



Microplastic and mesoplastic contamination in canned sardines and sprats



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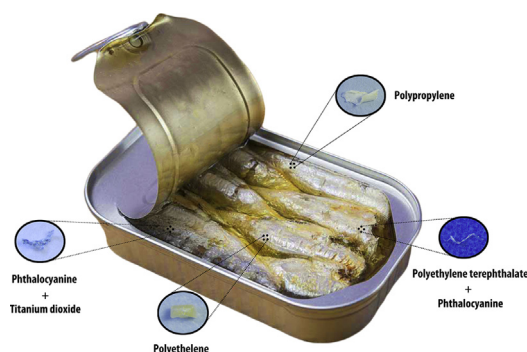
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HIGHLIGHTS

- Micro- and mesoplastics were detected in 4 brands of canned fish.
- The most abundant plastic polymers were PP and PET.
- Consumers may ingest between 1 and 5 anthropogenic particles per annum.
- The current plastic loads pose limited health risks to the consumers.

GRAPHICAL ABSTRACT



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ABSTRACT

No report was found on the occurrence of microplastics in processed seafood products that are manufactured for direct human consumption. This study investigates the potential presence of micro- and mesoplastics in 20 brands of canned sardines and sprats originating from 13 countries over 4 continents followed by their chemical composition determination using micro-Raman spectroscopy. The particles were further inspected for their inorganic composition through energy-dispersive X-ray spectroscopy (EDX). Plastic particles were absent in 16 brands while between 1 and 3 plastic particles per brand were found in the other 4 brands. The most abundant plastic polymers were polypropylene (PP) and polyethylene terephthalate (PET). The presence of micro- and mesoplastics in the canned sardines and sprats might be due to the translocation of these particles into the edible tissues, improper gutting, or the result of contamination from the canneries. The low prevalence of micro- and mesoplastics sized $> 149 \mu\text{m}$, and the absence of potentially hazardous inorganic elements on them, might indicate the limited health risks associated with their presence in canned sardines and sprats. Due to the possible increase in micro- and mesoplastic loads in seafood products over time, the findings of this study suggest their quantification to be included as one of the components of food safety management systems.

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1. Introduction

The increasing disposal of plastics into water bodies coupled with their progressive fragmentation have led to the distribution of small

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plastic particles termed mesoplastics (1–10 mm), microplastics (0.001–1 mm) (Cole and Galloway, 2015; Karami et al., 2016; Karami et al., 2017c), and nanoplastics (<0.001 mm) throughout various aquatic environments (e.g. Imhof et al., 2017). Since the first definition of microplastics by Thompson et al. (2004), many studies have reported their presence in different environmental compartments (e.g., Cincinelli et al., 2017; Frias et al., 2016; Kanhai et al., 2017). Several studies have investigated the occurrence of micro- and mesoplastics in the alimentary tract of a few fish species (e.g. Jabeen et al., 2017; Tanaka and Takada, 2016). So far, several extraction protocols have been developed for this purpose including the use of KOH (Karami et al., 2017b; Rochman et al., 2015). However, despite recent reports on the ability of microplastics to translocate from the digestive tract into other organs (e.g. Lu et al., 2016), none of the field studies were focused on potential micro-/mesoplastic loads in edible fish tissues.

Microplastics have been shown to cause toxicity in organisms (Karami et al., 2016; Rochman et al., 2013; Watts et al., 2015). Hence, the presence of plastic particles in the edible tissues of aquatic biota may put the health of seafood consumers at risk, possibly through carrying a significant amount of hazardous compounds (Karami, 2017). No information, however, was found on micro-/mesoplastic loads in processed seafood products like canned fish that are directly consumed without any further cleaning process. This could be due to lack of a reliable protocol for extracting anthropogenic particles from the whole fish. Therefore, from a human health perspective, it is of paramount importance to assess the occurrence of anthropogenic particles in these products. So far, however, investigating foodstuffs for micro-, meso-, or macroplastics (> 10 mm) has not been a part of International Standardization Organization (ISO) or any other management scheme that are responsible to test the safety and quality of end products (e.g., ISO 22000; ISO 22004, February 2017).

Although different methods might be followed in preparation of canned sardines and sprats in various countries, however, two methods namely, traditional Mediterranean and Norwegian methods, are generally proposed in production of canned sardines or sprats. In canneries, depending on the method used, sardines and sprats are pre-cooked (through steaming and drying processes) or smoked, and sterilized at a temperature between 95 and 140 °C each lasting for about 30–60 min (Warne, 1988). Therefore, in a preliminary experiment, the impact of high temperature and pressure on the morphology, mass, and Raman spectra of the main plastic polymers were investigated. Next, a total of 20 brands of canned sardines and sprats produced in 13 different countries over 4 continents were examined for the presence of micro- and mesoplastics. Little is known about the presence of other classes of contaminants like heavy metals on micro- and mesoplastics. Therefore, as the final objective of this study, the main atomic composition of isolated particles was identified.

