Extractable Pollutants from Microplastics to Vegetables: Incorporation to the Food Chain

Using static headspace GC/MS with the Agilent 8890 GC and 5977B GC/MSD

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Abstract

The presence and impact of microplastics (MPs) are being extensively researched and reviewed, especially in the marine environment. However, mobility, transportation routes, and accumulation of leaching compounds (such as additives) in plastic waste such as MPs are scarcely studied, and information regarding their ecotoxicity is limited. This application note investigates the levels of leachates from plastic materials in vegetables with edible- and nonedible-roots. Samples were analyzed by static headspace gas chromatography/mass spectrometry (SHS-GC/MS) using a static headspace (SHS) coupled to an Agilent 8890 GC and Agilent 5977B GC/MSD. A total of 92 compounds were identified in the 114 analyses, which corresponded to 42 and 72 edible- and nonedible-root vegetables, respectively. The application of chemometrics to the SHS-GC/MS dataset allowed differentiation between the levels of plastic-related compounds in edible- and nonedible-root vegetables—the former showing a higher content of plastic leachates. The developed SHS-GC/MS methodology was shown to be straightforward, reliable, and robust, and allowed the quantification of plastic-associated compounds in the samples. Several of the quantified compounds indicated potential contamination of polystyrene (PS) and/or polyvinyl chloride (PVC) MPs.

Introduction

MPs, defined as plastic particles smaller than 5 mm[1,2], have emerged as pervasive pollutants with potential negative effects on the environment and human health.[3–5] Their ability to easily migrate between air, soil, water, and living organisms has raised concerns regarding their toxicity and impact on the food chain. MPs contain various additives such as plasticizers, flame retardants, and stabilizers, which can release toxic substances into the environment, affecting soil quality and plant health.[6–8]

The contamination of agricultural systems with MPs poses a significant risk, as these particles and their associated toxic compounds can be absorbed by plant roots and migrate through the plant, affecting vegetable quality and entering the food chain.[7,9] Understanding the identity and distribution of MPs and their impact on food crops is crucial for assessing potential risks to human health. This application note explores the methodologies for detecting MPs and associated compounds, emphasizing the need for further research and regulation in this area.

Most studies of organic pollutants in vegetables focus on persistent organic pollutants (POPs)[10,11], which are extracted from samples and usually monitored by liquid chromatography (LC).[12] However, the accumulation of MP-associated pollutants in vegetables has hardly been evaluated.

This application note presents:

1. A developed and validated approach to the targeted and untargeted analyses of MP-associated pollutants found in vegetables
2. Quantitation of MP pollutants
3. Assessment of the influence of vegetable type (edible- versus nonedible-roots) on the intake of MP-associated pollutants
4. The accumulation and environmental migration pathways of the MP pollutants

In this application note, an SHS-GC/MS method involving a static headspace coupled to an 8890 GC and 5977B GC/MSD was used for the evaluation of volatile extractables from plastic residues present in vegetable matrices. This technique is well suited to extract volatile compounds from different samples due to its simplicity, automation, speed, low cost, and minimal sample preparation requirements.[13–15] Additionally, the application of MS in combination with a spectral deconvolution and retention indices (RIs) enabled advanced detection and identification of volatile MP-associated pollutants in vegetable matrices.

Experimental

Reagents

Analytical standards of cyclohexane, pentanal, hexanal, ethylbenzene, *p*-xylene, styrene, *o*-xylene, cumene, benzaldehyde, octanal, phenylacetaldehyde, acetophenone, *p*,α-dimethylstyrene, nonanal, 2,4-dimethylphenol, 4-methylacetophenone, 2-*tert*-butyl-4-methylphenol, and 2,4-di-*tert*-butylphenol were obtained from Sigma-Aldrich (St. Louis, MO, U.S.). Toluene and naphthalene (Sigma-Aldrich) were selected as internal standards (IS). The purity of the standards was greater than 99.9%. For identification purposes, a mixture of alkanes from C_8 to C_{40} at 500 µg/mL in heptane was purchased from Sigma-Aldrich. Acetonitrile (ACN) of 99.9% chromatographic quality was also obtained from Sigma-Aldrich. Individual stock solutions were prepared in ACN at 1,000 µg/mL and placed in amber bottles at −20 °C. Daily standard solutions were prepared with all the compounds at 10 µg/mL in ACN and kept at 4 °C. To mitigate possible interferences from the use of plastic material, glassware devices were applied alternatively and washed with acetone and Milli-Q water. The cleaned glassware was dried at 60 °C in an oven.

Instrumentation

The 8890 GC and 5977B GC/MSD equipped with an inert electron ionization (EI) source were used for the analyses. SHS sampling was carried out by a multipurpose sampler (MPS) with direct and headspace (HS) injection approaches from Gerstel (Mülheim, Germany). The MPS was coupled to the GC/MSD system.

Two Agilent J&W HP-5ms GC columns (5% diphenyl/95% dimethylpolysiloxane) of 15 m length × 0.25 mm id, 0.25 µm film thickness were used and connected by the Agilent purged ultimate union (PUU) for chromatographic separation. The Agilent pneumatic switching device (PSD) for the 8890 GC was used for flow and pressure control at the midpoint and to sweep the linkage. Once the latest analyte was released from the second column, the pressure at the midpoint was increased above 50 psi and the carrier gas flow was reversed through the first column to ensure that the GC columns did not have any sample residue related to heavy compounds. During the analysis, the helium carrier gas flow was maintained for the first column at 1 mL/min and for the second one at 1.2 mL/min. The GC program was initiated at 40 °C (5 minutes) and was raised to 130 °C at 3 °C/min, then it was increased to 250 °C at 6 °C/min, and, finally, the temperature was raised to 280 °C (1 minute) at 20 °C/min. The quadrupole, ion source, and transfer line temperatures were 150, 230, and 300 °C, respectively. The MS detector functioned in full scan mode in the range of masses from *m/z* 35 to 500. The retention times and the monitored ions in selected ion monitoring (SIM) mode are displayed in Table 1.

