

MASTER'S THESIS

# Development and testing of methods for extraction and characterization of microplastics from food products

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Máster Universitario en Ciencia e Ingeniería Avanzada de Materiales

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## List of Abbreviations

CA	Cellulose Acetate
CE	Circular Economy
CIT	Centre of Technological Innovation
DLS	Dynamic Light Scattering
DSC	Differential Scanning Calorimetry
EPS	Expanded Polystyrene
FEDIT	Federation of Technological Centres
FTIR	Fourier Transform Infrared
GHG	Green-House Gases
HDPE	High Density Polyethylene
HEHS	Human and Environmental Health and Safety
MPs	Microplastics
OM	Organic Matter
PA6	Polyamide
PET	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
SEM	Scanning Electron Microscopy
$T_c$	Crystallization temperature
TGA	Thermogravimetric Analysis
$T_m$	Melting temperature

## Abstract

The increase in the use of products containing plastic and the use of both intentionally added microplastics (MPs) and microplastics from degraded plastics, are raising concerns for the potential negative impact toward the human population and the environment. Moreover, microplastics could enter into the human body through the food chain. In the present study, the presence of microplastics in table sea salt was studied. This commercial and widely used food product is quickly dissolving in water, without matrix and it reported to contain microplastics. An extraction method for the microplastics contained in sea salt was tested and further quantification was studied. Furthermore, in this project a microplastics generation method was developed by milling under cryogenic conditions. Samples were from six different plastics products, namely polyethylene terephthalate (PET), polypropylene (PP), polyvinyl chloride (PVC), high density polyethylene (HDPE), expanded polystyrene (EPS) and polyamide (PA6) which were used as positive control samples. Knowing the content and chemistry of microplastic, a variety of common characterization techniques for plastics were performed to comprehend which is the best method for size characterization and distinction between plastic families.

## Resumen

El aumento en el uso de productos hechos a partir de plástico, juntamente con el uso de microplásticos añadidos intencionadamente y los microplásticos debido a degradación, están haciendo incrementar la preocupación del impacto negativo contra la población y el medio ambiente. Estos microplásticos podrían ser incorporados en el cuerpo humano mediante los alimentos. En este proyecto, ha sido estudiada la presencia de microplásticos en sal marina comercial. Este producto alimenticio ampliamente utilizado, según la literatura, contiene microplásticos. Se ha probado y estudiado un método de extracción de microplásticos contenidos en sal marina y también cómo facilitar su cuantificación. Además, en este proyecto, se ha desarrollado un método de generación de microplástico por molienda bajo condiciones criogénicas. Las muestras son provenientes de seis tipos diferentes de plásticos: *Polyethylene Terephthalate (PET)*, *Polypropylene (PP)*, *Polyvinyl Chloride (PVC)*, *High Density Polyethylene (HDPE)*, *Expanded Polystyrene (EPS)* y *polyamide (PA6)*, todas como muestras de control positivo en los ensayos de extracción y cuantificación. Sabiendo el contenido y química de los microplásticos, se han hecho varias caracterizaciones con la finalidad de entender cuál es el mejor método para conocer el tamaño de partícula y la diferenciación entre familias de plásticos.

## Resum

L'augment de l'ús de productes fets a partir de plàstic, juntament amb l'ús de microplàstics afegits intencionadament i microplàstics deguts a la degradació, están fent incrementar la preocupació del possible impacte negatiu en la població i el medi ambient. Aquests microplàstics podrien ser incorporats al cos humà mitjançant els aliments. En aquest projecte s'ha estudiat la presència de microplàstics en sal marina comercial. Aquest producte, àmpliament utilitzat, ha estat reportat que conté microplàstics. En aquest treball, ha estat provat un mètode per l'extracció de microplàstics continguts en sal marina i també la seva quantificació. A més, en aquest treball s'ha desenvolupat un método de generació de microplàstics per molta sota condicions criogèniques. Les mostres son provinents de sis tipus diferents de plàstics: *Polyethylene Terephthalate (PET)*, *Polypropylene (PP)*, *Polyvinyl Chloride (PVC)*, *High Density Polyethylene (HDPE)*, *Expanded Polystyrene (EPS)* i *Polyamide (PA6)*, totes com mostres de control positiu durant els experiments d'extracció i quantificació. Sabent el contingut dels microplàstics, diverses tècniques de caracterització han estat realitzades als plàstics amb la finalitat d'entendre quin és el millor mètode per conèixer la mida de partícula i la família entre els plàstics.



## 1. Introduction

### 1.1. Problematic with the plastics

One of the most worldwide used material is plastic. Plastic is a term used in many fields to describe both physical properties and behaviour of materials, as well as the name of a class of materials. The term “plastic” is used to define a sub-category of larger class of materials called polymers. Polymers consist in large molecules with long chain-like molecular architecture and very high molecular weights [1]. In addition, they are sub-divided into thermoplastics, thermosets, and elastomers, so the correct terminology in this case should be thermoplastics. Since the 1950s, the production and use of thermoplastics have been growing rapidly due to the exceptional properties of this material such as durability, versatility, malleability and low cost. In 2010, the global primary plastic production was 270 million tonnes per year, however the global plastic waste was 275 million tonnes per year, taking into account the incorporation of the other plastic sources from previous years [2]. Seven years later, in 2017, the global plastic production approached to 350 million of tonnes [3].

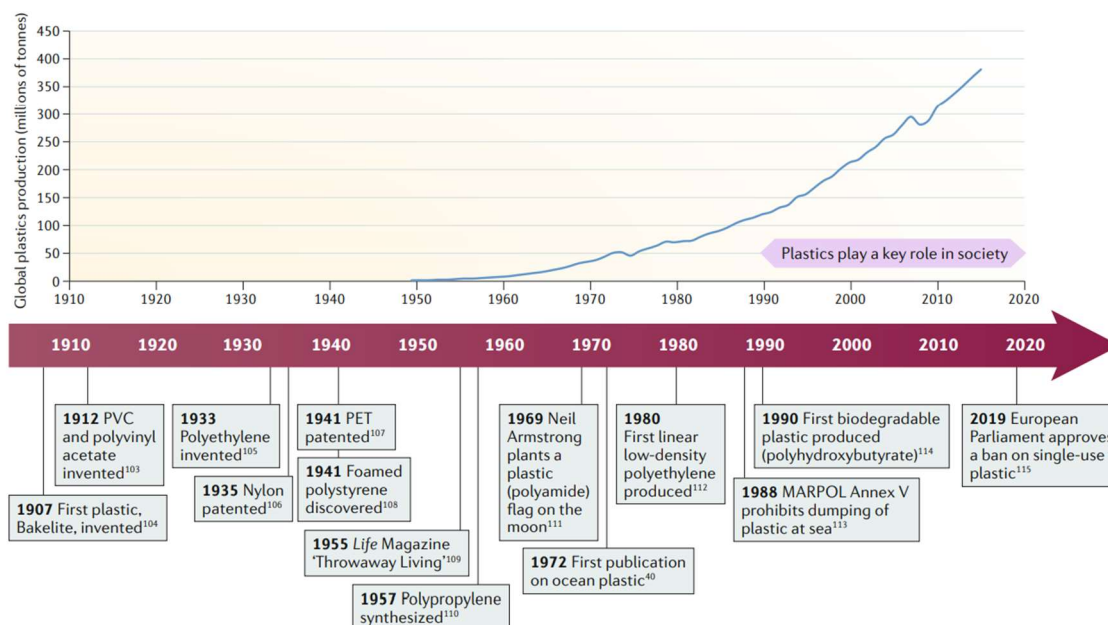
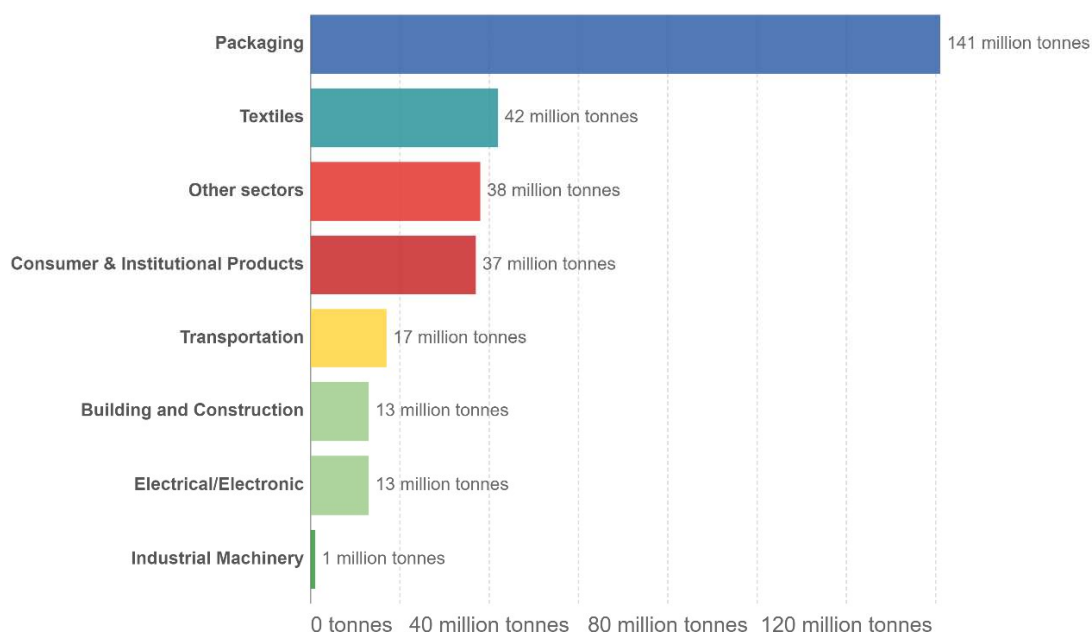


Figure 1. A timeline of plastic innovation, discovery and pollution [3].

