ORAL COMMUNICATIONS

**Food Products and Microplastics: A Call for Qualification and Quantification**

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This PhD thesis dealt with the assessment of microplastic’s contamination of some food products by developing an analytical methodology for the pre-treatment of the food matrices by evaluating digestion efficiencies as well as the impact of digestion agents on microplastics. Furthermore, qualification and quantification of microplastics by means of pyrolysis – gas chromatography – mass spectrometry (Py-GC-MS) has been conducted.

Alimenti e Microplastiche: Una Necessaria Qualificazione e Quantificazione

Questa tesi di dottorato ha trattato la valutazione della contaminazione da microplastica di alcuni prodotti alimentari sviluppando una metodologia analitica per il pretrattamento delle matrici alimentari valutando l'efficienza della digestione e l'impatto degli agenti di digestione sulle microplastiche. Inoltre, sono state condotte la qualificazione e la quantificazione delle microplastiche mediante pirolisi - gascromatografia - spettrometria di massa (Py-GC-MS).

**Key words**: Microplastics; foods; pollution; digestion; pyrolysis – gas chromatography – mass spectrometry;

# **1. Introduction**

In accordance with the PhD thesis project previously described, this oral communication reports the main results of the following four activities directed to:

A1) investigation of the best experimental conditions (i.e., time/temperature, sample/digestion reagent ratio) for digestion of organic matter of foods, necessary for visually analyse microplastics shape, color, size;

A2) assess the impact of digestion agents on microplastic particles, in terms of shape, size and color;

A3) determination of microplastic’s recovery rate;

A4) solubilization of plastic polymers for quantification of microplastics using Py-GC-MS;

# **2. Digestion Efficiency and Quantification of Microplastics**

Digestion efficiency (DE) is the parameters used for assessing the percentage of organic matter removed. Digestions are generally required in microplastic’s research field, especially when the matrix is rich in biological materials (e.g., food products) (Prata *et al*., 2019). Isolation of microplastics from matrices still poses a major challenge, but its optimization is necessary to identify microplastics in a sample (Pfeiffer *et al*., 2020). Digestions have been applied to fish for human consumption, but experimental parameters (i.e., time/temperature, sample/digestion reagent ratio) (Table 1) varied among the different research’s group (Makhdoumi *et al*., 2023).

**Table 1** *Experimental conditions applied to fish for human consumption*.

|  |  |
| --- | --- |
| **Sample** | **Experimental conditions** |
| **Edible tissues** | Potassium hydroxide 10% at 60°C 24 h (Daniel *et al*., 2020) |
|  | Potassium hydroxide 10% at 50°C 48 h (Mistri *et al*., 2022) |
|  | Hydrogen peroxide 30% 65°C 24-48h (Li *et al*., 2015) |
| **Sample/reagents ratio** |  |
|  | N/A (Daniel *et al*., 2020) |
|  | N/A (Mistri *et al*., 2022) |
|  | N/A (Li *et al*., 2015) |

Papers which aim to assess the occurrence of microplastics in seafood do not carefully investigate the digestions conditions as well as data on digestion efficiencies achieved are not reported. Digestion reaction are influenced by the temperature (Karami *et al*., 2017). Likewise, optimizing the sample-to-reagent ratio can reduce the time needed for digestion and minimize the potential damage to microplastics from the digestion agent. This highlights the importance of identifying the best experimental conditions.

Other food matrices (such as pasta, meat and cheese, mozzarella) are not documented in scientific literature in terms of occurrence of microplastics and so, digestion protocols. Scientific hypothesis seems to suggest the contamination of these foods. Particularly, Food and Agriculture Organization (FAO) suggested that plastic-packaging is a remarkable source of microplastics in foods (Gamarro and Costanzo, 2022). Optimisation of sample preparations is fundamental to assess microplastic’s contamination in foods and so, for assessing the recovery rate (Cai *et al.,* 2019). These treatments should be standardised to obtain results as comparable as possible among researchers’ groups.

