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Occurrence of common plastic additives and contaminants in raw, steamed and canned mussel samples from different harvesting areas using MSPD-HPLC methodology

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Highlights

- Analysis of plastic additives in mussels from two different harvesting areas.

- Plastic additives contamination in raw, steamed and canned mussels were compared.

- MSPD methodologies were validated for steamed and canned mussel samples.

- Processed have a positive impact in terms of dietary exposure to plastic additives.

- Food safety implications of processing food commodities contaminated with microplastics.

Abstract

Microplastics are a complex mix of chemicals containing polymers and certain plastic additives such as bisphenols and phthalates. These particles are porous materials that can also sorb contaminants from their surroundings, and leach chemicals from the particle under certain circumstances. Aquatic animals can ingest microplastic particles, which mostly bioaccumulate in the gastrointestinal tract of animals. In terms of dietary exposure, small animals consumed whole such as mussels, contribute more to the dietary intake of microplastic particles. Plastic additives and contaminants are not chemically bound to the polymers, and certain processing methods or cooking processes result in the release of these chemicals that leach from the plastic particles, leaving them more available for absorption when ingested. Analytical methods are crucial for a better understanding of the occurrence of plastic additives and contaminants in aquatic products, and to know certain circumstances and treatments that influence human exposure. This study uses an MSPD-HPLC methodology for the simultaneous determination of 9 analytes (BPA, BPF, BPS, DEP, DBP, DEHP, DDD, DDT, and DDE) analyzing, for the first time, the occurrence of these chemicals in raw, steamed and canned mussels of two different harvesting areas (Atlantic and the Mediterranean), becoming one of the most efficient methodologies for determining the presence of these analytes in very complex food matrices, able to define the changes in cooking and processing activities. The results showed that the heat and pressure treatment could influence the migration of plastic additives from microplastic particles present in mussels to the cooking liquids.

Keywords: Microplastic additives; Bisphenols; Phthalates; Pesticides; HPLC-MS; MSPD; Processed food

Graphical abstract

1. Introduction

Plastic production has steadily increased over the last half-century. Plastics have changed to meet the needs of a variety of sectors and consumers and to enable technological improvements and solutions (Geyer et al., 2017). Depending on their specific use, polymeric materials with different physical and chemical properties can be mixed and plastic additives (plasticisers, colorants, UV-stabilizers, flame-retardants, and antioxidants, etc.) can be added to improve the performance of the final products.

Recycling the complex mixture of chemicals used for plastic production can be difficult, as efficient waste management systems are required, but most countries do not have the capacity to develop them, which has an impact on the environment and human health (FAO, 2023a). In the aquatic environment, plastics degrade, break, and interact with their surroundings, creating microplastic pollution, which is a complex mix of unknown polymers, plastic additives, and environmental contaminants (Galloway et al., 2017). Plastic additives, added to give specific features and improve the functional characteristics of plastics, are not chemically bonded to plastic polymers and can therefore migrate from the particles (Bolgar et al., 2015). Their leaching behaviour can be predisposed by external factors, such as temperature, UV radiation, salinity, or turbulence (Suhrhoff & Scholz-Böttcher, 2016). In addition, microplastics are porous materials that can interact with pollutants present in the aquatic environment, sorb, and concentrate them (Rochman et al., 2015). For this reason, microplastics have become a potential food safety threat that is especially relevant for fisheries and aquaculture products. Although microplastics have been reported in products such as meat, honey and sugar (Liebezeit & Liebezeit, 2013) beer (Liebezeit & Liebezeit, 2014), or eggs (Liu et al., 2022), aquatic products and water seem to be the best-studied source of dietary intake of microplastics (Lusher et al., 2017). Despite the fact that microplastics have been observed in many important aquatic commercial species, most of them have been detected in the gastrointestinal tract of aquatic animals, where most particles seem to concentrate after ingestion (Garrido Gamarro & Costanzo, 2022). Even though several studies in aquatic animals have shown that smaller microplastics and nanoplastics could be translocated in other organs such as the liver (Collard et al., 2017), exposure can be higher through the consumption of small aquatic species such as crustaceans, echinoderms, bivalves, and small-sized fish that are commonly eaten whole (Garrido Gamarro et al., 2020). Mussels are among the most consumed bivalve molluscs and the occurrence of microplastics in these products has been reported in several studies (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). In addition, mussels are filter feeders and are known to be one the best bioindicators of marine pollution.