Table 1. Retention times and characteristic ions of analytical standards.

Compound	Retention Time (minutes)	Target Ion (*m/z*)	Qualifier Ions (*m/z*)
Cyclohexane	2.9	56	41, 84
Pentanal	3.4	44	29, 58
Toluene (IS)	5.1	91	65, 92
Hexanal	6.3	44	41, 56
Ethylbenzene	9.2	91	51, 106
p-Xylene	9.3	91	105, 106
Styrene	10.3	104	78, 103
o-Xylene	10.5	91	77, 106
Cumene	12.1	105	77, 120
Benzaldehyde	13.9	77	105, 106
Octanal	16.3	43	44, 56
Phenylacetaldehyde	18.3	91	92, 120
Acetophenone	20.2	105	51, 77
p,α-Dimethylstyrene	20.6	117	115, 132
Nonanal	21.5	57	41, 43
2,4-Dimethylphenol	23.5	122	107, 121
Naphthalene (IS)	25.1	128	102, 130
4-Methylacetophenone	25.5	119	91, 134
2-*tert*-Butyl-4-methylphenol	33.2	149	121, 164
2,4-Di-*tert*-butylphenol	39.0	191	57, 206

Software

Agilent MassHunter Workstation software was used for data acquisition and analysis. For statistical data analysis, Simca-P 14.1 (Umetrics, Malmö, Sweden) and StatGraphics (Statistical Graphics, Rockville, MD, U.S.) were used. Quantification was carried out using analyte peak area divided by the corresponding internal standard peak area of the extracted ion chromatogram (EIC) of the target ion.

Additionally, MS-DIAL software, version 4.80, was used for compound identification, based on a match criterion of over 70%.

Samples and analytical procedure

A total of 38 vegetables were bought from different suppliers at a market. Those included potato, radish, carrot, onion, sweet potato, garlic, turnip, parsley, parsnip, mushroom, ginger, fennel (edible-root vegetables) and broccoli, red pepper, tomato, cucumber, zucchini, pumpkin, chard, lettuce, leek, eggplant, artichoke, celery, red cabbage, asparagus, spinach, iceberg lettuce, cauliflower, and green beans (nonedible-root vegetables). For some of the commodities, several varieties were analyzed. To mitigate possible interferences from plastic packaging, the selected samples were not packaged. From the samples, 14 were classified as vegetables with edible roots and 24 as vegetables with nonedible roots. The nonedible parts (except the roots) of the vegetables were discarded, and the samples were not peeled. An IKA A 11 basic mixer (Wilmington, NC, U.S.) was applied to crush and homogenize the vegetable samples (30 g) before storing them in the freezer at –20 °C until analysis. A vortex mixer (Heathrow Scientific, IL, U.S.) was also used for sample treatment.

For SHS sampling, 3 g of homogenized vegetable were placed in a HS vial of 20 mL capacity, and toluene and naphthalene (IS) were added at 500 ng/g. Samples were shaken at 1,500 rpm for 10 seconds in a vortex agitator to be homogenized, then incubated at 130 °C for 35 minutes and shaken at 750 rpm. A 2 mL volume of gas from the HS above the sample was taken by a syringe (2.5 mL) and automatically injected into the GC system at 280 °C in split mode (10:1 ratio). The measurement was performed as described previously.

Results and discussion

Selection of chromatographic parameters

Several oven temperature conditions were assessed to achieve the best separation between the different compounds isolated from the carrot sample. The oven temperature program providing the best results was: from 40 °C (5 minutes) to 130 °C at 3 °C/min, then to 250 °C at 6 °C/min, and finally to 280 °C (1 minute) at 20 °C/min. This oven temperature program provided an analysis time of approximately 58 minutes, with a selected carrier gas flow of 1 mL/min for the first column and 1.2 mL/min for the second to enable most effective chromatographic separation. Even though all compounds released from the carrot sample were separated at 250 °C, the oven temperature was additionally raised to guarantee the elution of possible species contained in other vegetable samples that have high boiling points and a higher interaction with the stationary phase. Several injection split ratios were evaluated (1:30, 1:20, and 1:10). The 1:10 ratio provided the best sensitivity and peak resolution and was therefore selected. To avoid air entry into the GC/MS equipment during injection, higher split ratios are not recommended. Volumes between 0.75 and 2 mL were injected, and 2 mL was selected because it gave the highest signal for all the compounds. Higher injection volumes were not recommended by the supplier for the HS injection approach.

Optimization of sample treatment process

An SHS sampling technique was used for collecting volatile species released from the vegetable sample into the gas phase. To determine the possible influence and interaction of incubation time and temperature (both assessed at three levels) on SHS extraction efficiency, a response surface methodology (face centered) applying peak area as the analytical signal was carried out for every analyte. For this purpose, 1 g of nonspiked carrot was used. Eleven measurements were performed, in which incubation time ranged from 5 to 35 minutes and temperature ranged from 100 to 130 °C. Both parameters affected the analyte signal of all the detected volatiles. The determination coefficient (R^2) considering the analytical response of all analytes was 97.8%, indicating the suitability of the design. As shown in Figure 1, the optimal incubation conditions were 130 °C for 35 minutes.