Most plastics are dedicated to packaging and construction, and in a smaller proportion, to other applications such as textile, automotive or agriculture (Figure 2). EU is taking action to tackle the problem with plastic pollution and marine litter, emphasising the transition to a circular plastics economy. It is aiming: to transform the way plastic products are designed, produced, used and recycled in the EU, to transition to more sustainable plastics economy and to support more sustainable and safer consumption and production pattern for plastics, among others. Most plastics are made from fossil fuels, which contribute to increase greenhouse gas emissions and pollution. Alternative plastics, such as bio-based, biodegradable and compostable plastics may be a more sustainable alternative to fossil-based and non-biodegradable plastics [4].



Source: Geyer et al. (2017)

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Figure 2. Global plastic waste generation by industrial sector and measured in tonnes per year in 2015.

Around 80 % of marine litter is plastic [4]. Commonly, the end-point of plastic is marine environments. They can be found in two main different forms: litter and small particulates with size below 5 mm called microplastics. Specific policies also have been applied to address the growing volume of microplastics in these environment, such as to develop labelling, standardisation, certification and regulatory measure on unintentional release of microplastics or to further develop and harmonise methods for measuring unintentionally released microplastics [5].

## 1.2. Microplastics

### 1.2.1. Categorization

Plastic debris could be classified by size in mega-debris (>100 mm), macro-debris (>20 mm), meso-debris (20-5 mm) and micro-debris (< 5 mm) [6]. First observation of microscale plastic particles was in the early 1970s. It was not until 2004 that the term “microplastic” was started to be used as a result of a study done by Thomson et al. “*Lost at sea: where is all the plastic?*” [6], [7]. Microplastics are widely defined as synthetic polymers with an upper size limit of 5 mm and without specified lower limit. As is shown in Figure 3, the largest release of microplastics is from the synthetic textiles, followed by the tyres manufacturing [8]. These microplastics can be transported by rivers, discharged from water treatment plants, wind and surface run-off into either fresh water and seawater environments. Some of the microplastic sources are intentionally added and some of them are induced. Microplastics can be categorized into primary (originally manufactured to be at that size) or secondary microplastics (originate from fragmentation of larger items) [9]. Secondary microplastics are derived from the fragmentation of large plastic debris due to different processes such as physical (i.e. friction), chemical (i.e. photodegradation) or biological interactions [9].

The following list [10] separates by primary or secondary microplastics the most common sources of microplastics release:

Primary microplastics:

- Personal care products: A variety of personal care products contain intentionally added microplastics. These products wash down drains and represent 2% of total microplastic release into the marine environment.
- Plastic manufacturing: Pellets are virgin or recycled plastic particles, used as raw materials in manufacturing of most plastic products. However plastic pellets, flakes and powder loss occurs all along the plastic production, transport and recycling value chains.

Secondary microplastics:

- Textiles: Dispersion of synthetic fibres and fabrics from clothing is not only the result of washing and wearing, it also takes place during production. 70% of textiles produced today are synthetic and shed large quantities of microplastic into the environment, around 35% of total microplastics release.
- Road Transport: Tyre wear alone generates over 1.3 million tonnes of microplastics in Europe per year. Recycling of old tyres is often problematic, as their use in artificial reefs or sports pitches directly releases microplastics into the environment (28%).
- Fishing, Aquaculture and Shipping: Microplastics are released by ships' greywater flowing unfiltered into the ocean, degradation of lost fishing gear, single-use plastic waste from fishing and aquaculture, marine paints and coatings.
- Agriculture: A variety of agricultural sources contribute to dispersing microplastics into the ground, for example through the widespread use of cultivation films, and sewage sludge from wastewater treatment plants used as soil fertilizer.
- Tourism: Particular pressure is exerted by tourist's waste on local waste management systems or for example the massive use of single-use plastic products and packaging.

The impacts of microplastics on the climate and on the environment are still not well-known. Some studies suggested that they can also contribute to the emissions of green-house gases (GHG) due to the release of methane or ethylene after the gradual degradation and fragmentation of certain type of plastics, commonly associated to single-use plastics and packaging. Another possible impact would be the reduction of carbon sequestration because of plankton and its less effective fixation of carbon and transportation to the deep ocean [11].

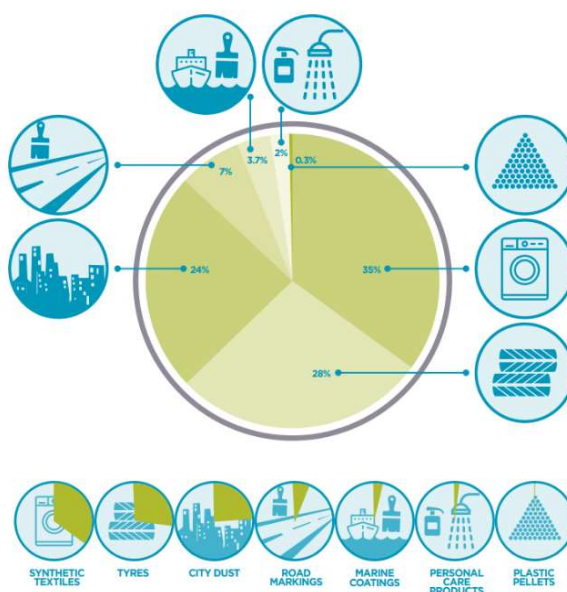


Figure 3. Global release of microplastics to the world oceans [8].

### 1.2.2. Microplastics in humans

Parallely to the concerns of microplastic in the environment, the concern about the potential toxicity in humans have been increasing as well, reflected in the number of publications about the topic through the last years. Current studies rely on laboratory experiments that expose human tissues, cells, and animals such as mice or rats to microplastics. Recently, is has been reported the discover of microplastic in human bloodstreams. The analysis of blood samples from 22 donors, all adults and in health condition, revealed a concentration of 1.6 µg/mL in 17 samples, and half of them contained PET plastic [12].

Microplastic particle can be a problem due to the accumulation in marine organism and its transfer through the food chain to higher trophic levels including humans [13]. In particular, the presence of microplastics in seafood products is often raised as a threat to human health. Furthermore, the European Commission's Rapid Alert System for Food Safety Authority's website reported the presence of microplastics contaminants in a wide variety of human food items [14]. A study conducted by the University of Newcastle, Australia, [15] estimates that an average person could be ingesting approximately 5 g of plastic per week. The detection of microplastics in human-related food such as seafood, drinking water, milk, beer, table salt, have been attracting the attention of the latest investigations [13]. No clear evidence of the impact of microplastic have been clearly assessed yet. The main reason lies in the difficulty of identification and quantification methods, they are costly and time-consuming [16]. Most of the samples contaminated with microplastic contain organic and inorganic matrix, from which the microplastics should be recovered [17]. Several approaches have been developed for the microplastic separation from complex matrices, some of them based on various mechanisms of leveraging plastic properties that differ from their surrounding media such as density, oil solubility, electrostatic, magnetism and solvent extraction separations [18]. Two of the most reported are density-based separation and digestion.

#### - Density-based separation

In presence of inorganic residues contained in the samples, differences in density can be used to separate plastics (0.8-1.6 g/cm<sup>3</sup>) from sediment (2.7 g/cm<sup>3</sup>), usually by carefully mixing the sediment with salt saturated solutions and collecting the supernatant containing microplastics for further filtration [19]. The popularity of the use of NaCl for density separation experiments relies on its inexpensiveness, accessibility and low chemical hazard. However, NaCl is only able to isolate low-density plastics (<1.2 g/cm<sup>3</sup>, e.g. PE, PS, PP), preventing the separation of almost 20 % of the plastics consumed in Europe [20].

To overcome this problem, many studies use ZnCl<sub>2</sub> or NaI to be able to collect common high-density plastics even if hazardousness (corrosive due to acid behaviour, harmful to the environment), bubbling and foam formation due to reaction with organic matter, as well as cost represent potential constraints to the widespread use of these solutions. ZnCl<sub>2</sub> and NaI economic and environmental cost can also be overcome by reducing initial sample size or recycling.

#### - Digestion

Digestion, when dealing with a bulk sample mainly composed of organic matter, as compost and sludge, contributes to remove most of the matrix and to reveal microplastics, which instead are

resistant to digestion treatment. Digestion of organic matter can be acid, alkali and with the use of oxidizing or enzymatic agents [17], however, it is only described here the two most common: acidic and alkaline [20].

Acidic approaches, particularly when using acids at high concentrations (e.g. 65–69 %  $\text{HNO}_3$ , 15 %  $\text{HCl}$ ) are not suitable for microplastic analysis. Therefore, the relationship between the efficiency on organic matter (OM) reduction and the effect on polymers is one of the most important considerations when selecting the digestion approach. As an example, the popularity of using oxidative methods, particularly 30 %  $\text{H}_2\text{O}_2$  and Fenton's reagent (but also  $\text{NaClO}$ ), relies on being simple and rapid methods, efficient in OM degradation (especially in samples of vegetal origin) with limited impact on polymers, compared with the common acidic approaches used. This variation on the effects of oxidative methods may depend on the agent concentration, method temperature or duration [20].

Alkaline methods, particularly 10 %  $\text{KOH}$ , are also commonly used in complex samples, especially in samples of animal origin since they are an easy, accessible, inexpensive and relatively low chemical hazard option. The effects of 10 %  $\text{KOH}$  on microplastics ranged from no observable effects to mass changes in cellulose acetate (CA), PS, PC and PE (12–22 %), FTIR spectra changes in CA (e.g. decrease in peaks) or PET degradation [20].