Based on the above, there is clear evidence that the ingestion of aquatic commodities contaminated with microplastics containing organic pollutants could lead to higher exposure to toxic chemicals, with possible endocrine disruption and carcinogenicity effects. Exposure to microplastic particles, their additives, and their sorbed contaminants depends on several factors, such as particle size, shape, chemical changes that occurred during the processing and/or cooking steps of fisheries and aquaculture products, and consumption patterns (Garrido Gamarro & Costanzo, 2022).

From the food safety point of view, the evaluation of the impact of dietary exposure to microplastics is limited by the lack of toxicological data available for the complex chemical mixtures. For this reason, the selected analytes in this work were plastic

additives and other contaminants associated with microplastic. This decision will allow the extraction of further conclusions from the food safety perspective.

Bisphenol-A (BPA), bisphenol-S (BPS), bis(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP) are used during plastic manufacturing processes and have emerged as pollutants that result in serious environmental problems (Tumu et al., 2023). They have shown that their dietary exposure can have food safety implications (Basak et al., 2020). The European Union has set migration limits in products intended for food contact for BPA (0.05 mg of BPA per kilogram of food) (COMMISSION REGULATION (EU) 2018/213 of 12 February 2018 on the Use of Bisphenol A in Varnishes and Coatings Intended to Come into Contact with Food and Amending Regulation (EU) No 10/2011 as Regards the Use of That Substance in Plastic Food Contact Materials, n.d.), for BPS (0.05 mg of BPS per kilogram of food) (European Food Safety Authority (EFSA) et al., 2020), for DEHP (in 1.5 mg of DEHP per kilogram of food), and for DBP (0.3 mg of DBP per kilogram of food) (COMMISSION RECOMMENDATION (EU) 2019/794 of 15 May 2019 on a Coordinated Control Plan with a View to Establishing the Prevalence of Certain Substances Migrating from Materials and Articles Intended to Come into Contact with Food, n.d.). This compounds have been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO, 2023b) or the European Food Safety Authority (EFSA) (2023), and therefore their toxicities are well understood. On the contrary, other compounds such as bisphenol-F (BPF), and diethyl phthalate (DEP), commonly present in plastic manufacturing are still to be evaluated by JEFCA and EFSA and therefore are not regulated, but there is a growing concern about their toxicity and therefore were also included in the study, together with dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyltrichloroethane (DDT), which are known to be persistent, bioaccumulative, and toxic substances (PBTs), that could be sorbed from the surrounding environment by microplastic particles and could pose a food safety threat (Lusher et al., 2017).

Counting on good analytical methods, capable of determining the presence of a high number of plastic additives and contaminants in a food matrix is key. Efficient methods would allow exposure assessment exercises to be carried out to understand the impact of plastic additives and sorb contaminants on human health. So far, there are very few methods that allow the multiresidue determination of a high number of compounds related to the composition of microplastics and contaminants associated with them in raw and processed fisheries and aquaculture samples. Some of the methods only determine one of the analytes such as BPA (Zuo & Zhu, 2014) (Santhi et al., 2012) (Cerkvenik-Flajs & Šturm, 2021) (Niu et al., 2020) (da Silva et al., 2013) (Bonfoh et al., 2020) (Maragou et al., 2020) (Pedersen & Lindholst, 1999) (Podlipna & Cichna-Markl, 2007) (Ros et al., 2016) (Cunha et al., 2017) (Cunha et al., 2012) (Gu et al., 2014), some of them determine two such as BPA and BPS (Ademollo et al., 2018; Tian et al., 2020), but a limited number of studies determine three or more than three analytes such as BPA, BPS and BPF (Shaaban et al., 2022) (Alabi et al., 2014) (Liao & Kannan, 2013) or DEHP, DBP and DEP (He et al., 2015) (TAN & LIN, 2007), and even fewer evaluate the changes during processing and/cooking, being most of the focussed on the changes that the polymers suffer (Li et al., 2022), not in the plastic additives. In that sense, understanding the changes happening in the plastic particle, how the plastic additives and contaminants might leach, etc., give us the necessary information about the exposure to these analytes through mussel consumption.