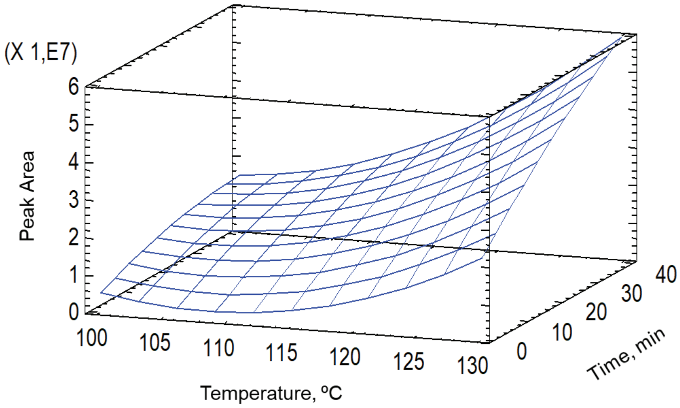

Figure 1. Estimated response surface obtained in the optimization procedure using a response surface methodology (face-centered).

Sample masses of 1, 2, 3, 4, and 5 g of carrot were analyzed to assess the effect of sample amount. For most of the compounds, the peak area increased with sample mass from 1 to 3 g; however, for higher masses, the analytical signal mostly decreased, probably due to a matrix effect. Therefore, a sample mass of 3 g was chosen.

Nontargeted approach for MP-associated compounds in vegetables

A total of 38 vegetable samples were measured in triplicate using SHS-GC/MS, with the detector operating in full scan mode to identify the highest number of volatile species that could be evolved from the vegetables.

The analysis of mass spectra (spectrum matching) together with the use of RIs by applying a mathematical deconvolution method (MS-DIAL) is a powerful tool for identification purposes that improves the evidence of the putative chemical structures.[16] This approach provided a very accurate identification. After removing duplicate entries and discarded compounds that were clearly associated with vegetable composition and chromatographic column bleeding, a total of 92 chemical structures were accurately identified with a score higher than 70% in all cases (Table 2).

Table 2. Compounds detected in the samples by SHS-GC/MS and identified with MS-DIAL (continued on the following page).

Compound	Retention Time (min)	Score (%)
n-Butane	2.773	84.6
Isobutane	2.774	90.0
Cyclohexane	2.873	95.0
2-Methyl-1,3-dioxolane	2.900	95.0
tert-Butyl Chloride	3.179	83.8
1,1,1-Trimethoxyethane	3.364	75.8
Pentanal	3.382	95.0
N,N-Dimethylethylamine	3.409	77.1
2,2-Dimethoxypropane	3.730	80.1
Ethyl Acetate	4.185	84.6
Isopentane	4.279	86.1
Isopentyl Alcohol	4.300	82.9
tert-Butyl Bromide	4.317	76.1
2-Chlorobutane	4.357	72.0
Bis(chloromethyl) Ether	4.410	80.1
Methyl Isobutyl Ether	4.484	83.2
Toluene (IS)	5.054	95.0
3-Methyltetrahydrofuran	5.089	82.0
Cyclopentane	5.228	81.5
Allyl Formate	5.322	84.7
Hexanal	6.287	95.0
Pentane	6.323	80.5
Methyl Butyl Ether	6.527	85.3
2-Propanol	7.285	83.8
Methacrylic Acid Methyl Ester	7.602	80.6
Cyclohexane Oxide	8.630	79.5
(3Z-) Hexenol	8.748	94.6
Ethylbenzene	9.243	95.0

Compound	Retention Time (min)	Score (%)
p-Xylene	9.257	95.0
(2Z-) Hexenol	9.312	93.6
Cyclohexane	9.490	87.5
Styrene	10.302	95.0
2,4-Hexadienal	10.467	72.7
o-Xylene	10.472	95.0
2-Heptanol	10.986	82.3
Isopentyl Formate	10.994	80.7
Cumene	12.125	95.0
Camphene	13.421	90.8
Benzaldehyde	13.878	95.0
Allyl propionate	14.104	80.7
4-Methyl-penten-3-one	15.029	71.9
1-Octen-3-ol	15.089	84.7
Benzonitrile	15.167	93.3
2,4-Dimethylthiazole	15.205	89.2
2-Ethylbutyraldehyde	15.486	84.8
2-Octanone	15.660	85.2
3-Octanol	15.898	84.4
Octanal	16.261	95.0
Cyclohexyl Formate	16.569	88.0
2,4-Heptadienal	16.724	88.3
Hexyl Acetate	16.908	79.4
Cyclooctane	17.231	76.7
Ethylhexanol	17.697	82.5
Phenylacetaldehyde	18.260	95.0
3-Heptyl Formate	18.439	75.2
Styrene Oxide	18.938	82.5
2-Octenal	19.117	91.0
Acetophenone	20.189	95.0
p,α-Dimethylstyrene	20.596	95.0
3-*tert*-Butyl-1-methylpyrrole	20.696	78.8
2-Methylmercaptopyridine	20.814	80.6
Isopentyl Methyl Ketone	20.918	87.5
4-Allyl-1,3,5-trimethylpyrazole	21.133	84.6
Undecane	21.255	90.1
Nonanal	21.492	95.0
1,2,3,5-Tetramethylbenzene	21.712	91.9
1,2,4,5-Tetramethylbenzene	21.917	83.1
tert-Butylbenzene	22.864	90.4
2,4-Dimethylphenol	23.496	95.0
2,6-Nonadienal	23.916	94.1
2-Nonenal	24.207	95.3
Nonanol	24.839	87.4
Naphthalene (IS)	25.071	95.0
m-Methoxyphenol	25.213	82.0
m-Toluoyl Chloride	25.271	88.6
4-Methylacetophenone	25.490	95.0
3-Chlorophenol	25.892	89.0
Ethyl Octanoate	26.117	86.4
tert-Pentylbenzene	26.359	76.6
Decanal	26.445	93.6
1-Methyl-4-isopropenylcyclohexene	27.504	91.6
p-*tert*-Butylphenyl Methyl Ether	27.798	71.2
2-Methylacetophenone	28.520	80.5
2-Ethylhexanol	29.065	82.2
Mesitaldehyde	30.110	83.6
Tridecane	30.826	87.3
2,4-Decadienal	31.506	81.2
1-(4-Methlphenyl)ethanol	32.402	82.4
2-*tert*-Butyl-4-methylphenol	33.175	95.0
Tetradecane	35.154	90.2
3'-Hydroxyacetophenone	36.185	73.3
2,6-Dimethylterephtalonitrile	36.391	87.4
(E)-3,7-Dimethyl-2,6-octadien-1-ol	38.694	82.6
2,4-Di-*tert*-butylphenol	38.950	95.7

In addition to species related to vegetable composition, many additive compounds and degradation products associated with plastics were detected in the samples. Some may be related to vegetable composition, polymeric materials, or both.

