MethodsX 12 (2024) 102777

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/methodsx

Set up and validation of a method to analyse microplastics in stool and small intestine samples

Saul Santini[a], Nora Exposito[b], Jordi Sierra[b,d], Alessandra Cincinelli[a], Joaquim Rovira[b,c,e,*]

[a] Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia 3-13, 50019 Sesto Fiorentino, Italy
[b] Environmental Engineering Laboratory, Departament d'Enginyeria Química, Universitat Rovira i Virgili, Paisos Catalans Avenue 26, 43007 Tarragona, Catalonia, Spain
[c] Laboratory of Toxicology and Environmental Health, School of Medicine, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain
[d] Faculty of Pharmacy and Food Sciences, Universitat de Barcelona, Joan XXIII Avenue s/n, 08028 Barcelona, Catalonia, Spain
[e] Institut d'Investigació Sanitària Pere Virgili (IISPV), 43204 Reus, Catalonia, Spain

ARTICLE INFO

Method name:
Analytical method for the determination of microplastics in human stools and pig intestine

Keywords:
Plastic
Biomonitoring
Faeces
Gut

ABSTRACT

The contamination of microplastics in humans is of increasing concern. Therefore, the aim of this study was to develop effective methods to determine the concentration and types of microplastics entering human digestive system. To study levels of MPs contamination in humans, an excellent indicator are stools. Indeed, stools, and thus the digestive system, can be an excellent indicator of the level of MPs contamination in humans. Hence, objective was to find effective methods to extract, quantify and characterize microplastics in stool and small intestine samples. The samples studied were human stools and pig jejunum (which has human-like characteristics). The methods were optimized by observing extraction efficiency, compatibility by Fourier-transform infrared spectroscopy (FTIR) characterization and non-deformation of the microplastics. The steps of the procedure were:

- Sampling to avoid plastic contamination
- Non-aggressive chemical and enzymatic digestion
- Counting and characterization

The methods were optimized and validated, observing recovery and repeatability. Therefore, two simple, effective methods with high analytical performance have been developed. The MPs present in the stool and intestine samples were counted by stereoscopic microscope and characterized by FTIR, finding several types of MPs such as synthetic cellulose, polyethylene, polypropylene, polystyrene, and polyethylene terephthalate, among others.

* Corresponding author.
E-mail addresses: saul.santini@unifi.it (S. Santini), joaquim.rovira@urv.cat (J. Rovira).

https://doi.org/10.1016/j.mex.2024.102777
Received 1 July 2023; Accepted 24 May 2024

Specifications table

Subject area:	Food Science
More specific subject area:	*Analytical methodologies for the analysis of emerging contaminants in biological samples*
Name of your method:	*Analytical method for the determination of microplastics in human stools and pig intestine*
Name and reference of original method:	Microplastics (levels and characteristics) determination in molluscs and water and sludge lines of a wastewater treatment plant. N. Expósito, J. Rovira, J. Sierra, G. Gimenez, J.L. Domingo, M. Schuhmacher, Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake, Mar Pollut Bull 181 (2022). https://doi.org/10.1016/j.marpolbul.2022.113843. L. Dronjak, N. Exposito, J. Sierra, M. Schuhmacher, K. Florencio, B. Corzo, J. Rovira, Tracing the fate of microplastic in wastewater treatment plant: A multi-stage analysis of treatment units and sludge, Environmental Pollution 333 (2023) 122,072. https://doi.org/10.1016/J.ENVPOL.2023.122072.
Resource availability:	N.A.

Method details

Background

Plastic is considered a persistent and ubiquitous pollutant, proposed as a new stratigraphic indicator of the Anthropocene [1]. Microplastics (MPs) are plastic fragments smaller than five millimetres (≤5 mm in diameter) that are found in various environmental compartments [2]. Plastics durability and resistance to degradation make it a very useful material but at the meantime difficult to dispose of, causing ever-increasing concern. Indeed, due to their persistence, MPs can be dispersed and accumulated in food and consequently in living organisms, including humans. Furthermore, to achieve the desired performance, chemicals such as plasticizers, antistatic agents, flame-retardants, heat stabilizers, acid scavengers, colorant etc. [3], are added during the production phase, which in turn can come into contact and accumulate them in humans. These chemicals if present in the food chain and absorbed by humans could cause many diseases related to hormonal disruption, reproductive problems, nervous tissue, liver and kidney damage, etc. [4]. To study levels of MPs contamination in humans, an excellent indicator is faeces. Indeed, faeces, and thus the digestive system, can be an excellent indicator of the level of MPs contamination in humans and the correlation with certain inflammatory states [5,6]. Moreover, MPs can be a good vehicle for other chemicals, as lipophilic organic substances in the environment can be absorbed by the material, transported and transferred within organisms [7,8]. The large number of methods used make it difficult to understand whether certain steps of the methods, such as chemical digestion, generate errors [9–11]. Therefore, it is very important to use sample treatment methods to extract microplastics from the sample without damaging them. To date, several analytical methods have been used to analyse microplastics in environmental matrix, indeed, there are no unique methods for the analysis of microplastics in human or other matrix. Therefore, the aim of this work was to find effective methods to extract, quantify and characterize microplastics in stool and intestine (jejunum) samples. To find the most effective analytical procedure, methods already present in the literature were optimized, taking into consideration not only extraction efficiency and compatibility by Fourier-transform infrared spectroscopy (FTIR) characterization, but also the simplicity and speed of the method to be replicated in other laboratories and applied in biomonitoring studies. (Fig. 1)