In this work, the method developed by *Cañadas* (Cañadas et al., 2021) for the determination of BPA, BPF, BPS, DEP, DBP, DEHP, DDD, DDT, and DDE in raw mussels was adjusted to understand the changes in the content of plastic additives and related pesticides in mussels from the Atlantic and the Mediterranean during cooking and processing. Because mussels are consumed whole and because they are good bioindicators of coastal microplastic pollution (Li et al., 2019), they seem to be the product that can bring more information and clarity to exposure assessment exercises. In addition, mussels are widely consumed (FAO/WHO, 2023), so the study is of global relevance.

2. Materials and methods

2.1. Standards and chemicals

All reagents were of analytical grade unless specified otherwise. Water (18 MΩ/cm) was purified with a Milli-Q water system (Millipore Ibérica, Madrid, Spain). Bisphenol A (BPA) (purity ≥ 99%), bisphenol F (BPF) (purity ≥ 98%), bisphenol S (BPS) (purity 98%), diethyl phthalate (DEP) (purity ≥ 99%), dibutyl phthalate (DBP) (purity 99%), bis(2-ethylhexyl) phthalate (DEHP) (purity ≥ 99.5%), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyltrichloroethane (DDT) were supplied by Sigma-Aldrich (Madrid, Spain). Solid phase materials used for MSPD were Florisil from Sigma-Aldrich (Madrid, Spain), sodium sulfate anhydrous (Na_2SO4) from Panreac (Barcelona, Spain), and washed sea sand (0.25-0.30 mm) from Symta (Madrid, Spain). The glass wool silanized used was supplied by Panreac (Barcelona, Spain). All solutions collected for the Matrix Solid Phase Dispersion (MSPD) procedure were filtered through 0.22 µm Teflon membrane disk filters from Merck (Madrid, Spain). Sodium hydroxide and organic solvents such as acetonitrile (ACN), methanol (MeOH), and hexane were purchased from Scharlab (Madrid, Spain).

Stock standard solutions (1000 mg/L) of each analyte were prepared by dissolving the adequate amount of each compound in ACN. They were stored in dark bottles at 4°C until use and remained stable for at least three months. Working standard solutions at adequate concentration were daily prepared by appropriate dilution of the mentioned solutions with the dilution mixture MeOH/H_2O (85:15, v/v).

2.2. Mussel samples pretreatment protocol

Mussels (*Mytilus galloprovincialis*) from aquaculture production, from the Atlantic (Arousa estuary in Galicia in Northwest, Spain) and the Mediterranean (south of France) were used, representing two different harvesting areas. Mussels were purchased from different local markets in Spain. The samples were immediately transported to the laboratory, protected from possible contamination, and frozen at -20ºC until analysis.

The raw samples were dissected with a clean scalpel blade to separate the mussels from their shells and drained on a metal mesh/drainer for 10 minutes, to remove the water content from the mussel. Following this procedure, it was possible to eliminate the excess water that is inside the shell, leaving the mussel completely drained. After that, all the whole raw mussels were homogenized using a blender with metal blades.

For the preparation of the steamed and canned mussels, the raw mussels were rinsed with tap water after the byssal threads of mussels were removed. Then, samples were

steamed using a minimal quantity of tap water to allow the production of steam. Once the mussels opened and the samples reached room temperature, their shells were removed. Steamed mussels were homogenized the same way.

Instead, canned mussels-were placed in a metal casserole, covered with tap water and gentle heating until they open their shells. Then, the mussels were located in a metal pan (without any type of non-stick lining) with a small amount of olive oil and fried for a minute. They were removed from the pan and placed in a glass jar with a metal lid to build up a vacuum inside. To do this, the jar was completely submerged in a saucepan and boiled for 25 minutes. After that, the jar was left at room temperature. When the jar was opened for homogenization and analysis, it was verified that a vacuum had been generated inside. All samples prepared (raw mussels and mussels under culinary treatments) were stored at -20°C until the analysis.