Detected substances that may be used as specific markers of MP contamination are chlorinated paraffins (*tert*-butyl chloride, 2-chlorobutane), which are characteristic products associated with PVC contamination.[17] PVC is commonly applied in agriculture to cover greenhouses, and its use has progressively expanded to replace paper or straw for soil mulching in many countries.[18] In addition, the presence of benzene derivatives such as ethylbenzene indicate contamination by PVC.[19] Phenolic species, which are UV-absorbing stabilizers[20], were also observed in the samples, specifically, 2,4-dimethylphenol, 2-tert-butyl-4-methylphenol, and 2,4-di-*tert*-butylphenol.[21] Although phenolic compounds are commonly present in the vegetable matrix, these are normally flavonoids and hydroxycinnamic acyl glycoside structures[22]; therefore, alkylphenols are potential markers of MP contamination. Alkylphenols are commonly added as additives to different polymers, being also specific degradation products of polycarbonate (PC).[23,24] PC is another polymeric material applied for agricultural practices; for example, as a cover during tomato production.[25] Moreover, in some of the analyzed vegetables, 2-methyl-1,3-dioxolane was found, which has been associated with plastic materials, specifically, polyethylene terephthalate (PET) bottles.[26] Ethyl acetate has been detected in PET samples previously[26]; however, this compound is also responsible for the aroma of several vegetables, and thus is also associated with vegetable matrices. Moreover, other benzene-derived species such as *p*-xylene and *o*-xylene are characteristic degradation products of PVC and PET, which are plastics commonly applied in agricultural practices.[27,28] Finally, tetramethyl benzene isomers, cumene, styrene, *tert*-pentyl benzene *p*,α-dimethylstyrene, and phenylacetaldehyde, which were detected in the samples, indicate contamination from PS—a plastic that is generally used in seedling trays in agricultural production processes.[24, 29–32]

Several saturated hydrocarbons (butane, cyclohexane, isopentane, cyclopentane, pentane, cyclooctane, undecane, tridecane, and tetradecane) were found in many of the studied samples. These compounds may be related to their use as plastic blowing agents[20], being also by-products generated during production processes of polyolefins such as polypropylene (PP) and polyethylene (PE)[33]—the latter being the most used plastic for agricultural applications. However, unsaturated hydrocarbons such as α,ω-alkenes, 1,4-pentadecadiene, and propylene, which are substances typically derived from PE, were not found in the samples.[18,21,31] In addition, several aldehydes (pentanal, hexanal, 2,4-hexadienal, octanal, nonanal, 2,4-decadienal, decanal, and benzaldehyde) were identified in the samples. These compounds may be present in plastic materials due to secondary processes associated to thermo-oxidation reactions, which may happen in polymer production.[34] Nevertheless, it is worth noticing that both families of detected compounds (aldehydes and saturated hydrocarbons) may be related to polymeric materials and/or vegetable matrix; therefore, it is difficult to use them as potential markers of MP contamination. For example, saturated hydrocarbons in plants are believed to be formed by elongation of a preformed fatty acid, followed by loss of the carboxyl carbon[35], and acetophenone derivatives, which were detected in the samples, may be related to plant origin.[36] Furthermore, volatile compounds like aldehydes, alcohols, isothiocyanates, sulfur compounds and nitriles have been shown to be associated with several Brassica vegetables[37], with benzaldehyde being found as the major flavor compound in some vegetables.[38,39] Compounds generated during plant metabolism, such as terpenes (linalool, camphene, and α-pinene), were also found in the samples.[40]

It is worth mentioning that adipic acid, butanediol, and terephthalic acid were not detected in the samples. These species are the main degradation products of polybutylene adipate-co-terephthalate polymer (PBTA) which is widely used in agriculture.[41,42]

Overall, the presence of plastic-associated compounds in the samples highlights that there is significant pollution of MPs and their related compounds in the agricultural environment where food is grown. Therefore, there is a clear need for regulation of MPs to ensure food safety.

Statistical study for discrimination between edible- and nonedible-root vegetables

Statistical analyses were performed to assess the relationship between the level of contamination by MP-associated pollutants and the type of vegetable sample (edible- and nonedible-root vegetables) using the peak area data of the 92 compounds identified in the 114 analyses, which corresponded to 42 in edible-root and 72 in nonedible-root vegetables. Chromatograms acquired by SHS-GC/MS analysis were applied. To explore the potential differentiation in pollutant levels in the samples, an orthogonal partial least squares discriminant analysis (OPLS-DA) was done by applying the unit variance scale.

The two defined classes (edible- and nonedible-root vegetables) showed some differences in their profile of extracted volatile compounds, producing two distinct regions in the principal component analysis score plot related to their compounds (Figure 2A). Due to the high number of compounds included in the statistical analysis, the unit variance scaling model was applied to simplify the loading plot. This model included the peak area of the plastic-related compounds with available standards to assess their contribution to the classification of the samples and support the obtained results.