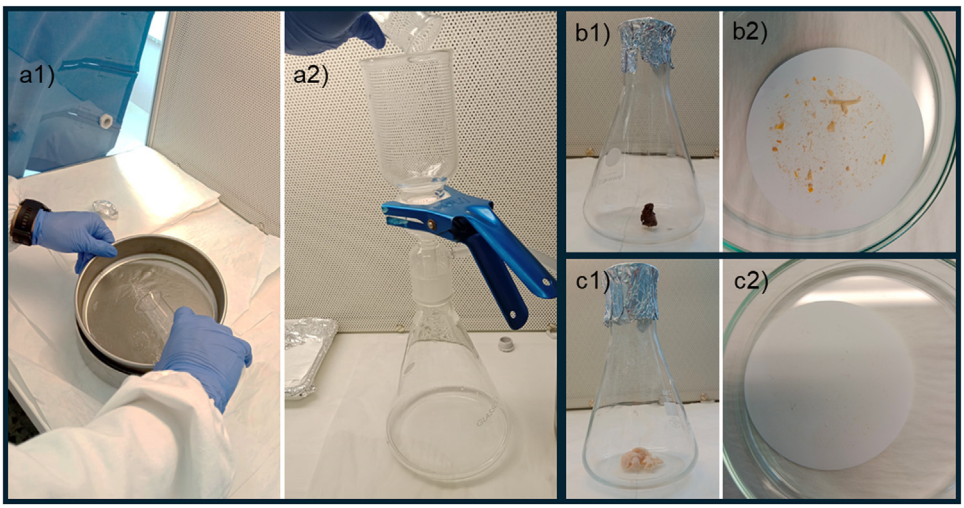

Fig. 1. Figure a1 shows the digested sample solution being filtered through the sieve for the digestion steps, figure a2 shows the filtration of the final digested sample, on a PTFE filter. The images b1) and c1) show the stool and intestine before the digestion procedure and b2 and c2 the stool and intestine filters after filtration.

Material and methods

Reagents and instruments

The material used for the digestion of stool and intestine samples is as follows: Hydrogen Peroxide 35 % pure solution (PanReac AppliChem), KOH solution 2 M prepared using KOH, 90 % (Scharlau), acetic acid solution 0.2 M prepared from acetic acid ≥ 95 % (Sigma-Aldrich), sodium acetate solution 0.2 M prepared from sodium acetate trihydrate (Scharlau), cellulase TXL (ASA) and Protease A-01 (ASA). For solutions preparation and to clean the glassware H_2O ultrapure was prepared using cellulose nitrate membrane filters, 0.45 μm mesh. To pass from one digestion step to the next, the sample was filtered through a 20 μm metal sieve and the final digested solution was filtered through PTFE 10 μm mesh filter. Samples were kept at constant temperature (40 °C) in a heating bath using a P-Selecta thermostat (Digiterm 200). At the same time, the sample solution was gentle agitated using a stir bar and placed on a Velp multistirrer instrument at low speed 80–90 rpm. Spherical polyethylene particles were used for method validation, in two different diameters: 53–63 and 425–500 μm with density 1.02–1.06 g/cm^3, purchased from Cospheric Inc, USA. The MPs were photographed and quantified by a LEICA MZ10 stereomicroscope equipped with a FLEXACAM C1 digital camera and the polymers were characterised using a Thermo Nicolet iN10 MX μ-FTIR microscope with Omnic Picta software.

Self-contamination

One of the problems in the analysis of microplastics is self-contamination, which can occur at all steps, starting with sampling. Indeed, it has been shown that analyses performed on various environmental matrices often have a large self-contamination component, for example for several matrices between 8–25 %, which can originate from the operators' clothing during sampling [12]. Moreover, before use, the glassware and instruments were washed with distilled water, ethanol and ultrapure water filtered through PTFE 10 μm mesh filter. Finally, they were covered with aluminium foil before use. To reduce external contamination, sample-processing step was performed in a Heraguard Eco (ThermoScientific) laminar flow hood. Finally, in parallel with sample preparation, laboratory blanks were also prepared in triplicate to estimate the error due to contamination.

Samples

The samples on which MP detection methods were developed were human faeces and pig small intestine (jejunum). For all samples, sampling was performed following laboratory protocols to avoid self-contamination. Stool samples were collected using metal spoons previously washed with ultrapure water and stored in glass containers covered with aluminium foil to avoid contamination by the cap and then placed in the freezer (−20 °C). Stool samples donors gave their written informed consent and the Ethical Committee of Clinical Research of Sant Joan de Reus Hospital (No 16–04–28/4aclaproj2) approved the study. To obtain a method compatible with the analysis of the human small intestines, pig jejunum was chosen as the sample, as the characteristics of this species are very similar to those of humans [13,14]. Indeed, the pig is one of the best models of human organ systems, including the gastrointestinal tract [15]. Pig small intestine samples were purchased from a slaughterhouse, were collected using metal instruments, and stored in aluminium foil. Each step was performed using white cotton coats and blue nitrile gloves.

Procedures

To improve existing protocols for MPs determination, new protocols were developed and adapted to the aims of this work. The analytical procedures used are the result of the optimization of methods used previously by some co-authors [16,17] incorporating steps and procedures from others protocols found in the literature [5,18–20] that have proved to be the most suitable for analysing intestine and stool samples.