2. Materials and methods

2.1. Materials and chemicals

Canned sardines and sprats manufactured in Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, and Vietnam were purchased from Australian and Malaysian markets. Sodium iodide (NaI), potassium hydroxide (KOH), and ethanol 95% were purchased from R&M Chemicals (UK). Solutions of NaI (4.4 M; density: 1.5 g/mL) and KOH (10% w/v) were prepared by dissolving the powder/pellet in ultrapure deionized water purified by a Milli-Q Gradient system (Millipore, Molsheim, France). GF/D microfibre filter membrane (pore size 2.7 µm) and filter membranes No. 540 and 541 (hardened ashless, pore size 8 µm and 22 µm, respectively) were supplied by Whatman. The 149 µm filter membrane was purchased from Spectrum Laboratories (USA). High-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC),

polyamide-6 (nylon-6, NY6) and -66 (nylon-66, NY66) virgin plastic fragments were supplied by Toxemerge Pty Ltd (Australia). About 90% (D90) of the particles were sized below 360 µm on their longest axis, 50% (D50) below 190 µm, and 10% (D10) were smaller than 75 µm.

2.2. Steaming experiment

Dead rainbow sardines (*Dussumieria acuta*) were purchased from a local market in Malaysia. The fish were eviscerated and the abdominal cavities were rinsed twice with distilled water. A 1 g of LDPE, HDPE, PP, PS, PET, PVC, NY6, or NY66 fragments in triplicate were then added to the abdominal cavity followed by tightly sewing the abdomen back together using a cotton string and a needle. Each fish was placed in a separate glass Petri dish filled with 30 mL of corn oil (Mazola). The Petri dishes were loosely capped and then transferred to an autoclave (Hirayama, model HA-305M; Amerex Instruments, Inc., Lafayette, CA) for steam cooking at 125 °C for 30 min. The plastic fragments were extracted according to the method of Karami et al. (2017b) as follows. The spiked fish was placed in a 500 mL DURAN glass bottle (Schott, Germany), filled with 200 mL of 10% (w/v) solution of KOH (1:10), sealed with a premium cap and a pouring ring (Schott, Germany), and incubated at 40 °C for 72 h. Afterwards, the digestates were vacuum filtered through a 22 µm-pore size filter membrane using a vacuum pump (Gast vacuum pump, DOA-P504-BN, USA) connected to a filter funnel manifold (Pall Corporation, USA). Next, the filter membrane (22 µm) was soaked in 10–15 mL NaI solution and was sonicated, agitated, and centrifuged. The supernatant of the mixture was then filtered through another filter membrane (8 µm) to isolate the plastic polymer fragments. Finally, the filter membrane was placed in a clean glass Petri dish and dried in an oven at 50 °C for 5 h. The extracted microplastics were weighed on a scale with 0.1 mg precision, and the recovery rate of each polymer was calculated as below:

$$\text{Recovery Rate (\%)} = \frac{W_a - W_b}{W_i}$$

where W_a = weight of dry filter membrane after filtration (filter membrane + microplastics), W_b = weight of dry filter membrane before filtration, and W_i = initial weight of spiked microplastics.

The microstructure of the fragments before and after the steaming treatment was studied using a scanning electron microscope (SEM) (Hitachi S-3400-II, USA) at an acceleration voltage of 5 kV. To prevent charging of samples with the electron beam, samples were first subjected to gold coating (~5 nm) using a coater (Quarum Q150R S) for 5 min before being examined with SEM. Ten particles were examined per treatment. Moreover, the polymers before and after steaming were analyzed with Raman spectroscopy for any changes in their spectra.

2.3. Contamination prevention

To minimize sample contamination, the extraction process was performed inside a pre-cleaned (with ethanol using SCOTT® paper towels made of cellulose) horizontal laminar flow cabinet (Model AHC- 4A1-ESCO). Also, procedural blanks were run in parallel with the samples. Cotton lab coat and nitrile gloves were worn during the experiment. All the glassware and forceps were washed with a detergent, rinsed with deionized water, once with ethanol, and dried in an oven at 50 °C for 5 h. To avoid contamination during the drying process, the caps of laboratory bottles were loose, and the forceps were placed in beakers covered with an aluminum foil. Lastly, all the solutions including deionized water, ethanol, and NaI and KOH solutions were filtered through 8 µm filter membranes (one filter membrane per ≈ 2 L of solution/solvent). The gloves were removed from the packaging and worn inside the laminar flow cabinet.

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