms which could affect their health (Wright et al., 2013; Karami et al., 2016b).

To quantify MP loads in seafood products, an efficient digestion step is required to eliminate the biological materials and tissues masking MPs. Albeit, there is no standard method that completely removes the tissues without affecting the polymer integrity. To date, four major classes of digesting agents have been employed to eliminate organic materials, namely acids (De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015), bases (Foekema et al., 2013; Rochman et al., 2015), oxidative agents (Nuelle et al., 2014; Collard et al., 2015), and enzymes (Cole et al., 2014). There are several concerns, however, over the experimental design used in the earlier studies to develop a MP isolation protocol. Firstly, the corrosiveness of digesting solutions against MPs was not tested through spiking the biological matrices with plastic polymers but, instead, the polymers were directly incubated with the digesting solutions (e.g., Dehaut et al., 2016). To develop a high-performance extraction protocol, it is necessary to simulate the conditions that the biological materials could mask MPs within organisms, since otherwise, this could alter the behaviour of the digesting solutions towards plastic polymers. For example, it was shown that polystyrene (PS) particles melted together after direct exposure to nitric acid (HNO_3; Claessens et al., 2013), whereas they were not fused when embedded in the soft tissues of blue mussel (*Mytilus edulis*) or Manila clams (*Venerupis philippinarum*) (Claessens et al., 2013; Davidson and Dudas, 2016). Secondly, the majority of the earlier studies employed plastic particles with either a wide size range or with different sizes across the treatments, which could thus increase the chances of committing Type I or II errors. This is because smaller sized particles possess higher surface area to volume ratio compared to the larger ones and such a difference could influence the impact of digesting solutions on the MPs. For example, Avio et al. (2015) reported an extraction yield of >90% for 100–5000 μm polyethylene (PE) and PS particles but 80% for those smaller than 100 μm. Thirdly, the impact of incubation temperature on digestion efficiency and recovery rate of plastic polymers has never been systematically investigated. This is believed to be particularly important since although higher temperatures can accelerate the digestion rate, it could potentially cause more damage to the plastic polymers (Thermo Fisher Scientific, 2016). For example, KOH solution has been shown to be more aggressive at higher temperatures (Dirkse and Timmer, 1969; Patil and Sharma, 2011).

Using an inappropriate isolation method could result in the underestimation of MP loads in aquatic organisms. Also, corrosive digesting solutions may cause particle fragmentation resulting in the overestimation of MP loads. In this study, a stepwise approach was taken to optimise an extraction protocol that is efficient in eliminating biological materials while, at the same time, being inert to plastic polymers. First the digestion efficiency of the main oxidative agents [sodium hypochlorite (NaClO) and hydrogen peroxide (H_2O_2)], bases [sodium hydroxide (NaOH) and KOH], and acids [hydrochloric acid (HCl) and HNO_3] were tested against biological materials at four different temperatures (25, 40, 50, or 60 °C). Subsequently, the integrity of the main classes of plastic polymers following the treatment with digesting solutions was assessed. Using a broad polymer range in this study ensures covering most of the polymer types that are found in the aquatic organisms collected in situ (Murray and Cowie, 2011; Van Cauwenberghe et al., 2015).

Muscle and skin were used as the representative biological matrices. The skin was selected since this the first barrier that digestive solvents need to dissolve before reaching the muscles which is one of the largest organs in fish body. Due to their large volume, partial digestion of these tissues could potentially mask the tiny MPs and, therefore, hinder their visual identification. Furthermore, fish muscle is the primary organ consumed by the humans. Therefore, from the human health perspective, it is more relevant to quantify MP loads in the fish muscle. However, tissues such as bones may not be removed after digesting the muscle tissues. Similarly, minerals or the shell fragments of bivalves are commonly found in the intestinal tract of fish and these may show resistance to the digesting solutions. With these taken into consideration, an extra step was developed to separate the bones and other high-density materials from the digestate of the whole fish before microscopical inspection of the membrane filters.

Fragments (i.e. irregular shape particles) were used in this study as the representative MPs because they were reported as the dominant form in different environmental compartments (Eriksen et al., 2013; Cózar et al., 2015). Some studies have relied on the morphological characteristics of MPs such as colour, shape, and size to characterize MPs in the environmental samples (Choy and Drazen, 2013; Lusher et al., 2013). Visual identification, however, is highly subjective and, therefore, advanced analytical methods are necessary to identify the chemical composition of MPs (Lusher et al., 2013). Fourier Transform-Infrared Spectroscopy (FTIR) and Raman spectroscopy have been successfully used to determine the chemical composition of plastic polymers in earlier studies (Imhof et al., 2012; Imhof et al., 2013; Qiu et al., 2015). In the present study, Raman spectroscopy was used to assess changes in the molecular integrity of plastic polymers across different treatments. There were two prime objectives of this study: 1) to develop a time-effective and low-cost protocol that efficiently digests soft tissues of the fish while protecting the integrity of plastic polymers, and 2) to assess an extra step of excluding bones and other high-density materials from the digestates before optical examination of the recovered MPs.

2. Materials and methods

2.1. Chemicals and other materials

The materials that included KOH, NaOH, sodium iodide (NaI), HNO_3 (69%), and HCl (37%) were purchased from R&M Chemicals (UK). Solutions of KOH (10% *w*/*v*), NaOH (5 M), and NaI were prepared by dissolving powder/pellet in ultra-pure distilled water. Solutions of stabilised H_2O_2 (35%) and NaClO (10–15%) were obtained from AppliChem (Darmstadt, Germany) and Sigma-Aldrich (USA), respectively. Solutions of 5% HNO_3, HCl, and NaClO were prepared by diluting the stock solutions with ultra-pure distilled water. Filter papers were supplied by Whatman Inc. (No. 540 and 541 hardened ashless, Florham Park, MI). Low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), PS, polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polyamide-6 (nylon-6) and -66 (nylon-66, NY66) fragments were purchased from Toxemerge Pty Ltd. (Australia). About 90% of the fragments were sized below 300 μm (D90 = 300) and 10% below 80 μm (D10 = 80).

2.2. Contamination prevention

To prevent contamination, all the solvents were filtered over a Whatman No. 540 filter paper (8 μm pore size). All the glassware was washed with a commercial dishwashing liquid, then with HPL-grade distilled water, and finally rinsed with ethanol and dried in an oven. Cotton lab coat, nitrile gloves, and face masks were worn during the entire experiment. The procedure was carried out in a horizontal laminar flow