To start the digestion, 1 g of stool and 2.5 g of intestine (jejunum) sample was weighed and placed in glass conical flasks. In the first step, 40 mL of H_2O_2 (35 %) were added to the samples and placed in a heating bath at T = 40 °C for 24 h. In the second step, the solutions were filtered through a 20 μm sieve, the solid phases were recovered with ultrapure H_2O and added to the same conical flask. 65 mL of KOH 2 M were added to the sample (checking that the pH remained 13) and placed the solutions at T = 40 °C for 24 h. Step 3 was performed following two different procedures for stool and intestine samples. The step 3 of the stool involves filtering the solution through a 20 μm sieve, recovering the solid phase with ultrapure H_2O and adding it to the same conical flask, adding 165 mL of acetate buffer (50 mL of CH_3COOH 0.2 M and 115 mL of CH_3COONa 0.2 M), adding 50 mL of cellulase and finally placed the stool samples at T 40 °C for 48 h. Instead, the intestine step 3 provides for filtered the solution through a 20 μm sieve, recover the solid phase with ultrapure H_2O and add it to the same conical flask, add 100 mL of Tris-HCL 1 M (pH=9 adding KOH), add 30 mL of protease and finally placed the intestine samples at 40 °C for 48 h. One of the problems during the procedure may be the incomplete digestion of the intestine. Indeed, observing by microscope, it is possible to note the presence of a transparent layer on the MPs surface, causing them to stick together. In these cases, to remove the intestine layer, the filter was placed in a Falcon, added enough H_2O_2 to cover it and shacked per 1 min by Vortex. Finally, the Falcon was placed in an ultrasonic bath (P-Selecta H-D) per 10 min and at 40 °C and then left to digest per 24 h. Once digestion was complete, the solution was filtered again through another 10 μm PTFE filter.

Table 1
Procedure validation for stool samples using particle standard with diameters between 53 and 63 μm and between 425 and 500 μm.

Samples	Particles diameters (μm)	Particles added (MPs/g)	Particles recovered (MPs/g)	Recovery (%)	Average (%)	RSD (%)
Stool 1	53–63	8268.9	5538.8	67.0	71.1	5.2
Stool 2	53–63	8586.1	6195.1	72.2		
Stool 3	53–63	9274.7	6877.9	74.2		
Stool 1	425–500	182.5	164.1	89.9	93.7	3.5
Stool 2	425–500	140.2	133.6	95.3		
Stool 3	425–500	200.0	191.6	95.8		
Intestine 1	53–63	5681.7	3075.9	54.1	61.3	9.7
Intestine 2	53–63	5572.2	3401.3	61.0		
Intestine 3	53–63	5264.4	3619.1	68.7		
Intestine 1	425–500	90.6	68.8	75.9	83.5	6.6
Intestine 2	425–500	86.1	74.0	85.9		
Intestine 3	425–500	85.8	76.0	88.6		

The digested stool and intestine samples were filtered through a 20 μm mesh sieve to remove the cellulase (or protease) solution and the unfiltered precipitate was transferred to a PTFE 10 μm mesh filter. Once the microplastics are present on the PTFE filter, they are ready to be observed first under a microscope for counting and then at FTIR for chemical-physical characterisation.

Schwabl et al. 2019 reported as a digestion method for human faeces the use of H_2O_2, NaOH and subsequent separation with Imidazolium salt [5]. Other studies on animal faeces showed that H_2O_2 and/or an alkaline hydroxide (KOH or NaOH) is often used for digestion, while in some cases Fenton's reagent or HNO_3 are used, but acid digestion can damage or fragment MPs, limiting the characterisation [21–24]. As far as the analysis of animal intestines, methods for the analysis of microplastics in marine mammals and larger animals have only recently been developed. To digest the intestine samples, the use of H_2O_2, KOH, SDS solution, with digestion times of up to 72 h and with a final separation by density using a saturated NaCl solution was performed [25–30].

Methods validation

To verify the repeatability and recovery, validation was performed for both methods. To validate the method, 3 repetitions were performed, adding a known number of polyethylene phosphorescent particles into the sample.

A known number of particles with diameters of 53–63 μm and 425–500 μm, approximately 1 mg (9790 particles) and 10 mg (193 particles), respectively, were added in all 3 repetitions. After weighing exactly the number of particles added to the 3 repetitions, each sample was subjected to digestion. Subsequently, the particles were quantified using a LEICA stereoscopic microscope. The recovery was calculated through the ratio of the recovered beads to those added before digestion, while repeatability, as RSD%, was calculated through the ratio of the standard deviation to the average of the recovered bead concentrations.

Table 1 summarize the recovery results for stool and intestine method. The validation results showed an average recovery higher than 60 % for the intestine and stools, for both 53–63 and 425–500 μm particles. The 53–63 μm particles recoveries showed a lower recovery than those at 425 μm whose results are over 80 %. Moreover, the methods were found to have a good repeatability (RSD <10 %) and in general, the method for stools showed a higher recovery and intraday repeatability than for intestine method, whose result was still satisfactory. Furthermore, due to the small size, particle counting at 53–63 μm was more difficult, which explains the lower repeatability.

To verify the complete digestion of the sample and to ensure that the digestion process did not deform the microplastics, the polyethylene particles were observed under a microscope. As can be seen from Fig. 2, with both the intestine and stools procedure, no deformation, degradation of the particles and no residual surface layer that can make difficult the FTIR investigation was observed.

Analysis of samples

Once the method was validated, stool and intestine samples were analysed. The analysis provides the count of the fibres and fragments present in each sample, size determination and finally characterisation of the MPs.

Fibres and fragments were distinguished and counted by stereomicroscope analysis at different magnification. MPs were classified as fibre, film or fragment if they presented one-, two- or three-dimensions on visual inspection under a stereomicroscope, the other dimensions being relatively negligible. The concentration (MPs/g) was determined for each stool and intestine repetitions, considering fibres and fragments together, after that a polymeric composition using ATR-μFTIR were done to confirm that particles and fibres are MPs or not. As can be seen from the results (Table 2), the stool samples (39.3 MPs/g) had a higher concentration of MPs than the intestine (23.5 MPs/g) once and in 5 out of 6 samples the fibres count was higher than the fragment count.