For the more specific case of salt samples, an easier procedure can be followed since the lack of organic matrix. However, some literature performs a digestion before the separation to remove sand (sea salt) or other possible residues contained on it. For the separation step, salt was mainly reported to be dissolved in a concentration of 200 g/L of distilled water. The density of this mixture was determined (by using a pycnometer) and give an average value of  $1.27 \text{ g/cm}^3$ . Then, the solution was placed in most of the cases in a centrifuge at 1900 rpm for 1 h, in order to separate the possible sand contained by the salt from the saline solution. Although the density of PET is quite similar to that of the saline solution (ca.  $1.3 \text{ g/cm}^3$ ), the fact that the plastics are in form of microfibers highly decreases their apparent density so all MPs are expected to remain at the upper part of the solution [21]. For the filtration step, the solution was normally filtered through a piece of  $5 \mu\text{m}$  pore size, 47 mm cellulose nitrate filter paper or a nylon filter at  $0.45 \mu\text{m}$  pore size, using in both cases a vacuum system [21]. Christiane Zarfl et al. [22] suggested that decreasing pore size is accompanied by increasing sample processing time and increased clogging of the pores, where a thick layer of natural particles may accumulate on the filters. To avoid this problem, filter cascades can be applied and sample volumes can be subdivided into smaller volumes before the data are pooled from each subsample.

Visual sorting is one of the most commonly used methods for the qualitative identification and quantification of microplastics. For the identification of the chemical composition and confirmation of microplastic's presence, infrared spectroscopy is a reliable technique. Another technique is the Raman spectroscopy which also gives information about the crystalline structure of the polymer [23]. Both of them, in many cases, coupled to an optical microscope to aim the particles or fibres to analyse.

### 1.2.3. Sea salt used for the study: obtention method

There are several types of table salt according to their origin, but the most sold and consumed salt is sea salt. To produce this salt, sea water is pumped into evaporation ponds, where the salt is concentrated by the action of sun and wind. After some time, the salt condenses and crystallizes. Before packing, it is cut and collected under controlled processes. This process induces the hypothesis that the salt from seawater may contains microplastics and there is a necessity to monitor the presence of such contaminant [21]. Recent studies point out the presence of microplastics in seas all over the world, with emphasis on those countries with the highest population (i.e. China or India) [24], [25].

### 1.3. Work site: LEITAT

LEITAT is a Technological Centre specialized in production technologies, developing R&D activities in the areas of engineering, material science, environment, biotechnologies and renewable energies with deep knowledge and experience in technological transfer to several industrial sectors. LEITAT is recognized by the Spanish Government as a CIT (Centre of Technological Innovation) and is one of the 5 Technological Centres that is accredited by the Regional Government of Catalonia. LEITAT is also a member of FEDIT (Federation of Technological Centres in Spain) and the IT Network of the Catalan Regional Government. Moreover, LEITAT has more than 2000 square meters of well-equipped laboratories and constituted of different research divisions, located in the headquarters in Terrassa and in two more premises in Barcelona and Vilanova del Camí. The centre offers the knowledge of professionals from different areas such as biomedicine, nanotechnology, biotechnology, smart systems, construction materials, energy harvesting, environment and health & safety, among others. The Circular Economy (CE) department provides innovative technologies and strategies for a sustainable production, efficient management of the natural sources, optimums treatment and valorisation of waste fluids. Into this department, is located the area of Human and Environmental Health and Safety (HEHS). The area is divided in four main knowledge fields: environmental toxicology, human toxicology, materials safety, and a transversal focused on regulation and legislation. This work was done within the activities of the Materials Safety group. The group is internationally renowned by its work on the assessment of (nano)material release from products during different stages of their life cycle and lately, the knowledge built on (nano)material is applied to other materials such as the microplastics.

## 2. Objectives

As reported in the Introduction part, there is a need to study the presence of microplastics in commercial food products. The table salt is a relatively simple food sample to study the possible contamination with microplastics because of its solubility in liquid medias. In literature it is reported many times the fact that one of the main endpoints of the plastic is the marine environment. Consequently, the sea salt, extracted from sea water, will likely contain microplastics of different chemistries, sizes, shapes, colours, etc. [13], [24], [26].

For these reasons, the objectives of the project will be:



1. Standardization of a method for the generation of microplastics from different polymers. The benefits of a controlled fabrication would be the generation of control samples of known polymer compositions, and at a known size for the present and future studies (i.e. ecotoxicology studies, human toxicology studies, degradability of bio-based polymers).
2. Study if the method for the conversion from plastic to microplastics affects in term of microplastics degradability or chemistry.
3. Quantification of the microplastic collected and how the different methodologies for the extractions affect the following quantification.

The two main work-flows of the thesis and the link between them are summarized in the following Figure 4:

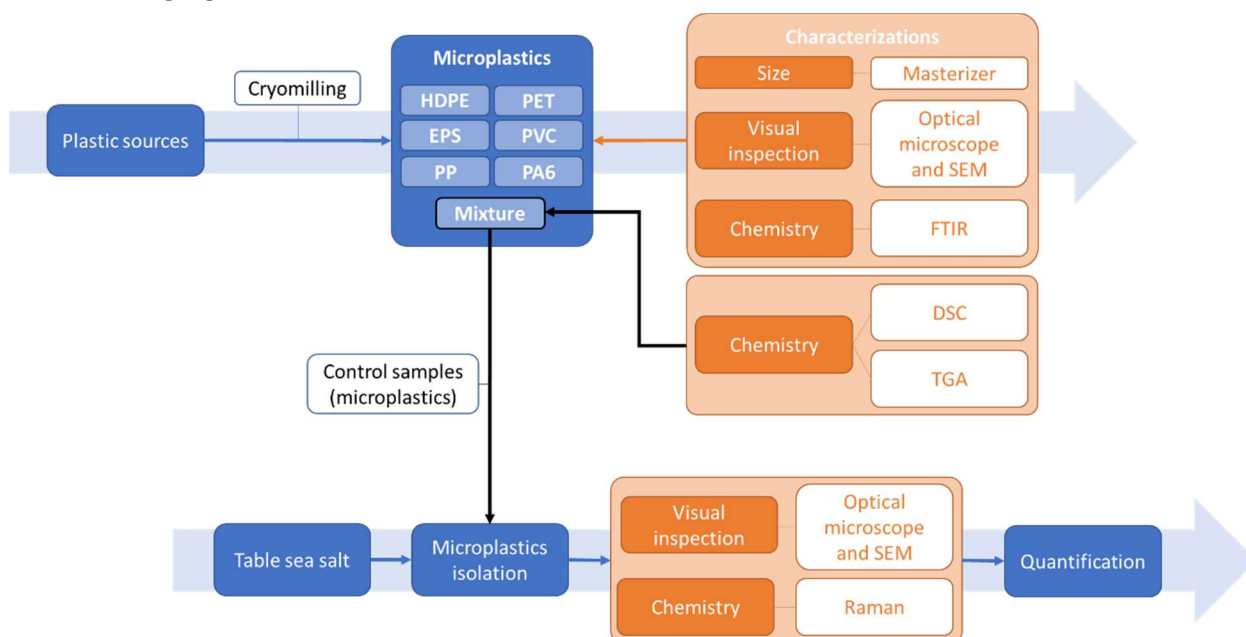


Figure 4. Thesis work-flow.

### 3. Materials

#### 3.1. Raw plastics

Six different plastics were used in this study to obtain 'standard' microplastic that could be used as control samples in the filtration and detection trials. The selection of the plastics was based on the percentage of each plastic found in the environment and the availability of plastic sources in a common supermarket. In this sense, the PET plastic is extracted from water bottles. The brand *Solan de Cabras* was selected for the blue coloration of the bottle, facilitating the observability and differentiability from other particles. PP was sampled from plastic containers (i.e. *Tupperware*) commonly made of this material. Some plastic material dedicated for laboratory use is made of HDPE. EPS was easy to find as protectors in shipment boxes. The PVC plastic was extracted from tubes commonly used for electrical installations. Finally, PA6 was selected due to the availability of this material in the group from other ongoing projects and its coloring. In this case, PA works as a matrix and contains a nanomaterial used as blue pigment.

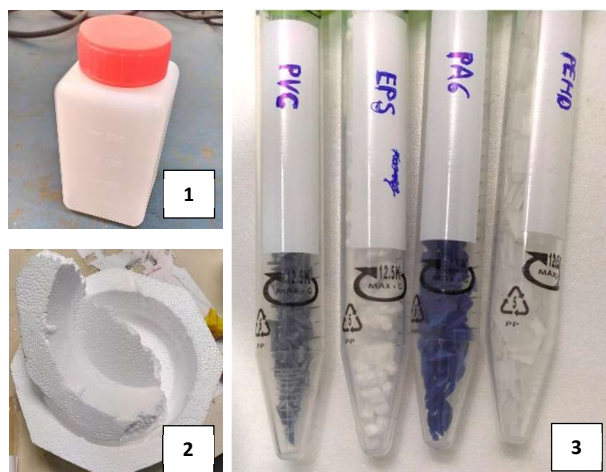


Figure 5. Examples of size reduction from debris to microplastics. 1) HDPE container; 2) EPS from a delivery box; 3) Result of different plastics after cutting and preparation for milling.

### 3.2. Characterization equipment

The equipment used for the size reduction of the plastic was the ultra-centrifugal mill (cryomilling) *Retch* model ZM200 (Figure 6). This mill pulverizes a great variety of soft to medium-hard and fibrous substances extremely fast, thus allowing for a fast sample production. The main reason for its choice as the equipment to reduce from plastic pieces to microplastic was because it allows the introduction of liquid nitrogen at the same time the equipment was performing the milling and, the interchangeable sieves at different mesh sizes to ensure the better homogeneity of the microplastic particles after the milling.