Another important issue in microplastic’s research field is their quantification. Currently, visual counting is the most used (Hamed *et al.,* 2019; Zeytin *et al.,* 2020). However, quantification by pyrolysis-gas chromatography- mass spectrometry (Py-GC-MS) is now a promising technique but, issues are still challenging. For example, for quantifying microplastics, the construction of calibration curves is required (Ishimura *et al.,* 2020). Furthermore, for food, where the contamination is supposed to be in the range of ppt, the method should be very sensitive. Therefore, for achieving low concentration in the calibration curves ‘construction, solubilization of polymers is a potential solution. Hansen Solubility Parameters were taken into account for studying the solubilization of plastic polymers. The theory as well as formula are based on the interactions between solvent and plastics polymers. Briefly, the degree of similarity between solvent and solute, in a given situation, determines the extent of interaction. The key factor, in fact, of this theory is to identify the affinities that the components of a system have among themselves. The Hansen parameters (i.e., δd, δp,δh) indicate specific characteristics of the polymer. Whether a solvent is capable of dissolving a polymer is indicated by the *Ra/R0* ratio. Specifically, if the ratio *Ra< R0*, then the probability that the solvent will dissolve the polymer is high.

# **3. Mathematical Modelling**

To design and optimise digestion efficiency, several parameters are be taken into account, such as time, temperature, type of agents, sample/reagent ratio.

Parameters were experimentally evaluated, to assess the best condition. Digestion efficiency (and the related standard deviation for each protocol) were assess with the following formula (1).

; => \*100 (1)

Furthermore, percentage recovery rate (RR) of microplastics were assessed, using the following formula (2).

where all symbols are given in section 7.

(2)

Regarding the solubilization of plastics polymers for the construction of calibrations curves, Hansen Solubility Parameters were taken into account (3).

(3)

which was calculated for each tested organic solvent, whereas for each polymer is experimentally determined.

For assessing whether a solvent was potentially able to dissolve a plastic polymer, the following formula was used:

If the ratio (4) was minor than 1, there were good possibility that the solvent dissolved the polymer.

All symbols used in this section are given in section 7.

# **4. Experimental Procedure**

In this PhD thesis several experiments were conducted for the assessment of the best experimental design for each food matrix. Precisely, for fish samples, the type of reagent was not investigated as KOH was the most used. Therefore, parameters such as sample-to-reagent ratio, temperatures, digestion efficiency were studied. On the contrary, for pasta, and meat, all parameters were tested, such as type of reagent for the digestion, sample-to-reagent ratio, temperature and digestion efficiency were evaluated. In this way, it was possible to identify the best condition for each matrix.

Afterwards, microplastic’s percentage recovery rate was assessed, by visual counting. Recovery rate was determined to determine whether conditions used for digestion are able to clearly quantify microplastics.

Regarding the preliminary solubility tests, solubilisation of powered polymer was tested using organic solvents suggested by the Hansen Solubility Theory, for each polymer.

For quantification of microplastics by assessing the mass content, dissolved polymers were spiked in a known concentration on real samples and digested. After that, filters were analysed by Py-GC-MS. Pyrolysis products were used for qualification of microplastics, as well as for quantify them by a comparison with calibration curves.

# **5. Materials and Methods**

For the digestion of organic matter, digestion solutions were prepared. Briefly, to ensure the absence of contamination, experimental solutions were prepared under a fume hood and workers wore blue nitrile gloves and cotton lab coat. Glassware used for the experiments were carefully washed and rinsed with MilliQ water, prior to use. Furthermore, solutions were filtered used filter paper for ensuring the absence of microplastic particles from the glassware or the air.

Stirring-plates were used for digesting organic matter. For evaluating the best sample/reagent ratio, for each matrix, three rations were tested, such as 1:20, 1:40 and 1:60, using 0.5 g of food and 10, 20 and 30 mL of digestion reagent. Temperatures tested were 50°C and room temperature. For testing the best type of digestion reagent, experiments were conducted in triplicate.