Furthermore, to determine the length of the fibers and the diameter of the fragments the stereomicroscope was used to photograph the MPs and then using the ImageJ programme the dimensions of each MPs were measured (Table 3). It was possible to note an average size under 1 mm, with an average of 0.324 mm for the stools, 0.410 mm and a maximum length of 4.811 mm in intestine samples. To verify the contribution due to contamination during sample processing, triplicates of procedure blanks were performed, the result being an error on the measurement of about 6 MPs (3 fibres and 3 fragments).

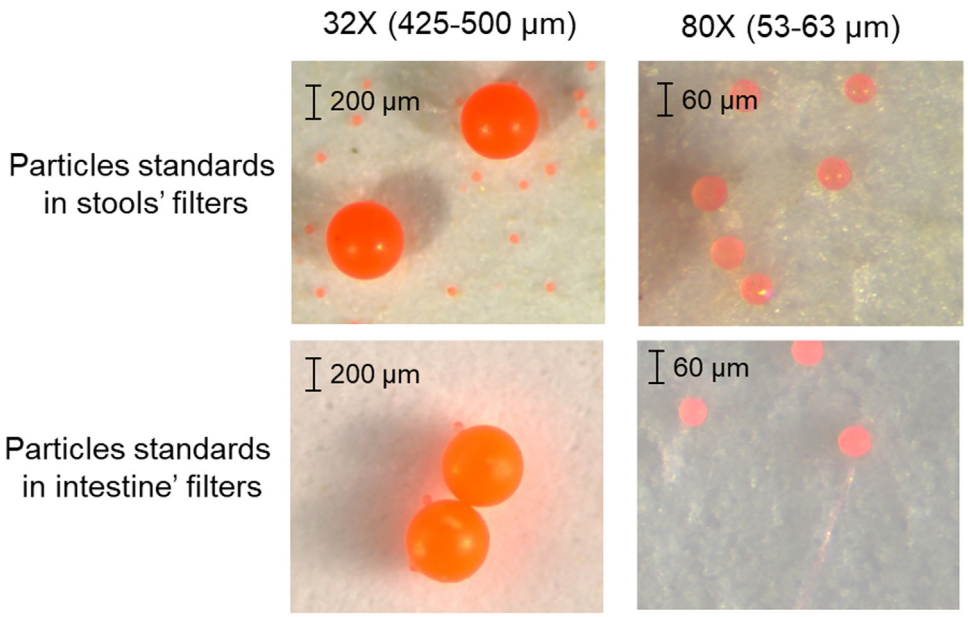

Fig. 2. The images show the particles used for validation, on the filters after digestion procedure, using two different LEICA microscope magnifications: 80X magnification for particles with a diameter of 53–63 µm and 32X magnification for particles 425–500 µm.

Table 2
Concentrations of MPs in the stool and intestine analysed samples and the error on the final average.

	Weight (g)	Total Fibers	Total Fragments	Concentration*	Average*	Standard deviation
Blank 1	–	2	4	6	5.7	0.6
Blank 2	–	2	3	5		
Blank 3	–	5	1	6		
Stool 1	1.03	38	6	42.7	39.3	6.3
Stool 2	1.22	20	19	32.0		
Stool 3	0.95	23	18	43.2		
Intestine 1	2.50	52	10	24.8	23.5	3.6
Intestine 2	2.52	31	18	19.4		
Intestine 3	2.33	28	33	26.2		

* the concentration for blank are expressed as MPs per blank sample, instead for stool and intestine sample as MPs per gram.

Table 3
Dimensions for the MPs found in stool and intestine sample.

	Length (mm)			
	Minimum	Median	Average	Maximum
Stool 1	0.033	0.255	0.388	1.777
Stool 2	0.015	0.088	0.305	1.344
Stool 3	0.027	0.079	0.281	1.352
Intestine 1	0.024	0.262	0.442	2.040
Intestine 2	0.021	0.172	0.491	4.811
Intestine 3	0.023	0.09	0.298	3.029

Finally, the MPs representative of the samples were characterised. To characterize the sample, the filter was placed under LEICA stereoscopic microscope and using scalpels, the MPs were picked up and placed carefully on a calcium fluoride (CaF_2) slide inside a 1 cm^2 square (Fig. 3). Three circumscribed areas were defined on the same slide, one for the gut, one for the stools and one for the blank. The slide was then covered with another slide, taking care not to move and contaminate the areas containing the MPs, and stored until spectroscopy characterisation. For MPs composition analysis was used an ultrafast mapping microscope Thermo Scientific Nicolet™ iN™10 with MCT detector with pixel aperture 25 × 25 µm in transmission mode, in the range of 850–4000 cm^{-1} with 4 scans at a spectral resolution of 4 cm^{-1}. The IR spectres were compared with OMIC software libraries database (Fig. 4). It includes HR Nicolet Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard Library, Willey's Know It All, Synthetic Fiber by Microscope and an own library (spectra from weathered environmental microplastics) with >80 IR spectra. Only match