As shown in the loading plot in Figure 2B, most of the plastic-associated compound species are in the negative region of the X-axis, which corresponds to the region of edible-root vegetables. The compounds that had a higher contribution to the sample discrimination were 2,4-di-*tert*-butylphenol, cumene, 2-*tert*-butyl-4-methylphenol, and 2,4-dimethylphenol. These compounds were present in higher quantities in the edible-root samples. Alkylphenols are common plastic additives used as antioxidant stabilizers or UV-absorbing stabilizers. Generally, these compounds are not linked to the backbone of the polymer, making possible their migration to agricultural soil and plants.[20] In addition, alkylphenol structures are known PC degradation products.[24] Therefore, although they are not specifically a type of polymeric material, their presence suggests potential contamination by PC. Alternatively, the presence of cumene indicates contamination by PS, as cumene is one of its degradation products.[19]

These results suggest that these pollutants accumulate mainly in the roots. Previous studies have described a simulation of the uptake of organic pollutants from the soil by the root and leaf parts of vegetables, indicating the importance of the diffusion route, which depends on soil parameters and the type of vegetable and chemical.[43] Root vegetables have longer exposure times and larger exposure areas to soil pollutants.[44] Therefore, more plastic-associated organic pollutants can be taken up by root crops, generating higher residue levels. In addition, the higher the lipid content of the vegetable roots, the higher the concentration of organic pollutant in the roots.[45] Contrastingly, other studies have shown a higher level of MPs in fruits compared to vegetables, although this may be due to the larger and more complex root system of fruit trees, which again highlights the key role of roots in the contamination of vegetables.[9] It is also worth noting that the pollutants may change during the transportation process by reacting with other plant compounds and, therefore, cannot be distinguished in the higher parts of the plant.[46]

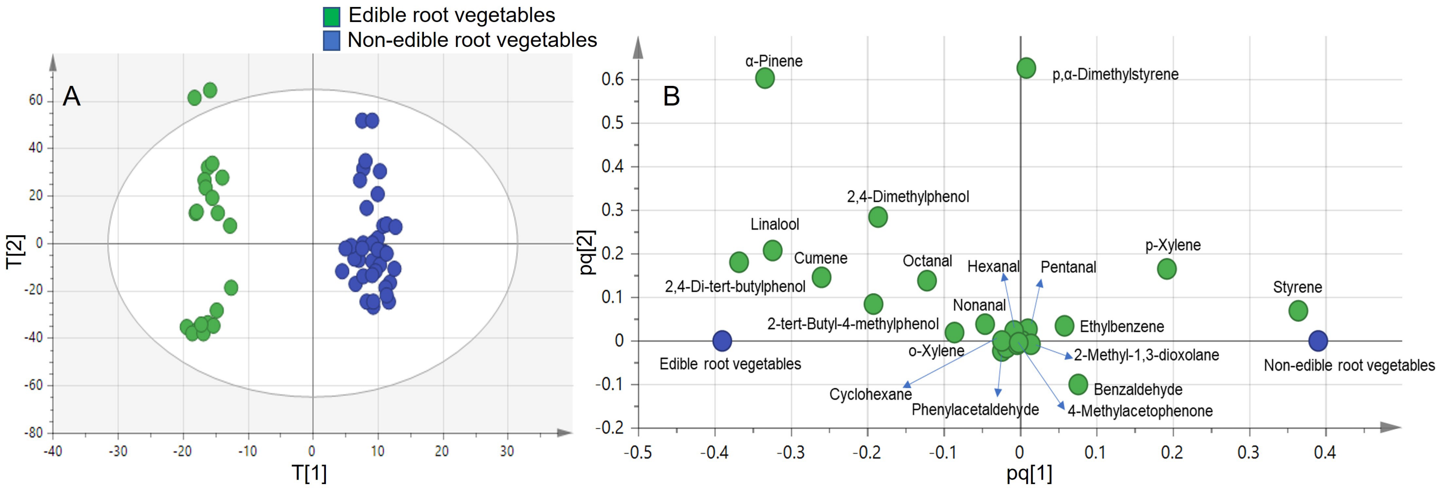

Figure 2. (A) Principal component analysis score and (B) loading plot of edible- and nonedible-root vegetables according to contamination by plastic-associated compounds.

According to the obtained results, it has been demonstrated that the analytical method developed here allows a distinction of MP-associated plastic contamination between edible- and nonedible-root vegetables based on the fingerprint of volatile compounds.

Quantification of plastic-associated compounds in vegetables

Validation of the described procedure was carried out to quantify the organic plastic-associated compounds found in the samples. The signals of a characteristic ion for every compound were measured to perform the quantitative methodology (Table 1). Aligned with usual practice, the target ion (the ion with the highest signal) was used for quantitative information; however, for specific analytes, another ion with lower signal in the high *m/z* area was used to mitigate interference noise. The standards contained aldehydes, benzene derivatives, and phenolic species that belong to several chemical families and may be related to additives, monomers, degradation products, and other impurities normally present in plastic materials.[6,34] The most likely source of each of these compounds in the vegetable samples is included in Table 3. To construct the calibration curves, standard solutions prepared at six levels, from 0.1 to 1,000 ng/g for the compounds, with ISs added at 500 ng/g, were applied. Toluene was used as IS for compounds with retention times from cyclohexane to octanal, both included, and naphthalene was applied for compounds from phenylacetaldehyde to 2,4-di-*tert*-butylphenol.

The slopes for all compounds were compared by direct calibration with ISs and by the standard addition method to three different vegetable samples. An ANOVA test was applied, and no significant differences were found (p-values > 0.05 for all analytes). Thus, the absence of matrix effect was proven and quantification was performed by direct calibration with the IS.