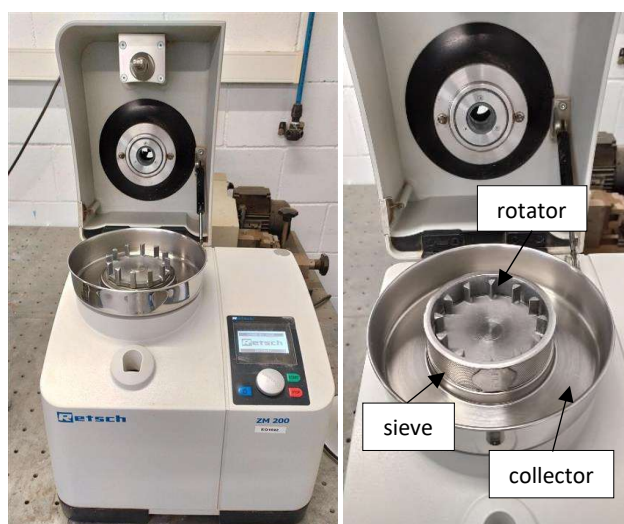


Figure 6. Ultra-centrifugal mill *Retch* model ZM200 (left); internal components of the mill in charge of the size reduction (right).

The equipment used for the particle size measurement was the model *Mastersizer 3000* from *Malvern*. It is based on the technique of laser diffraction to measure the particle size and particle size distribution of materials. It does this by measuring the intensity of light scattered as a laser beam passes through a dispersed particulate sample. This data is then analysed to calculate the



particle size that created the scattering pattern. Two different modules can be coupled to the equipment to measure both dispersion of particles in a liquid media and particles in powder.

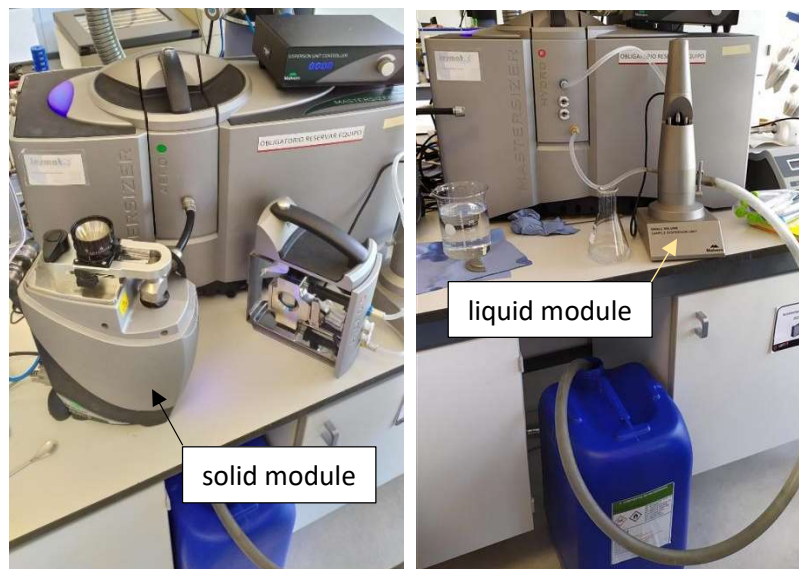


Figure 7. Mastersizer 3000 with the solid module connected (left) and with the liquid module connected (right).

Other complementary equipment used for the characterization of the samples were the Dynamic Light Scattering (DLS) (Malvern, model ZEN3600), Fourier Transform Infrared (FTIR) spectroscopy (Shimadzu, model IRAFFINITY-1S), Thermogravimetric Analysis (TGA) (TA Instruments, model Q500), Differential Scanning Calorimetry (DSC) (TA Instruments, model Q20), optical microscope (OLYMPUS, model DP71), confocal Raman microscope (Renishaw, model inVia Qontor), Scanning Electron Microscopy (SEM) (JEOL, model JSM-6010LV). Experimental conditions for the analysis with the equipments are detailed in the following section 4. *Methods*

## 4. Methods

### 4.1. Microplastics generation

Nowadays, there is no standardized method for the reduction of plastic into microplastics, so the process described below is an attempt to obtain microplastics with a homogeneous shape and size in the range of the micrometres. The first step is the cutting of the plastics into small pieces of approximately 1 cm<sup>2</sup> to facilitate the charging of the materials in the milling equipment. To standardize the protocol, 9 grams of each plastic were weighted and divided in 3 replicates of 3 grams. There was a pre-conditioning step to cool down the plastic pieces, placing them in a container with 250 mL of liquid nitrogen for some seconds. Parallely, in the cryomilling, it was fixed the 1 mm pore size sieve and set the equipment at 1600 rpm. The incorporation of small plastic pieces into the equipment should be done one by one to avoid the plugging of the equipment entrance during the milling and to try to control the increase of temperature that could melt the plastic. To decrease the internal temperature of the rotor, it is constantly incorporated liquid nitrogen during the process. The final volume of nitrogen liquid to ensure the temperature stability during the incorporation of plastic was 1 L approximately. Once the 3 g were ground, the same sample was subjected to a further milling but with a smaller cut-off the sieve of 80 µm. This gradual reduction of the size by different mesh size was done to ensure a

better homogeneity of the particles size. Finally, the microplastics obtained were collected and the equipment was cleaned and dried to avoid contamination between samples.

Since the microplastics found in real scenarios are usually a mixture of polymers, an additional sample of mixed microplastics was generated weighting and mixing together 10 mg of each polymer microplastic generated by cryomilling to try to simulate a real case sample.

#### 4.2. Particle size measurement

Two different methods were applied for the particle size measurements depending on the state of the sample: dispersed in a liquid or as solid materials. Both have advantages and disadvantages, that are reflected on the results obtained.

##### 4.2.1. Solid Module

Microplastic powder was deposited on the hopper of the equipment. The solid module coupled to the Mastersizer (Figure 7) uses a pump to create the vacuum needed to move the powder through the detector and to measure it. It is mandatory to control the feed rate to ensure a homogeneous drop of particles. The main advantage of the solid module is the short time needed to perform the measurement, however, on the other hand, there was no possibility of sample recovery once the 15 measurements are finished.

##### 4.2.2. Liquid Module

In the liquid module connected to the Mastersizer (Figure 7), the minimum concentration required to guarantee the proper measure of the particles was 1 g of sample in 1 L of dispersant. The amounts were adjusted to smaller quantities, 100 mg of plastic in 100 mL of ethanol 70% (ethanol:water; 70:30 v/v). Ethanol was considered a better dispersant than water to measure the size of the micro plastic particles due to the hydrophobic character of the plastic samples. The dispersion should be stirred to avoid the sedimentation or flotation of the particles. Stirring velocity also should be adjusted to avoid the presence of bubbles in the dispersion. Once the measurements (15 in total) were done, the sample was removed, the tubing of the dispersion module was cleaned with distilled water and it was rinsed again with ethanol.

##### 4.2.3. SEM characterization to individual microplastics

To observe the samples under SEM, each microplastic sample was fixed on a support. The supports had a carbon tape on the top surface to ensure the adhesion of the microplastic particles. Electrical conductivity of microplastics is very low so the samples were sputter coated with a gold layer of 5-10 nm for the properly observation under the electrical microscope.

##### 4.2.4. TGA characterization to the microplastic's mixture

For TGA analysis it was studied the mixture of microplastic generated to obtain a degradation profile. In this case, it was weighted 13.814 mg of mixture in an aluminium pan and it was set temperature ranging from 23 °C to 700 °C. To have an inert atmosphere and to avoid the combustion of the polymers, nitrogen gas was blown inside the equipment. Once the measurements were done, the blow of N<sub>2</sub> inside the chamber's equipment was stopped and it was started to be slowly replaced by air. Finally, the weight losses were highlighted doing the first derivate of the temperature in order to obtain the maximum degradation rates.

#### 4.2.5. DSC characterization to the microplastic's mixture

A heating-cooling method was performed in a Tzero aluminium hermetic pan. Two different DSC analyses were done. The first one was done with 6.408 mg at 2°C/min but after the heating cycle, the pan opened. In the second trial, less amount of sample was used, namely 2.231 mg and the conditions of the DSC were set with a cooling cycle to -20 °C and, from that temperature, a heating was done to 300 °C, because PET has one of the highest melting temperatures of the microplastics' mixture at 260 °C [27]. Then, a second cooling cycle was set to -20 °C. The ramp of temperature was decided at 5° C/min to ensure the stability of the measurements.

#### 4.3. Extraction of microplastics

Different methods were tested based on reported literature about the isolation of microplastics. The starting point for the study was the table salt due to the simplicity of the matrix and dissolution in liquid media. Since the expected concentration of impurities was low, no digestion was needed. Three different brands of commercial table salt were tested. In each trial, 20 g of salt was dissolved in 100 mL of distilled water by triplicate under stirring, shaking the salt container before weighting to homogenise the content. After dissolution, an extra step of bath sonication was included for 3 minutes to ensure the proper dissolution of all the salt grains. For the filtration of the salt solutions, it was used a full Pyrex filtration kit and both cellulose paper filters and nylon filters of different micropore sizes (5 µm and 0.45 µm). With these filters, only microplastics were aimed to study, however by literature it was also expected to have nanoplastics (from 1 µm to 100 nm [28]) in solution.

In addition, the effect of a centrifuge step after the dissolution of salt in water was also studied with the objective to remove the inorganic impurities (i.e. sand). After dissolving 100 grams of salt in 500 mL of distilled water, the solution was divided in vials of 50 mL to further centrifuge. The parameters of the centrifugation were 1900 rpm for 1 h, as reported in literature [21].

#### 4.4. Quantification of microplastics

The identification, characterization, and quantification of the microplastics needs further developments. Nowadays one of the most used methods, and the one used in this project, is the manual sorting. To facilitate the counting of microplastics, a grid of 0.5 x 0.5 cm per square was drawn on the filter before the filtration step. The observation of the plastic particles was performed by an optical microscope.

### 5. Results and discussion

In the following section are showed and discussed the two main work-flows of the master's thesis. In the first section (5.1 *Microplastics generation*) is it explained the results obtained from the characterization of the single microplastic families generated by cryomilling (Mastersizer and SEM) and also the characterization to distinct in a synthetic mixture how to distinct, if possible, the different already know families of polymers contained by FTIR, TGA and DSC. Then, in the second section (5.2 *Extraction and quantification of microplastics in salt*), it is discussed the methodology applied to isolate microplastic from real samples of commercial table salt and finally, the characterization done by µ-Raman to identify the possible families of the isolated microplastics.