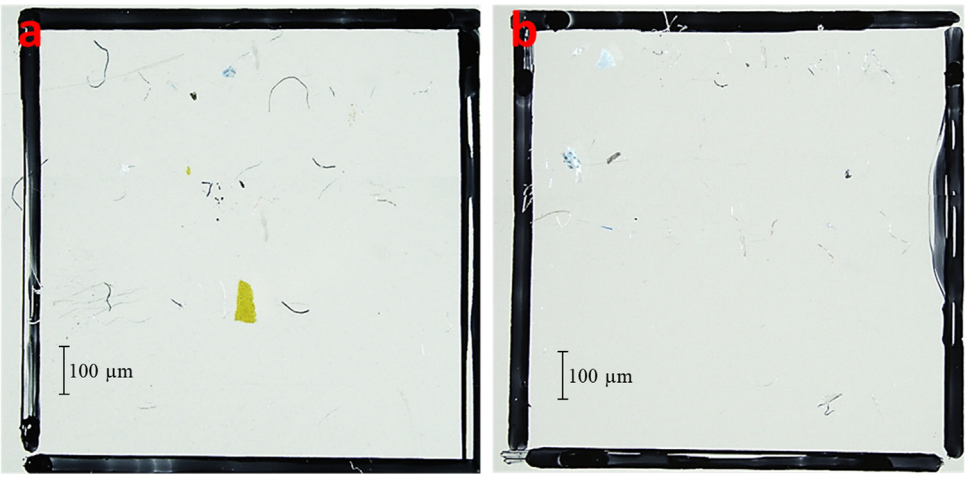

Fig. 3. Square areas (1 cm x 1 cm) where the MPs were located before the FTIR analysis of a) stool sample and b) intestine sample.

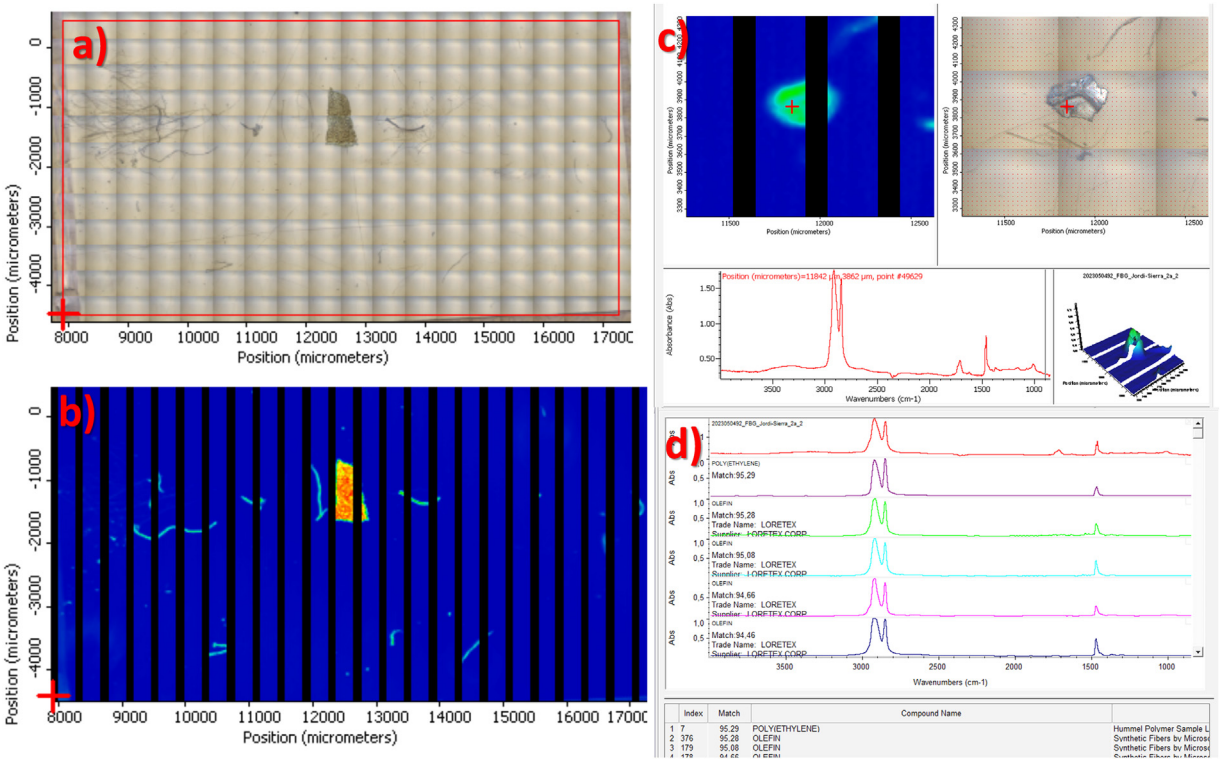

Fig. 4. -µFTIR map acquisition (a and b), isolated particle and fibre spectra acquisition (c) and comparison with commercial and in house library (d).

spectra with major -or equal- than 70 % of similarity with reference spectra were accepted. The rejected items were quantified as the temporary unidentified category.

The FTIR characterization allowed to understand if the digestion had not damaged the MPs in the sample and if there was any interference that did not allow the characterization. The spectra obtained did not show interferences, allowed the identification of the several types of MPs composition present in the samples (Figs. 5 and 6). Indeed, in the intestinal samples the presence of cellulose, polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polyester (PES), polyvinyl chloride (PVC), nylon, polysiloxane, Rayon and Teflon were found, while in the stool samples cellulose, PE, PET, PES, nylon and polyacrylate. In both matrixes, the most abundant composition characterize was synthetic cellulose (78.6 % in stool and 36.5 % in intestine) followed by PET in stool (10.7 %) and PP in intestine (20.8 %).