2.3. Matrix solid-phase dispersion procedure

The analytes were extracted from the raw mussel samples following the MSPD procedure developed by *Cañadas* (Cañadas et al., 2021) using spiked and blank mussel samples. Based on this previous procedure, this MSPD methodology was reoptimized for the extraction of the analytes from the processed mussel samples. The analytes were extracted from the mussel samples using spiked and blank mussel samples. For this, 0.1 g of sample mussels (steamed or canned) were homogenised and spiked with 100 µL of stock standard solution at 40 mg/L in a mixture MeOH/H_2O (85:15, v/v) and the mixture was maintained at 20ºC for 20 min to allow the solution to equilibrate with the mussel matrix. The spiked mussel sample was poured into a glass mortar containing 0.5 g of Florisil as dispersing sorbent (< 200 mesh), 0.5 g of Na_2SO_4 as anhydrous agent (purity ≥ 99%) and 0.2 g of washed sea. The mixture was blended during 5 min to complete its homogenization and then, it packed into a glass cartridge with a plug of glass wool at the bottom, placing on top of this a small amount of Na_2SO_4 (0.004 g). The MSPD cartridges were conditioned with 2 mL of ACN and the analytes were optimally eluted from the column dropwise by gravity with other 9 mL of this solvent, in three extraction steps at 0.5 mL/min flow rate. The collected extracts were evaporated under a nitrogen stream and reconstituted in 400 µL of MeOH/H_2O (85:15, v/v). The blank samples were prepared by following the same procedure, excluding the spiked analytes from the MSPD mixture. In addition, a solvent blank was prepared to check for background contamination due to the use of laboratory plastic material. All samples were analysed by HPLC-MS.

2.4. HPLC-DAD-MS conditions

Chromatographic separation of the analytes was performed using an Agilent Technologies model 1200 series liquid chromatograph (Agilent Technologies, Germany) equipped with an on-line degasser, a quaternary pump, autosampler, and a photo-diode array detector (DAD). The analytes were separated on an ACE 5 C18-PFP HPLC column (150 × 4.6 mm, 5 µm) from Symta (Madrid, Spain). The column and autosampler were maintained at room temperature. The mobile phase consisting of a mixture of at 1% formic acid in Milli-Q ultrapure water (solvent A) and ACN (solvent B) was set to a gradient program: 0–30 min: 45–80% B; 30–31 min: 80–100% B; and 100% B for 9 min, at a flow rate of 0.8 mL min. Subsequently, the column was reconditioned with 45% B under isocratic conditions for 10 min. The sample injection volume was 20 µL. All compounds were successfully separated within 40 min. Quantification was performed using external calibration and peak area measurements. Ionization of the compounds was

studied by using the ESI interface in positive for phthalates (DBP, DEP, and DEHP) and negative ionization modes for bisphenols (BPA, BPS, and BPF) and pesticides (DDD, DDT, DDE) under the following conditions: a nebulize pressure of 60 psig, the gas temperature 350ºC with a gas flow of 11,4 L min^{-1}, capillary voltage of -5000 V and a gain of 3 for all components.

Table 1 show the precursor ions which were selected in both positive and negative mode and the retention times (t_R) for all analytes.

3. Results and discussion

3.1. MSPD procedure optimization

A method based on MSPD was used for the simultaneous determination of 6 targeted plastic compounds and 3 pesticides in raw, steamed, and canned mussel samples by HPLC-MS detection. The MSPD methodology developed by *Cañadas* (Cañadas et al., 2021) was applied to raw mussels. However, when this methodology was applied to the processed samples, the extracts were not clean enough, therefore some variables affecting the extraction process were re-optimized. Two extraction solvents (ACN and MeOH) and a mix of MeOH:ACN at different ranges (80:20, 50:50, and 20:80, v/v) were tested. For all the processed samples (steamed and canned) higher recoveries were obtained when 9 mL of MeOH is using as extraction solvent, with values between 85 and 95%, with RSD< 10% for all analytes except DEP. In general, protic polar solvents such as ethanol and methanol (dielectric constants of 24 and 33, respectively) showed a better extraction efficiency than aprotic polar solvent as acetonitrile (dielectric constant 37.5) due to the protic polar solvents had been related to the fact that these solvents have a greater capacity for the solvation of anions than aprotic solvents due to the formation of hydrogen bonds (Collins et al., 2006). When the same volume of acetonitrile was used, recoveries of analytes were between 81-90% for steamed samples and 77-84% for canned samples were reached (see Figures 1 and 2).