The limits of detection (LODs) and quantification (LOQs) were calculated considering a signal-to-noise (S/N) ratio of 3 and 10, respectively (Table 3). LODs ranged from 0.03 to 15 ng/g and LOQs from 0.1 to 50 ng/g, depending on the compound. The precision of the method was calculated by 10 successive analyses of one carrot sample spiked at 5 and 100 ng/g, depending on the analyte, and with the ISs at 500 ng/g, applying the proposed SHS-GC/MS method. Relative standard deviations (RSDs) were lower than 13% for all the analytes.

Due to the lack of reference materials to calculate the trueness of the method, recovery studies were performed with two vegetable samples (carrot and zucchini) spiked at 5 and 100 ng/g, depending on the compounds, and the ISs

Table 3. Analytical characteristics of the SHS-GC/MS developed method.

Compound	Linearity Range (ng/g)	LOQ* (ng/g)	LOD** (ng/g)	RSD*** (%)	Source
Cyclohexane	1 to 100	1	0.3	12	Matrix/polymer
Pentanal	5 to 500	5	1.5	8.1	Matrix/polymer
Hexanal	5 to 500	5	1.5	3.8	Matrix/polymer
Ethylbenzene	1 to 100	1	0.3	5.9	PVC
p-Xylene	0.5 to 50	0.5	0.15	5.9	PVC/PET
Styrene	0.1 to 10	0.1	0.03	4.8	PS
o-Xylene	0.5 to 50	0.5	0.15	4.6	PVC/PET
Cumene	1 to 100	1	0.3	6.3	PS
Benzaldehyde	1 to 100	1	0.3	7.8	Matrix/polymer
Octanal	5 to 500	5	1.5	11	Matrix/polymer
Phenylacetaldehyde	50 to 1,000	50	15	9.0	PS
Acetophenone	50 to 1,000	50	15	8.7	Matrix/polymer
p,α-Dimethylstyrene	1 to 100	1	0.3	4.3	PS
Nonanal	5 to 500	5	1.5	13	Matrix/polymer
2,4-Dimethylphenol	50 to 1,000	50	15	11	Polymer/PC
4-Methylacetophenone	50 to 1,000	50	15	12	Matrix/polymer
2-*tert*-Butyl-3-methylphenol	0.1 to 10	0.1	0.03	10	Polymer/PC
2,4-Di-*tert*-butylphenol	0.1 to 10	0.1	0.03	8.8	Polymer/PC

* Calculated for a S/N ratio of 10.

** Calculated for a S/N ratio of 3.

*** Carrot sample spiked at 5 and 100 ng/g, depending on the analyte and the ISs at 500 ng/g (n = 10).

at 500 ng/g. For a good distribution of the compounds in the samples, the spiked samples were kept for 1 hour before the analyses in the HS vial at 25 °C. The recovery data varied from 90 to 110% (n = 72) for all the analytes. The analyses were carried out in duplicate.

For the quantification of the samples, the developed targeted approach by SHS-GC/MS was used. All compounds, except 2,4-dimethylphenol and acetophenone, were detected and quantified in the samples. The concentrations of the compounds quantified are shown in Tables 4A and 4B.

Table 4A. Analyte content (ng/g) in vegetable samples (ND = not detected).