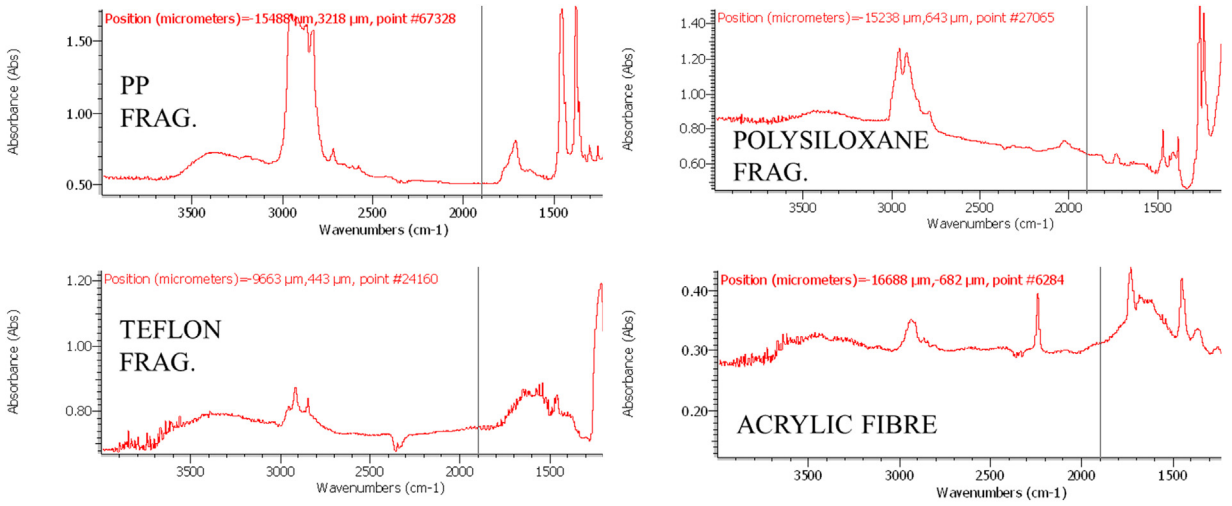

Fig. 5. IR spectra of fragments and fibres in stool samples.

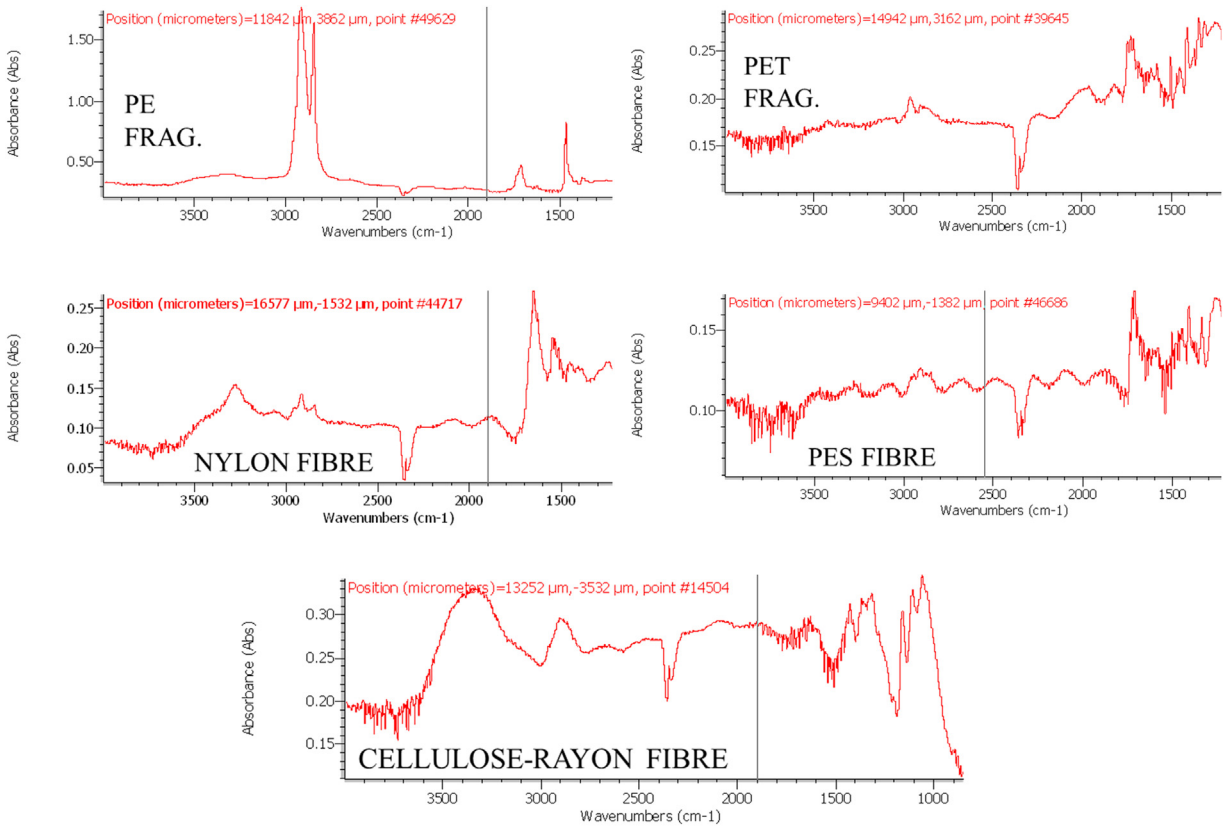

Fig. 6. IR spectra of fragments and fibres in intestine samples.