Figure 1. Recoveries obtained for bisphenols, phthalate esters, and pesticides using spiked steamed mussels after MSPD extraction and HPLC-MS detection (SD, standard deviation) using different extraction solvents.

Figure 2. Recoveries obtained for bisphenols, phthalate esters, and pesticides using spiked canned mussels after MSPD extraction and HPLC-MS detection (SD, standard deviation) using different extraction solvents.

However, though these recovery percentages with MeOH are somewhat higher compared to recoveries with ACN, this solvent allows quantifying the DEP compound, which is not extracted with MeOH, and obtaining cleaner chromatograms. For these reasons, ACN was selected as the optimum solvent for the extraction of the set of analytes of the MSPD mixture composed of 0.1 g of mussel samples and 0.5 of Florisil, as a solid phase for sample dispersion, 0.5 g of Na_2SO_4 as a drying agent and 0.2 g of washed sea sand, an inert material, also like solid support. The samples extracted were subjected to solvent evaporation and reconstituted in adequate solvent MeOH/H_2O (85:15, v/v) for further analyse by HPLC-MS.

Additional experiments were performed to evaluate different sample masses between 0.05-0.1 g of cooked mussels. Assays carried out under the conditions previously selected, allowed to choose 0.1 g for future assays since the obtained samples were clean enough to be analysed and the recoveries for all analytes using this amount of sample and 9 mL of ACN as extraction solvent were between 75 and 100% with an RSD lower than 12% for all analytes in the whole samples. Decreasing the sample masses to 0.05 or 0.075 g did not improve the recoveries, being between 70 and 90%, with RSD< 15%.

3.3. Validation of the methods

The MSPD methods for steamed and canned mussel samples were validated in terms of linearity, precision, recovery, and limits of detection (LODs) and quantification (LOQs), using spiked mussel samples. The linearity of the methods was tested for all the plastic additives and contaminants studied. Calibration curves were constructed in the range of 2.0–120 µg/kg by preparing spiked samples. As can be seen in Table 2, the results showed good linearity for all the analytes, and for both types of processing, with determination coefficients (R^2) between 0.989-0.999. Precision was calculated in terms of intra-day repeatability (n= 3) and inter-day reproducibility (three consecutive days) and was determined by triplicate assays at three spiking levels (2, 40, and 80 µg/kg). The intra-day repeatability and inter-day reproducibility were evaluated as relative standard deviation (RSD) ranged from 5.6-10% and 2.6-7.6%, for steamed samples, respectively. In the case of canned mussel samples, reproducibility (day-to-day variability) values ranged from 3.4-7.2% and the intra-day repeatability was lower than 10% for all analytes. These data are indicators of good accuracy. The limit of detection (LOD) is defined as the smallest amount of an analyte that can be detected upon signal-to-noise. The limit of quantification (LOQ) is the value that corresponds to the smallest amount of analyte in a sample that can be determined quantitatively with acceptable accuracy and precision (RSD< 7%) under the established experimental conditions. This limit depends on the relationship between the magnitude of the analytic signal and the value of the statistical fluctuations of the zero signal. Unless the analytical signal is greater than the zero signal plus a multiple k of the standard deviations of this zero-signal due to random errors, it is not possible to identify with certainty the analytical signal. It has been considered a factor k= 3 in the calculation of LOD and k = 10 for LOQ. The obtained results were calculated according to the FDA Guidance for Industry (FDA, 2015). The LOQs for all the analytes, and for two food processing techniques applied, calculated as the lowest concentrations where the RSDs were less than 5%, were between 0.21-4.63 µg/kg (Table 2). The LODs obtained for all analytes were between 0.06-0.86 µg/kg and 0.47-1.39 µg/kg for steamed and canned samples, respectively.