For the assessment of the dissolution of plastic polymers into organic solvents, analytical grade solvents (> 98%) were used. Powered polymers such as polystyrene (PS, 150 μm), polyethylene terephthalate (PET, 300 μm) and polyethylene (PE, 150 μm) (Goodfellow, UK) were used. Therefore, polymers were weighted (0.005 g) and placed into a clean bottle. Afterwards, appropriate solvent (5 mL) (or mixture of solvents) was added and the solution was held in rotation for 24h at room temperature. Briefly, for PS, dichloromethane (DCM), chloroform, toluene, tetrahydrofuran (THF) and mixture of DCM: toluene (1:1; 1:2; 2:1) and DCM: *n*-hexane (1:1; 1:2; 2:1) were tested as were suggested by the theory. Likewise, THF: DMC (1:1; 1:2; 2:1) and hexafluoro2-propanol (HFIP) (even though the latter was not suggested by the theory) were evaluated. For PE, the theory suggested a mixture of n-hexane: toluene (1:1; 1:2; 2:1).

For investigating the recovery rate of microplastics, a solution of fluorescent microbeads (diameter,10 μm) was used. About 10 μL of the dilute solution 1/1000 were spiked on the samples. Microbeads were counted thrice using a visual microscope. Afterwards, samples were digested with the appropriate techniques for each matrix analysed. The solution was filtered (Whatman, pore size 1.6 μm) and the recovered particles were counted thrice.

For a preliminary quantification of microplastics using Py-GC-MS, samples were spiked with a known concentration (100 ppb) of the polymer solution. Samples were digested and filtered. Filters were analysed by Py-GC-MS.

# **5. Results and Discussion**

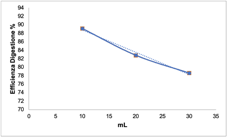
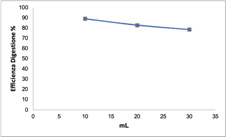
## **5.1 Digestion Efficiency**

Digestion efficiencies for matrices analysed are reported in Table 2.

**Table 2** *Digestion efficiencies of pasta, fish, meat investigated in this PhD thesis. \*S/R indicates the sample-to-reagent tested; \*\* T indicated the temperature used in the experiments; ° indicated the mean calculated on three tests; § SD indicated the standard deviation;*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **S/R\*** | **T\*\*** | **Time** | **Concentration** | **DE**  Mean° | SD§ |
|  |  | (°C) | (h) | (M) | (%) |  |
| Fish | 1:20 | RT | 48 | 5 | 84.0 | 5.2 |
| 1:40 | RT | 48 | 5 | 82.8 | 8.5 |
| 1:60 | RT | 48 | 5 | 79.3 | 0.6 |
|  |  |  |  |  |  |  |
| Fish | 1:20 | 50 | 48 | 5 | 97.4 | 0.4 |
| 1:40 | 50 | 48 | 5 | 95.4 | 3.0 |
| 1:60 | 50 | 48 | 5 | 93.2 | 4.2 |

## In both cases, as the volume of reagent increases, other conditions being equal, the digestive efficiency is reduced (Figure 1). It clearly emerged that tests at 50°C gave a higher digestion efficiency.

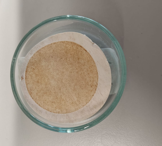
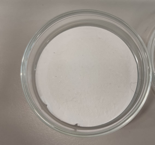
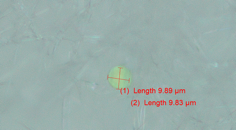
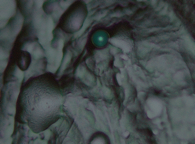


**Figure 1** *Trend of digestive efficiency with varying volume (sample/reagent ratio) used for digestion. In order to clarify the potential dependence of digestive efficiency with the volume of reagent used, the graph is proposed with both full scale (left) and reduced scale (right).*

## For meat and pasta, the type of digestion agents was investigated. KOH 5M showed the highest digestion efficiency, which was of 98.0±0.5%, whereas KOH 1M, Fenton’s reagents and H2O2 (30%) showed digestion efficiencies of 88.4±2.2. 80.0±4.5 and 80.1±5.7, respectively. Regarding pasta, Fenton’s reagent showed the highest efficiency 98.0±0.6. For other digestion agents, digestion efficiency was not calculated as the solutions were not able to be filtered.

## **5.2 Recovery rate**

Recovery rate of microplastics were determined. For fish samples, recovery rate of microplastics was of 97±0.6%, by using KOH 5M (1:20) at 50°C for 48 h. For pasta samples, recovery rate was lower than fish one. It was, in fact, of 78±1.7. This is potentially due to the fact that, even though digestion efficiency was high (98.0%), organic matter was still present on the filter (Figure 2, left). Red meat recovery rate was of 98±01%.

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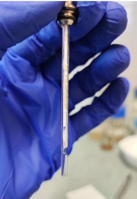
**Figure 2** *Filters after digestion and filtration of pasta, using Fenton’s Reagent (left) and meat sample, using KOH 5M (centre) and polystyrene fluorescent microbead before (on meat sample) and after digestion and filtration (right).*

## **5.3 Polymer solubilization into organic solvents**

For polymers solubilization, results showed that PS was dissolved using by DCM or a mixture of DCM: toluene, as it was suggested by the theory. Even though chloroform was suggested by theory, it did not work during the experiments. Likewise, for PET, HFIP was the only organic solvent able to dissolve PET polymer. Other solvents emerged from the calculation based on the theory did not work. Finally, PE was tested with the mixture of solvents (n-hexane: toluene) suggested by the theory. However, solubilization did not occur.

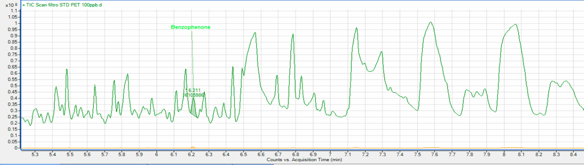
## **5.4 Microplastic’s quantification by Py-GC-MS**

PS solubilised in DCM and PET in HFIP was spiked on fish samples in a final concentration of 100 ppb. Likewise, solutions at known and increasing concentrations were prepared and filtered for the calibration curves ‘construction. After sample preparation steps, filter was cut and around 1 mm of the filter was placed into the liner and then placed in the pyrolysis cup for the analysis (Figure 3).

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**Figure 3** *Filter placed into the liner of the pyrolysis cup for the Py-GC-MS analysis.*

For the spectra obtained from the analyses, a strong background emerged. Basically, it can depend on the very important complexity of the food matrices.



**Figure 4** *Chromatogram of a fish sample spiked with 100 ppb of PET. Benzophenone is the main pyrolysis product of PET, which can confirm the identification of the polymer in the sample.*

As for the calibration curves, results are still being validated. The main issue is the liner. It is, in fact, not able to host the entire surface of the filters, meaning that several analyses are necessary for obtaining reliable results.

# **6. Conclusions and Future Perspectives**

The present PhD thesis would like to standardize the analytical methodologies currently applied in the microplastic’s research field. Discrepancies are, in fact, still present in the field, and so, results obtained by different groups can be difficultly compared. Basically, in the scientific literature, the preparation of the samples is really poorly investigated. Most of papers stated the occurrence of microplastics, but several parameters such as quality control/quality assurance (QA/QC) as well as the digestion of the organic matter are under investigated. In this way, knowledges are required to fill the gap. In this thesis, results showed that even at high digestion efficiencies, the number of microplastics can be underestimated, as organic matter still present can totally hide them. At the same time, Py-GC-MS is a really promising techniques but, some investigation are still necessary to quantify microplastics reliably.

# **7. Nomenclature**

Wi initial weight of digested material; Wa Weight of dry filter membrane after filtration; Wb Weight of dry filter membrane before filtration; Na number of microplastics on the filter after extraction; Nb number of microplastics added to the sample before the extraction procedures; Ra is the solubility parameter; Ro is radius of the sphere of solubility; δd indicates the dispersion term of polymer and solvent; δp indicates the polar term of polymer and solvent; δh is the hydrogen bond term.

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