The described methods showed good performance for digestion and FTIR characterization for both the intestinal and faecal matrix. Both methods allowed the polymers present in the samples to be counted and not damaged, causing no alteration to the composition and allowing the exact evaluation of the type of microplastic, as demonstrated by the identification of polymers with extreme accuracy through the comparison of spectra in the software library. Furthermore, the method involves the use of non-aggressive digestion solutions, which allow the MPs not to degrade and deform. Indeed, many procedures involve the use of acids, such as sulphuric or nitric acid, and high temperatures, which aid digestion, speeding up the process and making it more efficient, but with the risk of altering the shape and characteristics of the MPs [20,31]. Moreover, both procedures do not include the use of ultrasound to improve

the separation of the MPs from the matrix, which could risk breaking the microplastics, resulting in an error in the assessment of their number and size. In summary, these two methods can be considered as good procedures for the preparation of human stool samples and, considering that the pig intestine share characteristics with the human intestine, an excellent starting point for the development of methods to analyse MPs in the human intestine. The composition of the stools can change considerably from one individual to another, due to the different diet and intrinsic digestion capabilities of each, such as the different composition of the human intestine depends on several factors, which directly or indirectly influence the characteristics of the intestine. For this reason, the methods studied try to be the simplest and easiest to reproduce and, even if they are not effective for all types of faeces or intestines, they can still provide an excellent basis on which to adapt methods according to specific cases.

Ethics statements

Stool samples donors give their written informed consent and the Ethical Committee of Clinical Research of Sant Joan de Reus Hospital (No 16–04–28/4aclaproj2) approved the study. The pig intestine was bought from a slaughterhouse that regularly sells its products to the public.

CRediT author statement

Saul Santini: Conceptualization, Methodology, Writing – original draft. **Nora Expósito**: Conceptualization, Methodology, Validity tests, Writing – review & editing. **Jordi Sierra**: Visualization, Investigation, Writing – review & editing. **Alessandra Cincinelli:** Supervision and Writing – review & editing. **Joaquim Rovira:** Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Grant PID2022-140866OB-I00 (MP-DiGuLi project) funded by MICIU/AEI and by the “European Union”. J.R. was supported by a Miguel Servet contract (CP22/00062) funded by Instituto de Salud Carlos III (ISCIII) and cofounded by Fondo Social Europeo Plus (FSE+). The authors wish to thank Pilar Hermo from the Scientific and Technological Centers of the University of Barcelona for the realization of the IR maps.

References

[1] A. Abeynayaka, F. Kojima, Y. Miwa, N. Ito, Y. Nihei, Y. Fukunaga, Y. Yashima, N. Itsubo, Rapid sampling of suspended and floating microplastics in challenging riverine and coastal water environments in Japan, Water (Basel) 12 (2020) 1903. https://doi.org/10.3390/w12071903.
[2] L. Cozzolino, K.R. Nicastro, G.I. Zardi, C.B. de los Santos, Species-specific plastic accumulation in the sediment and canopy of coastal vegetated habitats, Sci. Total Environ. 723 (2020) 138018, doi:10.1016/J.SCITOTENV.2020.138018.
[3] K.J. Parton, B.J. Godley, D. Santillo, M. Tausif, L.C.M. Omeyer, T.S. Galloway, Investigating the presence of microplastics in demersal sharks of the North-East Atlantic, Sci. Rep. 10 (2020) 1–11, doi:10.1038/s41598-020-68680-1.
[4] S.M. Iliff, E.R. Wilczek, R.J. Harris, R. Bouldin, E.W. Stoner, Evidence of microplastics from benthic jellyfish (Cassiopea xamachana) in Florida estuaries, Mar. Pollut. Bull. 159 (2020) 111521, doi:10.1016/j.marpolbul.2020.111521.
[5] P. Schwabl, S. Koppel, P. Konigshofer, T. Bucsics, M. Trauner, T. Reiberger, B. Liebmann, Detection of various microplastics in human stool: a prospective case series, Ann. Intern. Med. 171 (2019) 453–457, doi:10.7326/M19-0618.
[6] Z. Yan, Y. Liu, T. Zhang, F. Zhang, H. Ren, Y. Zhang, Analysis of microplastics in human feces reveals a correlation between fecal microplastics and inflammatory bowel disease status, Environ. Sci. Technol. 56 (2022) 414–421, doi:10.1021/acs.est.1c03924.
[7] B. Rios-Fuster, C. Alomar, M. Compa, B. Guijarro, S. Deudero, Anthropogenic particles ingestion in fish species from two areas of the western Mediterranean Sea, Mar. Pollut. Bull. 144 (2019) 325–333, doi:10.1016/j.marpolbul.2019.04.064.
[8] M.S. Hossain, M.S. Rahman, M.N. Uddin, S.M. Sharifuzzaman, S.R. Chowdhury, S. Sarker, M.S. Nawaz Chowdhury, Microplastic contamination in Penaeid shrimp from the Northern Bay of Bengal, Chemosphere 238 (2020) 124688, doi:10.1016/j.chemosphere.2019.124688.
[9] C.G. Avio, L. Pittura, G. d’Errico, S. Abel, S. Amorello, G. Marino, S. Gorbi, F. Regoli, Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: general insights for biomonitoring strategies, Environ. Pollut. 258 (2020) 113766, doi:10.1016/j.envpol.2019.113766.
[10] M.L. Pedrotti, S. Petit, B. Eyheraguibel, M.E. Kerros, A. Elineau, J.F. Ghiglione, J.F. Loret, A. Rostan, G. Gorsky, Pollution by anthropogenic microfibers in North-West Mediterranean Sea and efficiency of microfiber removal by a wastewater treatment plant, Sci. Total Environ. 758 (2021) 144195, doi:10.1016/J.SCITOTENV.2020.144195.
[11] A. Karimah, M.R. Ridho, S.S. Munawar, D.S. Adi, Ismadi, R. Damayanti, B. Subiyanto, W. Fatriasari, A. Fudholi, A review on natural fibers for development of eco-friendly bio-composite: characteristics, and utilizations, J. Mater. Res. Technol. 13 (2021) 2442–2458, doi:10.1016/J.JMRT.2021.06.014.
[12] C. Scopetani, M. Esterhuizen-Londt, D. Chelazzi, A. Cincinelli, H. Setälä, S. Pflugmacher, Self-contamination from clothing in microplastics research, Ecotoxicol. Environ. Saf. 189 (2020), doi:10.1016/j.ecoenv.2019.110036.
[13] A. Ziegler, L. Gonzalez, A. Blikslager, Large animal models: the key to translational discovery in digestive disease research, CMGH 2 (2016) 716–724, doi:10.1016/j.jcmgh.2016.09.003.
[14] L.M. Gonzalez, A.J. Moeser, A.T. Blikslager, Porcine models of digestive disease: the future of large animal translational research, Transl. Res. 166 (2015) 12–27, doi:10.1016/j.trsl.2015.01.004.
[15] Q. Zhang, G. Widmer, S. Tzipori, A pig model of the human gastrointestinal tract, Gut Microbes 4 (2013) 193–200, doi:10.4161/gmic.23867.