3.4. Determination of contaminants in raw and processed mussel samples from two different harvesting areas

For the evaluation of the impact of the different processing techniques in the presence of the analytes in mussels, different studies were carried out for raw, steamed, and canned samples.

A mesh of mussels (*Mytilus galloprovincialis*) was used for each growing area: Atlantic and Mediterranean, respectively. Specimens from each mesh were divided into three parts and underwent different treatments (raw, steam, and canned) The validated method was applied to samples of mussel samples raw, steamed, and canned. Five samples per treatment and area were processed (n= 30), and analyses were carried out in triplicate. The analyte concentration is expressed as μg of contaminant per gram of drained mussel (μg/g), considering the exact weight of the sample in each assay. In Table 3, the results of the additives and pesticides detected in the samples are shown. The analysis of the pesticides (DDD, DDT, DDE) in all the mussel samples (raw, steamed, and canned) are below the detection limits of the mass spectrometer. Given the good sensitivity of the method in previous studies with raw mussels, it can be deduced that there is no significant contamination of these compounds in samples from these harvesting areas.

Regarding the contents of bisphenols (BPS, BPF, BPA), and phthalates (DEP, DBP, DEHP) used in the plastics industry for the provision of robustness and flexibility to polymeric materials, respectively, it is observed that, in general, the concentration of these compounds decrease after the cooking process, and by extreme processing conditions, which might be due to interactions with boiling water or cooking oil. In both, mussels from the Atlantic and the Mediterranean, the concentrations of all analytes are higher in raw samples than in steam ones, highlighting the BPF content in the raw species from both sources (4.976 and 11.470 μg/g). BPA was detected only in raw mussels from the Atlantic in a small concentration and bisphenols in canned samples were not detected in any of the harvesting areas.

Phthalates DEP and DBP appear in raw and in all processed samples from both harvesting areas. A slight decrease in concentrations of these analytes is observed when the mussel samples are processed, being again slightly higher in the steamed mussels than in the canned mussels. DEHP was not observed in any samples except in steamed samples from the Mediterranean, in which a small amount was detected.

The comparison of the results of the two harvesting areas (Atlantic and Mediterranean) does not reflect a significant difference between the content of these plastic derivatives in general, except for the content of BPF and BPS to a lower extent, which contents are higher in samples from the Mediterranean than in samples from the Atlantic in both, raw and steamed samples. Given these results, the decrease in the concentration of contaminants in the processed samples with respect to the raw samples could be explained by a transfer from the mussel to the processing liquid and this transfer is higher in the case of canned samples than steamed samples, which might be due to the nature of the olive oil, where organic compounds are more easily soluble.

As an example, Figure 3 shows the chromatograms of HPLC-MS for a Mediterranean steamed sample.

Figure 3. HPLC-MS chromatograms of canned mussel samples spiked at 20 μg/kg (−) and blank (−) after MSPD extraction.

Those analytes for which the European Union has set migration limits in products intended for food contact (BPA (0.05 mg of BPA per kilogram of food) (COMMISSION REGULATION (EU) 2018/213 of 12 February 2018 on the Use of Bisphenol A in Varnishes and Coatings Intended to Come into Contact with Food and Amending Regulation (EU) No 10/2011 as Regards the Use of That Substance in Plastic Food Contact Materials, n.d.), for BPS (0.05 mg of BPS per kilogram of food) (European Food Safety Authority (EFSA) et al., 2020), for DEHP (in 1.5 mg of DEHP per kilogram of food), and for DBP (0.3 mg of DBP per kilogram of food) (COMMISSION RECOMMENDATION (EU) 2019/794 of 15 May 2019 on a Coordinated Control Plan with a View to Establishing the Prevalence of Certain Substances Migrating from Materials and Articles Intended to Come into Contact with Food, n.d.)), the results do not exceed the set limits.

4. Conclusions

This paper studies the effect of cooking in the presence of compounds derived from microplastic contamination in mussels. To our knowledge, it is the first time that this research has been performed. The extraction of the targeted analytes from steamed and canned samples was based on the matrix-solid phase extraction (MSPD) method coupled with HPLC-MS. The extraction methodologies of 6 plastic additives were validated for both, steamed and canned mussels.