Sample	Mean Analyte Content ± Standard Deviation (ng/g) (n = 3)									
	Cyclohexane	Pentanal	Hexanal	Ethylbenzene	*p*-Xylene	Styrene	*o*-Xylene	Cumene	Benzaldehyde	Octanal
Chard	27 ± 3	1,130 ± 130	62 ± 1	ND	ND	25 ± 6	ND	ND	180 ± 20	ND
Garlic	37 ± 3	80 ± 10	3,350 ± 120	18,140 ± 350	ND	29 ± 1	ND	7.4 ± 0.1	220 ± 30	ND
Artichoke	10 ± 2	1,750 ± 30	8,880 ± 750	ND	ND	1.5 ± 0.1	ND	ND	550 ± 40	ND
Celery 1	1.9 ± 0.1	140 ± 10	300 ± 30	ND	ND	0.70 ± 0.05	ND	ND	ND	140 ± 30
Celery 2	4.0 ± 0.5	240 ± 10	181 ± 5	ND	ND	4.2 ± 0.1	ND	ND	ND	60 ± 30
Eggplant	9.1 ± 0.3	140 ± 10	772 ± 6	ND	ND	ND	ND	ND	90 ± 120	ND
Sweet Potato	11 ± 2	400 ± 20	1,900 ± 200	ND	ND	ND	ND	ND	320 ± 30	27 ± 3
Broccoli	15 ± 1	9,220 ± 70	813 ± 2	ND	ND	1.3 ± 0.2	ND	ND	600 ± 50	ND
Zucchini	19 ± 2	1,000 ± 50	242 ± 2	ND	ND	0.87 ± 0.01	ND	ND	940 ± 40	ND
Pumpkin	17 ± 1	310 ± 30	232 ± 1	ND	ND	31 ± 7	ND	ND	442 ± 7	ND
Onion	38 ± 3		990 ± 70	ND	30 ± 10	1.4 ± 0.1	ND	175 ± 3	650 ± 20	ND
Mushroom 1	50 ± 4	40 ± 5	190 ± 15	ND	ND	18 ± 1	ND	ND	1,000 ± 30	ND
Mushroom 2	56 ± 6		220 ± 30	ND	ND	8 ± 1	ND	ND	830 ± 60	ND
Parsnip 1	15 ± 2	230 ± 27	574 ± 4	ND	ND	ND	ND	ND	151 ± 9	3,530 ± 190
Parsnip 2	12 ± 1	90 ± 10	140 ± 8	ND	ND	ND	ND	ND	ND	2,440 ± 90
Red Cabbage	14 ± 3		135 ± 6	930 ± 74	ND	0.16 ± 0.02	ND	ND	ND	ND
Cauliflower	30 ± 2	1,710 ± 220	250 ± 20	40 ± 7	ND	0.35 ± 0.05	ND	ND	350 ± 30	ND
Asparagus	26 ± 1	970 ± 110	3,680 ± 270	ND	ND	1.2 ± 0.2	ND	ND	330 ± 20	ND
Spinach 1	21 ± 1	1,040 ± 140	119 ± 6	ND	ND	5 ± 1	2.1 ± 0.1	ND	269 ± 1	ND
Spinach 2	21 ± 1	820 ± 80	130 ± 20	76 ± 9	ND	13 ± 1	2.2 ± 0.1	ND	280 ± 7	ND
Fennel	11 ± 1	160 ± 22	350 ± 30	17 ± 3	ND	0.70 ± 0.02	ND	ND	180 ± 15	200 ± 10
Ginger	19 ± 4	290 ± 30	8,550 ± 460	ND	ND	6 ± 1	2.6 ± 0.4	ND	310 ± 20	ND
Green Beans	11 ± 3	2,810 ± 120	1,040 ± 120	ND	ND	76 ± 9	ND	ND	280 ± 12	ND
Common Lettuce	19 ± 1	190 ± 10	78 ± 1	ND	ND	0.30 ± 0.04	ND	ND	320 ± 15	ND
Iceberg Lettuce	6 ± 3		450 ± 20	ND	ND	2.2 ± 0.1	ND	ND	290 ± 10	ND
Turnip	13 ± 1	1,690 ± 55	310 ± 30	ND	ND	ND	ND	ND	70 ± 8	80 ± 8
Potato	9 ± 1	280 ± 10	1,130 ± 110	ND	ND	ND	ND	ND	180 ± 12	ND
Cucumber	12 ± 6	1,550 ±110	940 ± 20	13 ± 1	ND	0.39 ± 0.01	ND	ND	950 ± 40	90 ± 10
Parsley	38 ± 2	9,660 ± 170	1,170 ± 70	3,439 ± 8	220 ± 20	81 ± 0.1	10.4 ± 0.1	ND	1,170 ± 50	ND
Red Pepper	17 ± 3	140 ± 10	260 ± 10	ND	370 ± 30	1.4 ± 0.2	ND	ND	660 ± 70	40 ± 5
Leek	43 ± 3	5,240 ± 280	3,620 ± 20	ND	ND	0.30 ± 0.05	0.70 ± 0.01	36 ± 2	260 ± 11	ND
Radish 1	15 ± 1	340 ± 140	110 ± 10	ND	ND	8 ± 1	ND	ND	88 ± 5	ND
Radish 2	18 ± 1	1,000 ± 200	190 ± 10	ND	ND	6.7 ± 0.6	ND	ND	176 ± 7	ND
Tomato 1	9 ± 1	290 ± 50	260 ± 15	70 ± 9	21 ± 8	0.40 ± 0.05	ND	ND	420 ± 10	ND
Tomato 2	5.3 ± 0.1	320 ± 10	350 ± 10	32 ± 1	19 ± 1	ND	ND	ND	220 ± 4	ND
Carrot 1	6.1 ± 0.5	90 ± 7	980 ± 30	ND	ND	ND	ND	ND	131 ± 4	700 ± 20
Carrot 2	7 ± 4	150 ± 12	343 ± 6	ND	ND	1.2 ± 0.2	ND	ND	92 ± 7	570 ± 20
Carrot 3	7 ± 2	230 ± 13	360 ± 10	ND	ND	0.80 ± 0.08	ND	ND	71 ± 6	270 ± 20

Table 4B. Analyte content (ng/g) in vegetable samples (ND = not detected).

Sample	Phenylacetaldehyde	4-Methylacetophenone	2-*tert*-Butyl-4-methylphenol	2,4-Di-*tert*-butylphenol	*p*,α-Dimethylstyrene	Nonanal
Chard	1,170 ± 150	ND	13 ± 2	ND	ND	ND
Garlic	3,340 ± 480	ND	1,040 ± 20	ND	ND	160 ± 20
Artichoke	1870 ± 80	ND	ND	ND	ND	57 ± 5
Celery 1	290 ± 40	ND	ND	0.30 ± 0.10	ND	14 ± 1
Celery 2	310 ± 10	ND	ND	0.28 ± 0.01	ND	9 ± 1
Eggplant	870 ± 90	ND	ND	ND	ND	ND
Sweet Potato	1,400 ± 50	ND	ND	ND	14 ± 4	33 ± 4
Broccoli	6,600 ± 160	ND	ND	0.20 ± 0.01	ND	108 ± 7
Zucchini	6,190 ± 240	ND	ND	ND	32 ± 2	29 ± 2
Pumpkin	3,590 ± 80	ND	39 ± 1	ND	ND	29 ± 1
Onion	10,900 ± 100	ND	420 ± 40	ND	ND	5,400 ± 80
Mushroom 1	4,400 ± 50	ND	ND	0.23 ± 0.01	ND	ND
Mushroom 2	5,060 ± 220	ND	ND	0.14 ± 0.01	ND	ND
Parsnip 1	3,330 ± 240	ND	ND	ND	ND	160 ± 20
Parsnip 2	2,340 ± 80	ND	ND	ND	ND	39 ± 4
Red Cabbage	190 ± 15	ND	ND	0.29 ± 0.01	7.0 ± 0.5	33 ± 2
Cauliflower	1,080 ± 40	ND	ND	0.30 ± 0.20	36 ± 6	150 ± 3
Asparagus	1,920 ± 252	ND	ND	ND	11 ± 1	50 ± 1
Spinach 1	700 ± 70	ND	18± 1	ND	ND	ND
Spinach 2	1,260 ± 150	ND	21± 1	ND	ND	ND
Fennel	1,250 ± 160	ND	ND	0.60 ± 0.01	71 ± 3	91 ± 7
Ginger	3,790 ± 70	ND	ND	ND	15,220 ± 920	ND
Green Beans	4,360 ± 560	ND	ND	ND	ND	32 ± 1
Common Lettuce	580 ± 10	ND	ND	ND	ND	36 ± 4
Iceberg Lettuce	680 ± 10	ND	ND	ND	5.0 ± 0.1	75 ± 7
Turnip	930 ± 54	ND	ND	ND	ND	141 ± 1
Potato	4,430 ± 170	ND	ND	ND	ND	ND
Cucumber	1,970 ± 280	ND	ND	ND	ND	183 ± 9
Parsley	4,560 ± 140	1,460 ± 270	ND	ND	28,800 ± 500	100 ± 10
Red Pepper	4,250 ± 104	ND	ND	0.43 ± 0.06	ND	210 ± 5
Leek	2,650 ± 340	ND	ND	ND	180 ± 10	ND
Radish 1	1,210 ± 70	ND	ND	ND	ND	84 ± 3
Radish 2	2,364 ± 8	ND	ND	ND	ND	55 ± 2
Tomato 1	1,710 ± 53	ND	7.7 ± 0.1	ND	ND	15 ± 1
Tomato 2	750 ± 50	ND	7 ± 2	ND	ND	19 ± 4
Carrot 1	1,300 ± 60	ND	190 ± 30	ND	270 ± 5	105 ± 5
Carrot 2	1,000 ± 140	ND	ND	ND	990 ± 100	86 ± 7
Carrot 3	1,230 ± 170	ND	ND	ND	940 ± 140	33 ± 1