[16] N. Expósito, J. Rovira, J. Sierra, G. Gimenez, J.L. Domingo, M. Schuhmacher, Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: human intake, Mar. Pollut. Bull. 181 (2022), doi:10.1016/j.marpolbul.2022.113843.
[17] L. Dronjak, N. Exposito, J. Sierra, M. Schuhmacher, K. Florencio, B. Corzo, J. Rovira, Tracing the fate of microplastic in wastewater treatment plant: a multi-stage analysis of treatment units and sludge, Environ. Pollut. 333 (2023) 122072, doi:10.1016/J.ENVPOL.2023.122072.
[18] B. Park, S.K. Kim, S. Joo, J.S. Kim, K. Jo, N.S. Song, J. Im, H.J. Lee, S.W. Kim, S. Bin Lee, S. Kim, Y. Lee, B.Y. Kim, T.W. Kim, Microplastics in large marine animals stranded in the Republic of Korea, Mar. Pollut. Bull. 189 (2023) 114734, doi:10.1016/j.marpolbul.2023.114734.
[19] N. Zhang, Y. Bin Li, H.R. He, J.F. Zhang, G.S. Ma, You are what you eat: microplastics in the feces of young men living in Beijing, Sci. Total Environ. 767 (2021) 144345, doi:10.1016/j.scitotenv.2020.144345.
[20] J.C. Prata, J.P. da Costa, A.V. Girão, I. Lopes, A.C. Duarte, T. Rocha-Santos, Identifying a quick and efficient method of removing organic matter without damaging microplastic samples, Sci. Total Environ. 686 (2019) 131–139, doi:10.1016/j.scitotenv.2019.05.456.
[21] X. Zhang, Q. Feng, X. Li, L. Guo, D. Ma, X. Cheng, Y. Qi, Microplastics in household fecal sewage treatment facilities of rural China, J. Hazard. Mater. 448 (2023), doi:10.1016/j.jhazmat.2023.130925.
[22] Z. Yan, H. Zhao, Y. Zhao, Q. Zhu, R. Qiao, H. Ren, Y. Zhang, An efficient method for extracting microplastics from feces of different species, J. Hazard. Mater. 384 (2020), doi:10.1016/j.jhazmat.2019.121489.
[23] F. Pérez-Guevara, G. Kutralam-Muniasamy, V.C. Shruti, Critical review on microplastics in fecal matter: research progress, analytical methods and future outlook, Sci. Total Environ. 778 (2021), doi:10.1016/j.scitotenv.2021.146395.
[24] N. Zhang, Y. Bin Li, H.R. He, J.F. Zhang, G.S. Ma, You are what you eat: microplastics in the feces of young men living in Beijing, Sci. Total Environ. 767 (2021), doi:10.1016/j.scitotenv.2020.144345.
[25] B. Park, S.K. Kim, S. Joo, J.S. Kim, K. Jo, N.S. Song, J. Im, H.J. Lee, S.W. Kim, S. Bin Lee, S. Kim, Y. Lee, B.Y. Kim, T.W. Kim, Microplastics in large marine animals stranded in the Republic of Korea, Mar. Pollut. Bull. 189 (2023), doi:10.1016/j.marpolbul.2023.114734.
[26] J.M. Donkers, E.M. Höppener, I. Grigoriev, L. Will, B.N. Melgert, B. van der Zaan, E. van de Steeg, I.M. Kooter, Advanced epithelial lung and gut barrier models demonstrate passage of microplastic particles, Microplast. Nanoplast. 2 (2022), doi:10.1186/s43591-021-00024-w.
[27] C. Philipp, B. Unger, E.K. Fischer, J.G. Schnitzler, U. Siebert, Handle with care—Microplastic particles in intestine samples of seals from German waters, Sustainability (Switzerland) 12 (2020) 1–15, doi:10.3390/su122410424.
[28] J. Carlin, C. Craig, S. Little, M. Donnelly, D. Fox, L. Zhai, L. Walters, Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA, Environ. Pollut. 264 (2020), doi:10.1016/j.envpol.2020.114633.
[29] M. Haave, A. Gomiero, J. Schönheit, H. Nilsen, A.B. Olsen, Documentation of microplastics in tissues of wild coastal animals, Front. Environ. Sci. 9 (2021), doi:10.3389/fenvs.2021.575058.
[30] C. Philipp, B. Unger, U. Siebert, Occurrence of microplastics in harbour seals (Phoca vitulina) and grey seals (Halichoerus grypus) from German waters, Animals 12 (2022), doi:10.3390/ani12050551.
[31] H.B. Lee, K.S. Lee, S.J. Kim, B. Il Choi, B.R. Go, C.J. Rhu, T.H. Han, Effect of chemical agents on the morphology and chemical structures of microplastics, Polymers. (Basel) 14 (2022) 4353, doi:10.3390/polym14204353.