MSPD sample treatments provided good sensitivity, accuracy, and repeatability for the simultaneous determination of the 9 analytes studied for raw, steamed, and canned mussel samples coming from the Atlantic and the Mediterranean. For all the analytes, satisfactory recoveries were achieved, being 81–90% and RSD between 1.7-8.4% for steamed samples, and 77-84% and RSD between 1.3-19% for canned samples and LOD, and LOQ were ranged between 0.06-4.63 µg/kg.

The analysis of real samples revealed interesting results related to the occurrence of plastic additives in mussel samples before and after the cooking process. It has been observed a decreased content of the analytes in processed samples with respect to raw samples. The differences in the concentration of compounds in the different processing steps suggest that the heat and pressure treatment might influence the migration of bisphenols (BPS, BPF, BPA), and phthalates (DEP, DBP, DEHP) from the microplastic particles present in mussels to the processing liquid. The interaction of these plastic additives with the water and/or oil where they have been processed or cooked might have had as a result the decrease in the concentration of analytes in the matrix. Analytes were always found to be higher in raw samples, followed by steamed. Only DEP and DBP were detected in canned samples. DDD, DDT, and DDE were not detected in any of the cases, so taking into consideration the good sensitivity shown in previous occasions using this methodology for the analysis of raw mussels, it can be assumed that there is no significant contamination of these compounds in samples from any of the harvesting areas.

The results show that in the case of mussels, cooking, and processing have a positive impact in terms of dietary exposure to these compounds, always if the boiling water and/or oil is not consumed. The determination of plastic additives in the oil or water would be necessary to get to further conclusions.

CRediT authorship contribution statement

E. Garrido: Methodology, Data acquisition, writing-original draft preparation. D.L. Soliz: Data acquisition. R.M. Garcinuño Martínez: Methodology, Validation, Writing - review & editing, Funding acquisition. G. Paniagua González: Resources, Methodology, Validation, Writing - original draft preparation. P. Fernández Hernando: Supervision, Writing - review & editing, Funding acquisition.

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.

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Tables

Table 1. Characteristics of mass transitions for the studied analytes.

Analyte	Ion SIM	Cone voltage, eV	Retention time (t_R), min
BPS	92, 108, 156, 249	120	3.376
BPF	98, 183, 199.1, 200	120	4.563
BPA	119, 213, 228	120	6.244

DEP	149, 177	100	10.489
DBP	149, 205	100	22.679
DEHP	149, 167, 279	120	34.976
DDD	165, 235, 237	100	27.673
DDT	165, 199, 235	100	31.819
DDE	176, 246 248	120	33.000

Table 2. Data from analytical validation methods for the determination of analytes in steamed and canned mussel samples.

Analyte	Linearity µg/kg	Spiking level µg/kg	Stained mussel samples					Canned mussel samples				
			R^2	Recovery ± RSD %		LOD µg/kg	LOQ µg/kg	R^2	Recovery ± RSD %		LOD µg/kg	LOQ µg/kg
				Inter-day	Intra-day				Inter-day	Intra-day		
BPS	2.0–120	2.0	0.991	86.9 ± 4.2	89.3 ± 8.3	0.06	0.21	0.997	81.5 ± 3.9	79. ± 7.5	0.47	1.57
		40.0		88.2 ± 3.8	80.6 ± 9.5				79.9 ± 4.1	78.8 ± 8.2		
		80.0		84.3 ± 4.5	81.5 ± 8.6				82.5 ± 3.7	80.2 ± 9.1		
BPF	2.0–120	2.0	0.999	83.8 ± 6.4	79.9 ± 9.2	0.13	0.44	0.998	82.7 ± 6.9	79.3 ± 9.2	0.53	1.77
		40.0		84,4 ± 4.8	83.5 ± 8.3				80.5 ± 5.1	78.1 ± 9.0		
		80.0		81,6 ± 5.3	80.3 ± 8.9				82.3 ± 4.7	81.7 ± 8.2		
BPA	2.0–120	2.0	0.996	84.3 ± 3.4	83.2 ± 5.9	0.68	2.28	0.990	74.5 ± 4.0	70.6 ± 6.2	0.70	2.33
		40.0		87.7 ± 2.6	86.9 ± 6.3				77.3 ± 3.9	73.2 ± 7.8		
		80.0		82.9 ± 4.3	84.3 ± 5.6				73.6 ± 3.4	70.2 ± 6.0		
DEP	2.0–120	2.0	0.991	88.6 ± 3.7	81.3 ± 6.4	0.10	0.32	0.999	79.2 ± 4.6	77.1 ± 7.5	0.31	1.03