Phenylacetaldehyde, cyclohexane, benzaldehyde, and many aliphatic aldehydes were found in more than 80% of the samples (Figure 3). Among these species, phenylacetaldehyde may be considered a potential marker of PS contamination[46], as previous studies have demonstrated phenylacetaldehyde from styrene degradation by microorganisms in PS materials.[47] Therefore, the presence of this compound with styrene, which was also present in 80% of samples (although in lower concentrations), suggests PS contamination of the agricultural soils in which the studied vegetables were grown. However, benzaldehyde and cyclohexane cannot be considered markers of polymeric contamination as they can also originate from the vegetable matrix, as described previously.

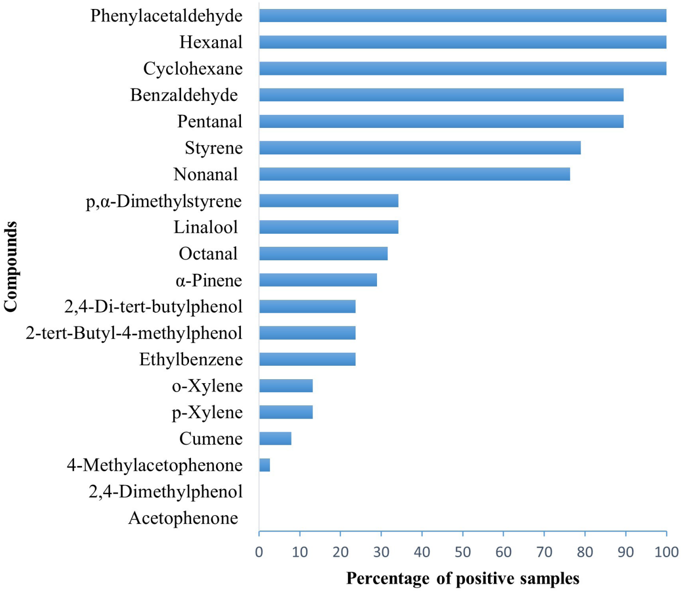

Figure 3. Percent positive detection of compounds in the vegetable samples.

Hexanal may be related to polymeric materials as it has been found, together with other aldehydes, in virgin and recycled PET samples.[1] However, aldehydes are also related to the flavor and sensory characteristics of vegetables[38], and thus aldehydes cannot be considered markers of polymeric contamination.

Other benzene derivatives such as *p*-xylene and *o*-xylene were found in approximately 10% of the samples. These analytes were also detected by other authors in polymer matrices, with hazard levels defined by the U.S. EPA for drinking water being 10 µg/g, a value higher than the levels found in the studied vegetables (the highest value being 370 ng/g for *p*-xylene in the sample of red pepper). The presence of these compounds indicate contamination from PVC or PET, which are both common plastics for agricultural applications.[27,28]

Conclusion

A straightforward, reliable, and robust SHS-GC/MS methodology using an Agilent 8890 GC and an Agilent 5977B GC/MSD was developed to identify and quantify plastic-associated pollutants in vegetable samples. The method represents a preliminary approach to assess the impact of agricultural MP contamination on food. The obtained data add new information to the mobility of MP leachates from agricultural soils to vegetables. The application of chemometrics to the dataset acquired from the SHS-GC/MS analysis has demonstrated that the accumulation of these organic pollutants was higher in edible-root than nonedible-root vegetables. The main contributors to this differentiation were 2,4-di-*tert*-butylphenol, cumene, 2-tert-butyl-4-methylphenol, and 2,4-dimethylphenol, which are chemicals associated with plastic contamination. Specifically, cumene may be related to polystyrene (PS) pollution.

A total of 92 putative compounds were accurately identified using spectral deconvolution retention indices (RIs). In the samples, plastic-associated compounds were quantified. Their contents varied from 0.14 to 28,800 ng/g for 2,4-di-tert-butylphenol and *p*,α-dimethylstyrene, respectively. The presence of phenylacetaldehyde in all the samples, as well as styrene, cumene, and *p*,α-dimethylstyrene in some of them, indicated PS contamination. Other compounds found in the studied vegetables, such as xylene isomers and ethylbenzene, highlighted potential contamination of other polymeric materials like polyvinyl chloride (PVC) or polyethylene terephthalate (PET).

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