### 5.1. Microplastics generation

After the cryomilling process for the plastic size reduction (4.1 *Microplastics generation* ) it was observed a loss of material. Initially, the amount of plastic was 3 g and the final weight measured after the cryomilling was around 0.5 - 0.6 g. In Figure 8 is highlighted in red where the plastic was stuck, and it was neither milled by the rotatory metal nor passed through the sieve. The collection of the microplastic was also difficult due to its strong adhesion to surfaces and low weight. Furthermore, by visual inspection it was possible to appreciate particles with an inhomogeneous size and bigger than the expected size of the mesh sieve. The particles were likely to be bigger than 80  $\mu\text{m}$  and at different size distributions.

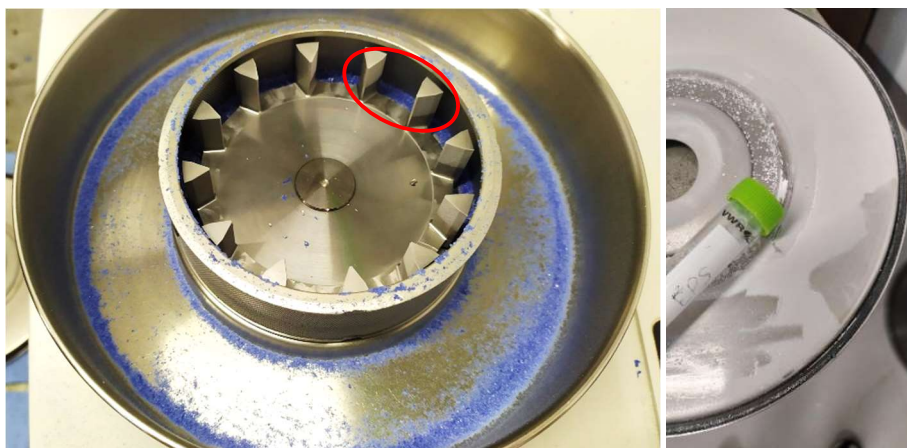
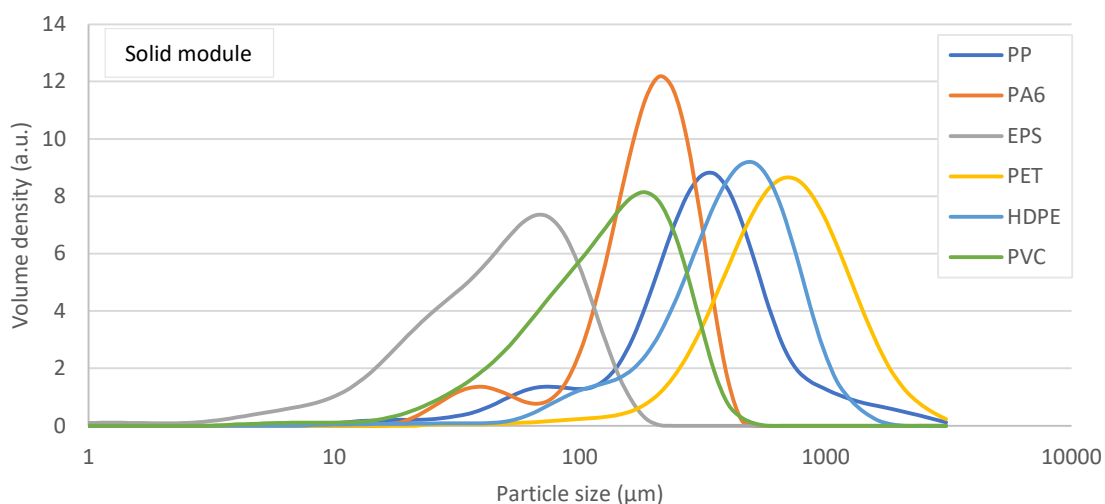


Figure 8. Cryomilling container after PET conversion into microplastic (left). High adhesion of EPS microplastic to the container lid (right).

The size distribution of the microplastic samples generated was studied by the Mastersizer, using both solid and liquid modules. In Figure 9 below is plotted the particle size distribution obtained after using the solid module.



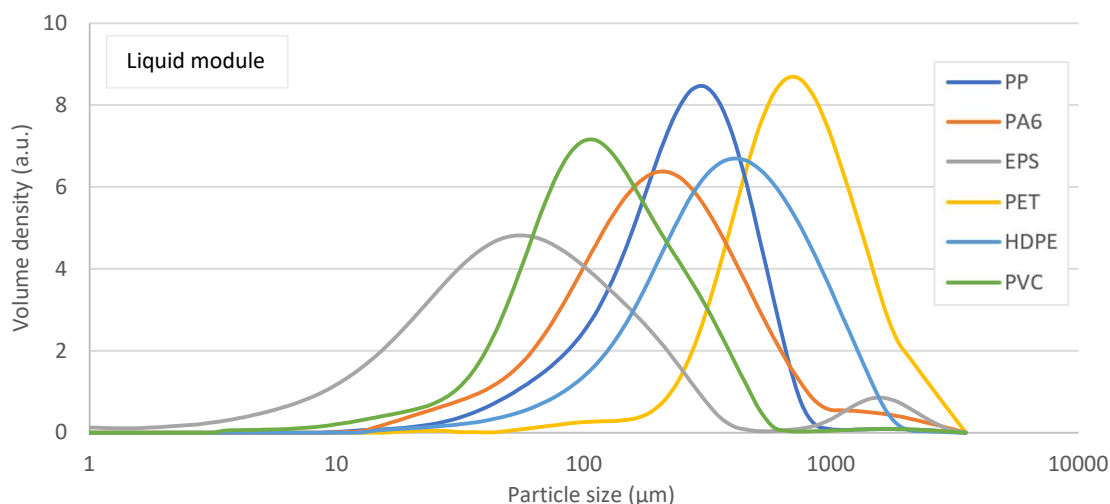


Figure 9. Average particle size distribution in Mastersizer solid module (upper) and liquid module (lower).

The observation of the different distributions shows a broad distribution in the results obtained. The particle size varies from 70 to 700  $\mu\text{m}$ . The only polymer obtained in a range of an expected size (80  $\mu\text{m}$ ) was the EPS at 76  $\mu\text{m}$ . However, the peak makes relevant the fact that the microplastics homogeneity is not reached. Similar effect of inhomogeneity in the size distribution is observable for the rest of polymers, but in the particle size range higher than 100  $\mu\text{m}$ . It is done a special emphasis in the PA6. The PA6 peak seems to demonstrate better homogeneity due to the highest volume density and the narrower distribution, however the same peak by liquid module shows higher polydispersity. Plastic particles, when it was used the liquid module, were not easily dispersable in water media, therefore it was decided to disperse the particles in ethanol. Because of this, high energetic stirring was needed before the introduction in the equipment. An advantage of the liquid module is the continuous flow of sample through the equipment and the results are similar to solid module, which means both module give comparable results. EPS measured in liquid module gives an average particle size in the range it was expected but the broad distribution of the results keeps high. Despite that, liquid module gives more stable results, with more repetitibility between the 15 technical measurements as compared to solid module.

The difference between the cut-off diameter of the sieve and the diameters obtained by Mastersizer could be related to two different hypotheses. One possibility could be the contamination of some not well-grounded particles that were collected and then measured. Another possibility could be related with the assumptions the software was doing. Mastersizer software converts the light scattered in an equivalent sphere diameter. These spherical particles could be assumed bigger than the reality of the microplastics, with a certain density different from the plastic bulk.

Images by SEM were taken of all the samples in order to confirm the result obtained by Mastersizer. The particle size for all the plastics samples was not homogeneous as well. About the shapes of the particles, in almost all the plastic families the particles could be considered roughly spherical but other shapes like discs, plates or fibres could be observed.



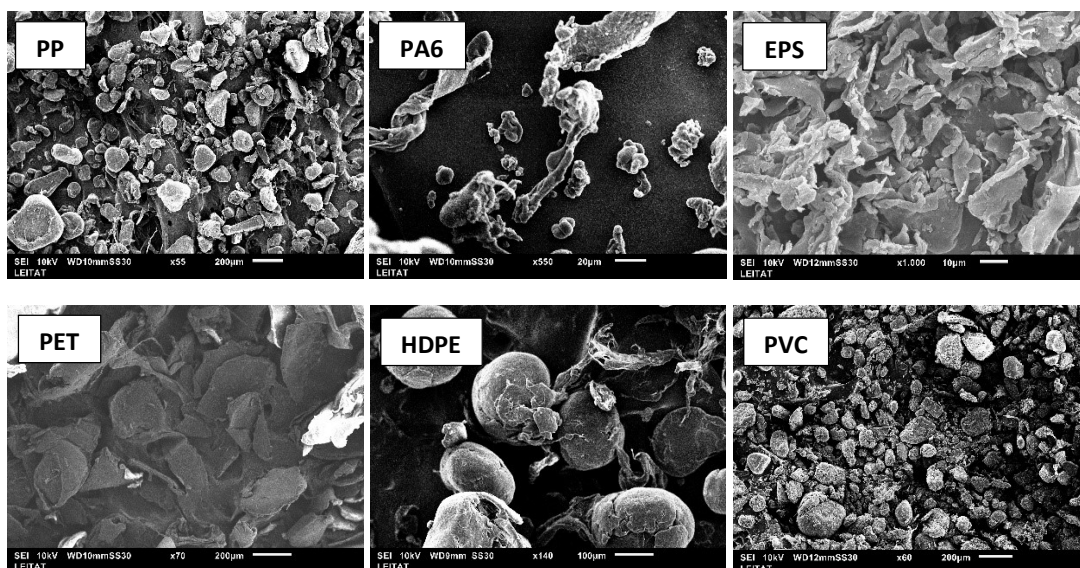


Figure 10. SEM images of the six different microplastic families.

Because the determination of the type of polymer is a key aspect for the microplastics studies, different characterization equipment's were used to try to identify the chemistry of the microplastics mixture that was previously prepared as positive control. The generation of the mixed microplastic sample was explained in 4.1. *Microplastics generation*. Fourier transform infrared was one of the characterization techniques used for the detection of the microplastics powder by the further distinction of peaks corresponding to each polymer in the mixture, if possible. In Figure 11 the individual spectrum of each microplastic generated is compared with the spectrum of the mixture.

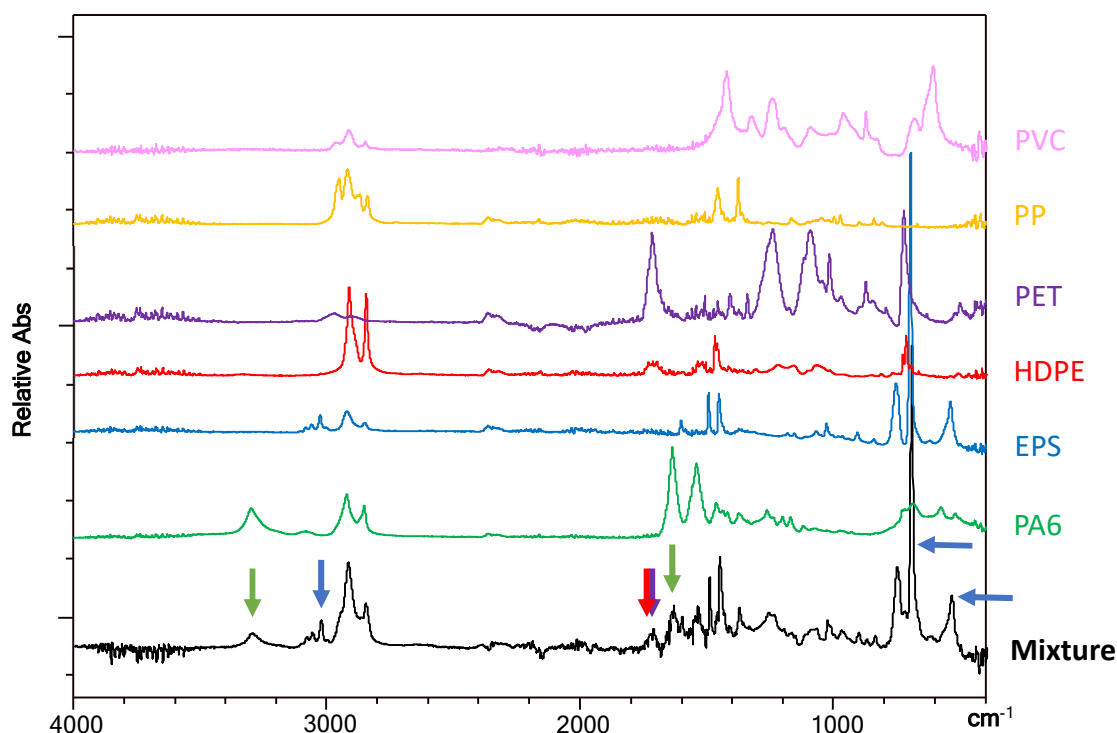


Figure 11. FTIR spectra of both the single microplastic generated and the spectrum of their mixture.

Observing the spectra of the mixture (in black), it is possible to distinguish some characteristic peaks [29] possibly corresponding to only one of the polymers. These peaks are highlighted by arrows in colours. In the range between 3500 - 3000  $\text{cm}^{-1}$ , two peaks could be differentiated. One in green at 3300  $\text{cm}^{-1}$  could be from the PA6 and correspond to the -C=O bond of the monomer. The next differentiative peak in blue arrow at 3000  $\text{cm}^{-1}$  could be associated to the EPS because the single spectrum of the EPS contains the same peak and none of the other polymers in the mixture have it. This peak could be representative of the benzene of the polystyrene (PS) monomer. Then, at 1750  $\text{cm}^{-1}$ , a peak is highlighted by two different arrows, red and purple. The peak could be associated to the HDPE or to the ester group of the PET. After this peak, the green arrow shows a peak at 1600  $\text{cm}^{-1}$  that could correspond to PA6 and its -N-H- bond. Finally, in the range between 500 – 400  $\text{cm}^{-1}$ , there are two intensive peaks possibly coming from the EPS and the impurities contained on it. This last range mainly corresponds to the bonds with heavy elements.

A summary of the previous discussions relating the frequency ranges and the candidates of the nature of vibration are included in the following Table 1. Unfortunately, different conclusions could be reached for the distinctive peaks, therefore no confirmation could be assessed to the previous assumptions.

Table 1. Summary of the possible candidates to the frequency ranges.

Frequency ranges ( $\text{cm}^{-1}$ )	Candidates to the nature of vibration
3300	-C=O bond (PA6)
3000	Benzene group
1750	Ester group (PET); Impurities (HDPE)
1600	-N-H- bond (PA6)
400	Impurities (EPS)

Degradation of the polymers by TGA was also assessed as an alternative method for the detection of the different microplastic families in a mixture generated by cryomilling. In Figure 12 are included both the weight loss due to the increase of temperature and the derivate of the curve for the better distinction of the maximum degradation rates.

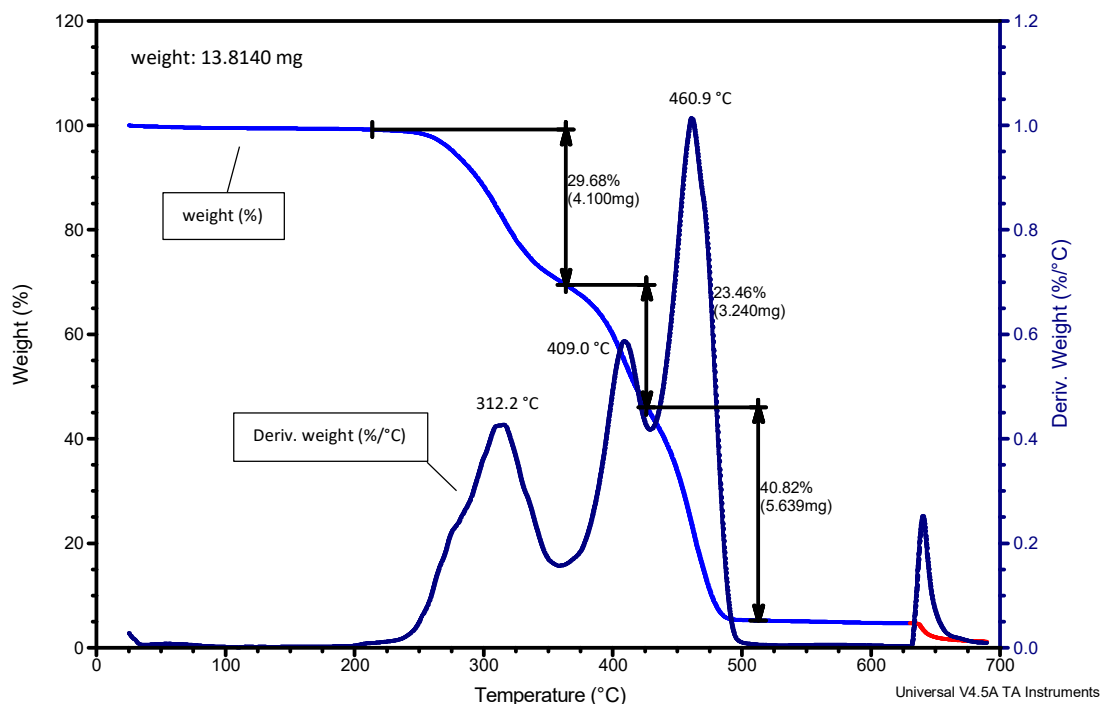


Figure 12. TGA analysis of the microplastic's mixture.

Three main steps could be differentiated, one at 220 °C with a weight loss of 29.7 %, another at 360 °C with a weight loss of 23.5 % and finally one at 425 °C with a weight loss of 40.8 %. On the range between 600–700 °C there is a smooth decrease of weight in red colour corresponding to the exchange from a N<sub>2</sub> atmosphere to an air atmosphere with O<sub>2</sub>. The weight loss related to this decrease is because the combustion with oxygen of the residual sample material after a TGA process. On the other side, in the derivate curve of the weight loss (%/°C), it is possible to distinct three main peaks. The first maximum peak with a degradation rate of 312.2 °C, second maximum is at 409.0 °C and third maximum is at 460.9 °C. This last maximum peak makes relevant the fact that there is some overlapping of peaks due to the similarity in the temperature range which the polymers degraded. For HDPE, degradation should occur between 400 and 500 °C. Degradation profiles as well as the basic degradation mechanism of PP is similar to PE, however the presence of methyl groups in every alternative carbon atom in the main PP backbone allows the formation of more unstable secondary free radicals which lead to its lower thermal stability. For PVC, the chemical structure and composition make this polymer even more thermally sensitive than PE and PP. Unlike PE, PP and PVC, degradation of PS results in the formation of monomer as the major product. In inert atmosphere, PET should show thermal stability up to 400 °C and then degradation should occur following an abrupt single mass loss between 400 °C and 460 °C. Similar process should occur for PA6 but with the abrupt single mass loss step between 400 °C and 475 °C [25].

Differences in the degradation temperature were expected to be observed between polymers at micron size, however the equipment was not sensitive enough to avoid overlapping of the peaks due to their similar thermal degradation range of temperatures. Also, different TGA conditions could be tested to improve the results and to try to avoid the overlapping. Considering the results obtained for this specific sample, TGA is not a suitable technique for the



determination of the polymer content in a known mixture of microplastics if there is overlapping of peaks. For this reason, its use is not recommended to further study real samples with a more complex and with an unknown mixture of microplastics.

For the mixture of known polymers, DSC was also used as a characterization method to identify by their melting temperature the already known microplastics in the generated mixture. In Figure 13, at the heating cycle (red line) four peaks are easily recognised and one more in the range between 40-60 °C that could be considered as a step in the heat capacity curve at the glass transition.

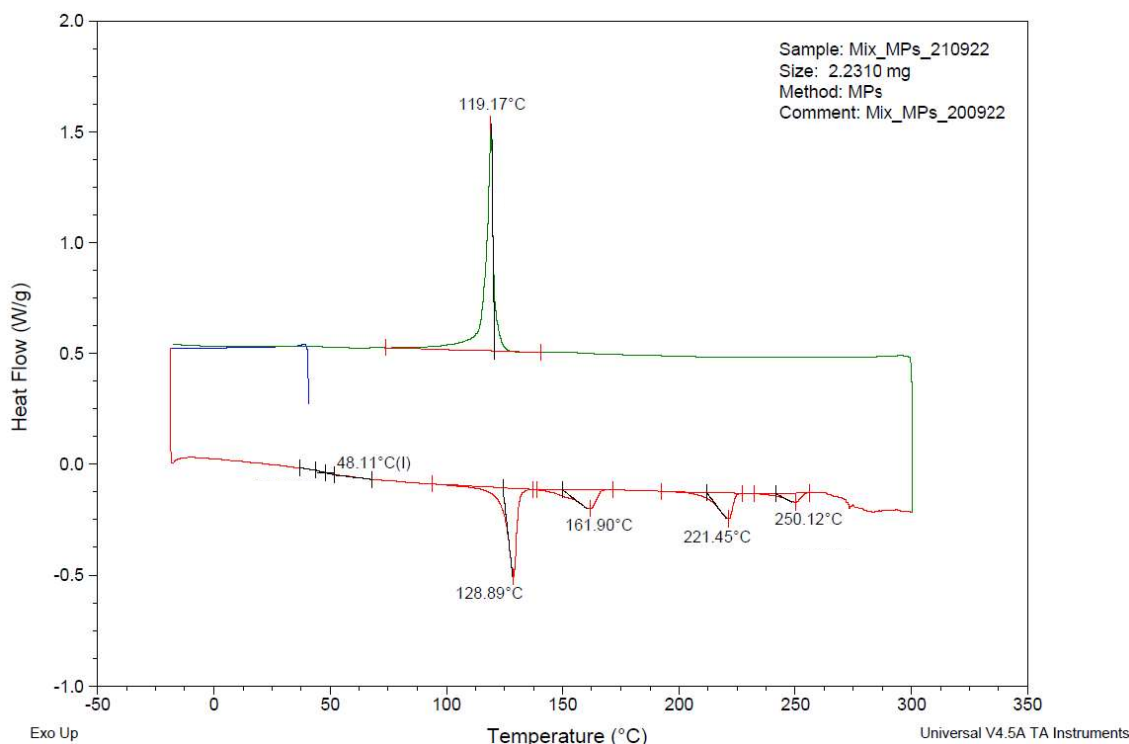


Figure 13. DSC analysis to microplastic mixture generated by cryomilling.

Starting by the well-observable peaks of the heating cycle, the first at 128.9 °C could correspond to the melting temperature ( $T_m$ ) of the HDPE (theoretically is around 125 °C [31]). The second peak at 161.9 °C could correspond to the  $T_m$  of the PP theoretical value at 160 °C [32]. Third peak at 221.5 °C possibly is PA6 (theoretical value of 217.5 °C [33]) and finally, the peak at 250.1 °C is the melting transition of the PET (theoretical value of 260 °C [34]). Four polymers in the mixture could be identified, however a peak for the PVC and a peak for the EPS is not observed because they are amorphous. On the other hand, no conclusions could be extracted from the cooling cycle (green line). Only one peak is observed at a temperature of 119.2 °C. Possibly, this peak could correspond to the crystallization temperature ( $T_c$ ) of the HDPE. No evidence of other polymers of the mixture are interpreted during the cooling. An explanation of the results could be the low amount of sample to analyse and for further analysis it should be increased.

No solid conclusions could be extracted from the DSC analysis due to its lack of peaks during the cooling cycle and not all the polymers could be distinguished during the heating cycle.

## 5.2. Extraction and quantification of microplastics in commercial salt

### 5.2.1. Filtration process

After the application of *Extraction of microplastics* methodology, filtrations using 0.45  $\mu\text{m}$  mesh size nylon filters were performed. At this mesh size, the majority of microplastics were expected to be collected, however an unexpected amount of inorganic impurities was observed and was hindering the proper observation and confirmation of microplastic presence. For this reason, it was decided to filtrate first at 5  $\mu\text{m}$  pore size cellulose filters to try to remove the inorganic impurities and then at 0.45  $\mu\text{m}$  pore size to ensure the collection of all the microplastics with less impurities content.

In Figure 14 is shown the filters after a first filtration in which there were high amount of inorganic material after a first filtration of the salt solution at 0.45  $\mu\text{m}$  pore size, the amount of impurities was too high. For this reason, it was decided to start with a filtration at 5  $\mu\text{m}$  pore size. Even the impurities were still present on the filter, their amount was reduced, allowing the proper inspection of microplastics for the microplastics sorting.



Figure 14. Filters after filtration process at 0.45  $\mu\text{m}$  pore size (left) and 5  $\mu\text{m}$  pore size (right).

Regarding the impurities, an extra step of centrifugation based on the literature was added to try to remove the inorganic particles. As it is possible to see in Figure 15, the impurities were deposited on the bottom of the vial (black arrow) but the presence of microplastic after the centrifugation process, observed by optical microscope, was also affected, for this reason the centrifuge step was discarded and it was replaced by only sequential filtrations.

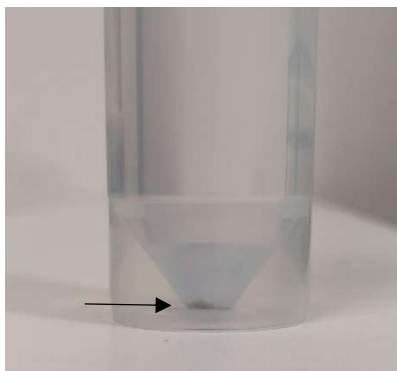


Figure 15. Impurities at the bottom of a 50 mL vial.

In all the filters analysed by optical microscope we observe particles with shape and colour typical of microplastics, however the amount of them was different depending on the brand of table salt studied. In Figure 16 are included some representative microscope images.

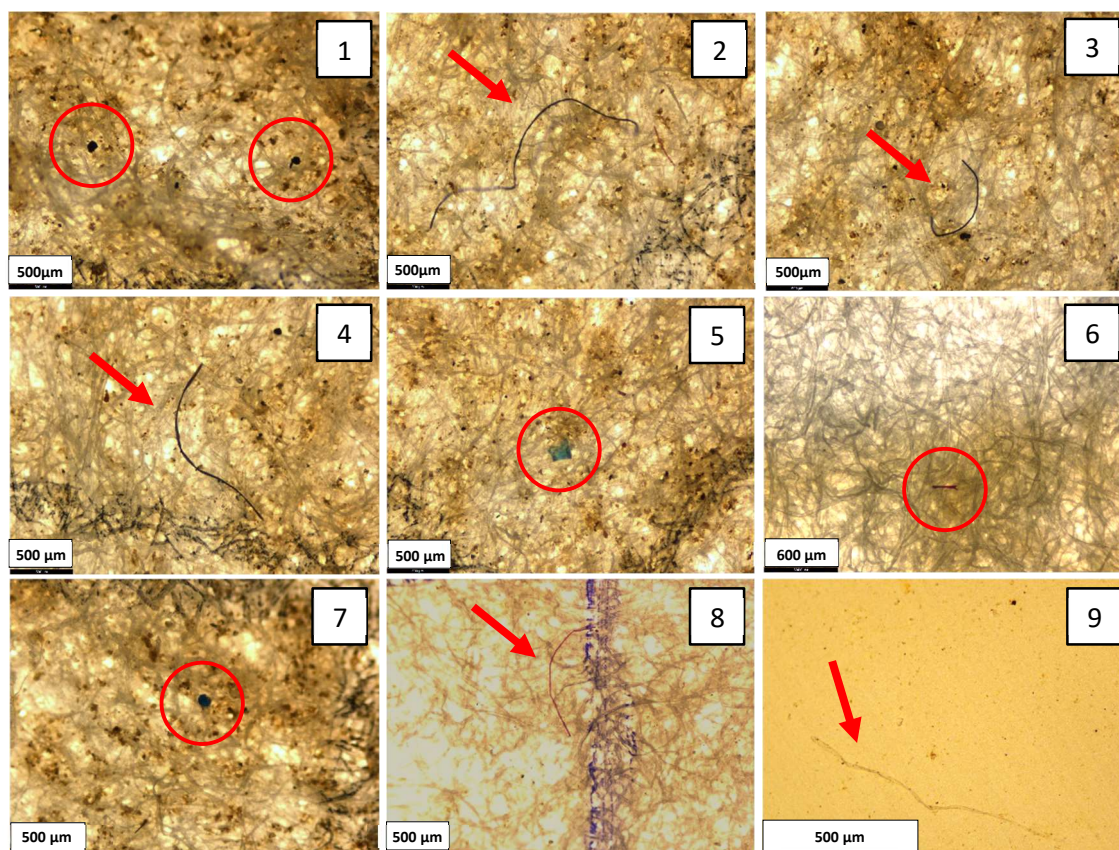


Figure 16. Images taken by optical microscope of the different paper filters.

In concordance with the reported studies, in most of the samples the particles supposed to be microplastics have fibre or roughly spherical shape. Differences between table the three salt brands were also observed. For only one of the brands, the number of supposed microplastics is higher than the rest. By manual sorting, around 5-10 possible microplastics were identified to this first brand with higher content of possible microplastics. Initially it was hypothesised that this was due to the grain size of the salt (coarse salt) but, this hypothesis was discarded because another brand of coarse salt contained a very low number of microplastics. For the other two other brands salt was investigated and these samples contained mostly microplastics with fibre shape but in a lower amount, between 2-5 microplastics, respect to the first coarse salt sample. No evidence of possible links between the grain size (coarse or fine) of the salt and the number of microplastics was found.

#### 5.2.2. $\mu$ -Raman characterizations

After filtrations of commercial table sea salt, analysis by  $\mu$ -Raman spectroscopy coupled to an optical microscope were done to those possible fibres and plastic particles. In Figure 17 it is shown two different Raman spectres together with an image of the particle and fibre observed.

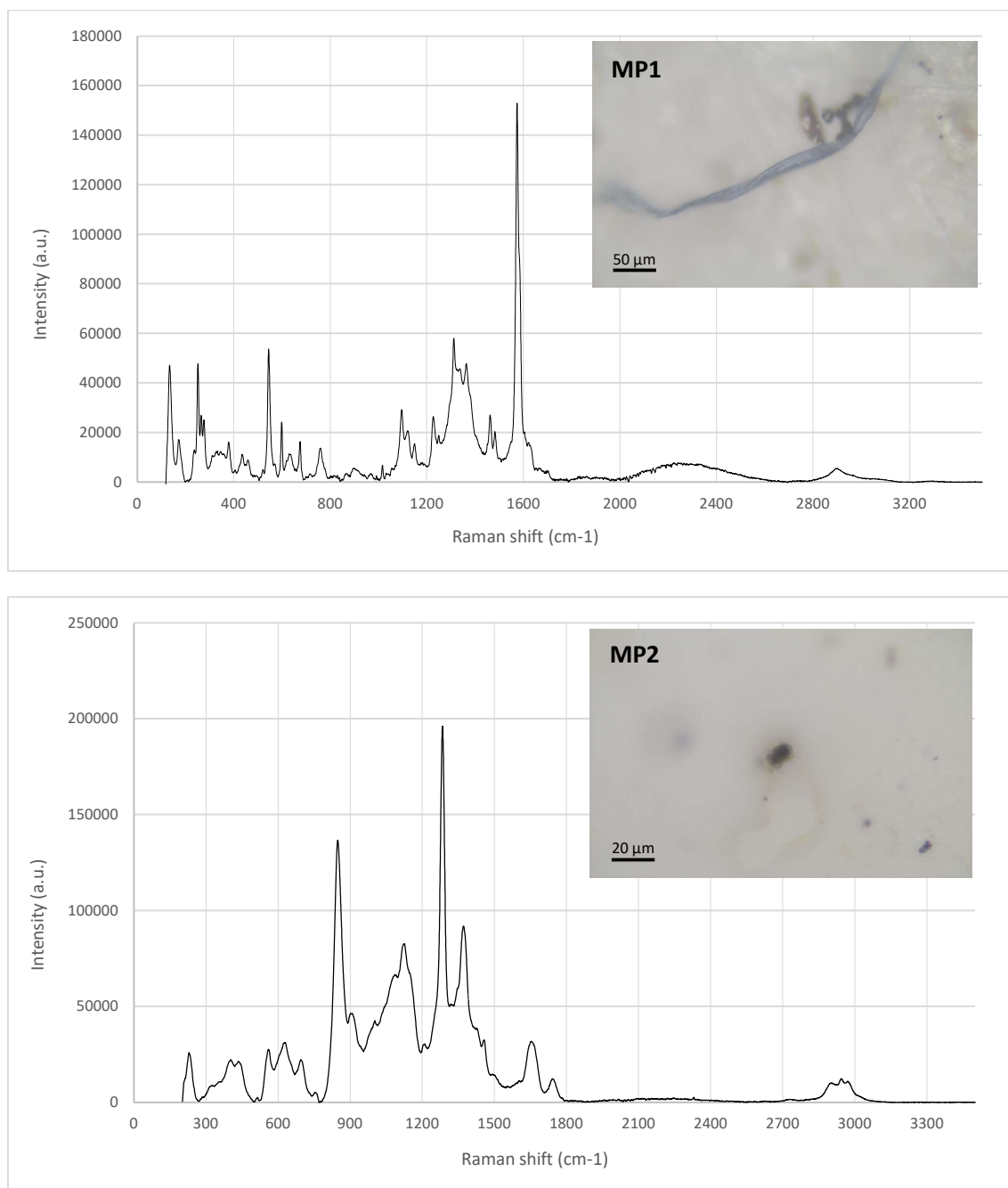


Figure 17. Raman spectra of a fibre (upper) and a particle (lower).

Observing both spectrums, no clear confirmation of the composition of the microplastics under study could be done. Based on literature about other studies of plastics using Raman [35], some approximations were assessed to relate the peaks with a functional group. For MP1, no clear candidates have been assessed. This spectrum contains a very intensive peak at 1574 cm<sup>-1</sup> and could be associated to a C=C bond but polymers such as PA6, PMMA or PET, among others. Comparing the MP2 spectra in the range between 200 to 1800 cm<sup>-1</sup>, one of the candidates could be the PP. Some similitudes have been hypothesized and they are highlighted on a red square (Figure 18) in the range between 750-900 cm<sup>-1</sup> and 1250-1500 cm<sup>-1</sup>, however there are no clear evidence which confirm that.

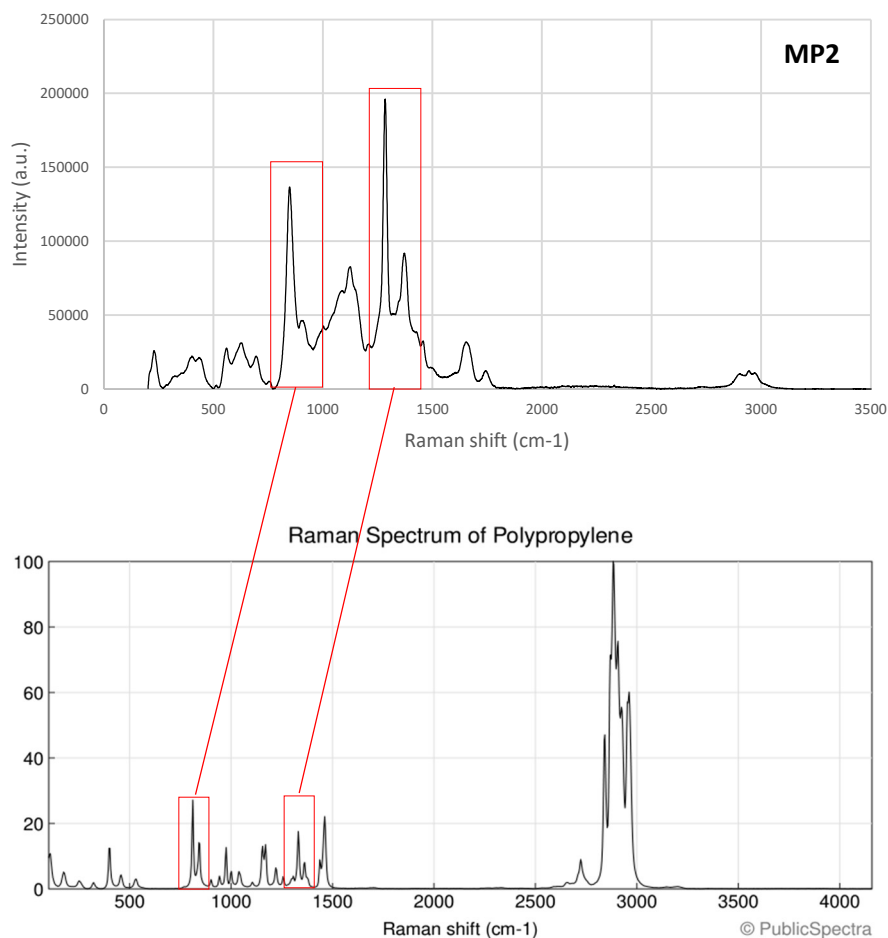


Figure 18. Comparison of MP2 with PP [36].

Intensive peaks in both spectrums are observed without any correlation comparing with pure polymers. This issue makes more relevant the fact that the microplastics observed could be copolymers or they contain other components such as pigments or additives. For the proper evaluation of the microplastics chemical structure, it would be necessary material data bases to correlate in which percentage there is a similarity between a sample and a known single structure.



## Conclusions

Plastic is one of the most widely used material and more and more research is studying the environmental contamination caused by microplastics. One of the most common environmental fate of microplastics is to reach and be accumulated in the marine environment. In this context, commercial table sea salt is reported to be one of the most contaminated food products food products by microplastics. Different methods and characterizations have been studied in this project to optimize the isolation of microplastics from table sea salt by subsequent filtrations. Common and accessible plastic characterizations (i.e., optical microscope, electron microscope, Raman spectroscopy) have been applied to identify the different chemistries of the microplastics in salt samples. Raman spectroscopy is a powerful technique for plastic characterizations, but it needs to be equipped with software and data bases for peak assignment and to obtain the most probable candidate materials. In fact, microplastics in reals scenarios are not only individual raw polymers, they are in most of the cases degraded polymers probably also containing pigments, additives and aggregated with other materials such as impurities and contaminant, which make more difficult the spectrum interpretation and the peak assignment. Regarding the quantification of microplastics in real samples, manual sorting is not accurate enough to assess a number of possible microplastics per sample.

In parallel, to facilitate the study of microplastics in commercial food samples, it was optimized a methodology to generate microplastic by cryomilling, that can be used as control samples and to can be easily characterized by FTIR, TGA and DSC. Some preliminary information could be obtained from the above-mentioned characterization techniques but it was difficult to obtained conclusive results after characterizations to discriminate the presence of specific polymer families.

This study highlights the necessity of standardized protocols for the isolation and characterizations of microplastics in food products, and even more in complex matrices. In this context, our next steps would be the extrapolation and improvement of the applied methodologies to food products with more complex matrices. In addition, the development of methodologies for the proper characterization and distinction of microplastics using widely available and non-expensive techniques.

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