		40.0		89.9 ± 4.8	88.7 ± 9.3				81.0 ± 3.5	78.5 ± 8.2		
		80.0		87.4 ± 5.6	86.5 ± 6.8				80.1 ± 3.9	78.3 ± 7.0		
DBP	2.0–120	2.0	0.990	89.7 ± 6.3	79.9 ± 9.9	0.86	2.88	0.990	79.5 ± 4.9	77.9 ±10.0	1.39	4.63
		40.0		87.4 ± 5.5	85.9 ± 9.7				78.1 ± 4.3	75.2 ± 8.9		
		80.0		89.3 ± 7.2	86.9 ± 9.6				79.8 ± 5.4	73.1 ± 8.4		
DHEP	2.0–120	2.0	0.989	83,9 ± 6.3	78,3 ±10.0	0.62	2.07	0.993	80,7 ± 4.5	79,2 ± 9.8	0.99	3.30
		40.0		81.4 ± 7.3	82.9 ± 8.9				84.2 ± 5.8	81.0 ± 9.3		
		80.0		83.5 ± 7.6	79,0 ±10.0				81.8 ± 5.2	78,3 ± 8.5		
DDD	2.0–120	2.0	0.999	81.2 ± 6.5	79.4 ±10.0	0.33	1.09	0.999	78.3 ± 7.2	73.8 ± 8.9	0.49	1.62
		40.0		89.8 ± 5.8	86.6 ± 7.3				76.4 ± 6.0	71.4 ± 8.3		
		80.0		87.8 ± 6.0	82.0 ± 8.1				75.5 ± 4.2	71.5 ± 6.5		
DDT	2.0–120	2.0	0.990	80.2 ± 5.1	79.0 ± 5.3	0.45	1.51	0.997	78.6 ± 4.8	74.9 ± 5.3	0.70	2.33
		40.0		83.6 ± 4.2	81.5 ± 6.2				80.9 ± 5.5	79.4 ± 7.8		
		80.0		85.4± 3.0	83.6 ± 5.9				81.3± 4.2	73.0 ± 9.5		
DDE	2.0–120	2.0	0.998	83.2 ± 3.2	81.2 ± 8.2	0.22	0.75	0.998	77.2 ± 5.7	72.8 ± 9.5	0.65	2.15
		40.0		85.7 ± 2.5	83.4 ± 6.4				79.9 ± 4.9	76.1 ± 9.0		

80.0	88.1± 3.6	86.1 ± 5.7	78.3 ± 5.2	75.9 ± 8.5

Table 3. Obtained results for the analysis of the raw and processed (steamed and canned) real samples (n=30) from the Atlantic and the Mediterranean. Analytical measurements were done by triplicate.

Analyte ppm (µg/g ± SD)	BPS ± SD	BPF ± SD	BPA ± SD	DEP ± SD	DBP ± SD	DEHP ± SD	DDD ± SD	DDT ± SD	DDE ± SD
Raw Atlantic	0.068±0.010	4.976±0.032	0.058±0.010	0.318±0.056	0.661±0.142	ND	ND	ND	ND
Steamed Atlantic	0.044±0.005	0.352 ±0.006	ND	0.244±0.078	0.080±0.003	ND	ND	ND	ND
Canned Atlantic	ND	ND	ND	0.138±0.027	0.065±0.002	ND	ND	ND	ND
Raw Mediterranean	0.531±0.144	11.470±0.319	ND	0.215±0.050	0.188±0.050	ND	ND	ND	ND
Steamed Mediterranean	0.628±0.075	7.686±1.080	ND	0.164±0.0145	0.058±0.004	0.017±0.001	ND	ND	ND
Canned Mediterranean	ND	ND	ND	0.029±0.003	0.057±0.012	ND	ND	ND	ND

ND: non detected

Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: