SUPPLEMENTARY MATERIAL

Plasticizers and BPA in spices and aromatic herbs of Mediterranean areas

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Abstract

This research is carried out in order to characterize the actual contamination by two ubiquitous environmental pollutants, plasticizers and Bisphenol A, in spices (black pepper, caraway and coriander) and aromatic herbs (fennel, laurel, mint, oregano, rosemary, thyme and verbena) from Algeria (n=26), Tunisia (n=65) and Italy (n=53). Algerian samples seem to contain fewer residues than Italian and Tunisian samples. Among the Italian samples, only aromatic herbs, precisely mint, oregano, and laurel, were contaminated. In general, all Tunisian samples showed five plasticizers residues: the caraway, among the spices, and the rosemary, among the aromatic herbs, are found to contain more residues. Also, dietary intake of these contaminants by spices and aromatic herbs under analysis seems not to constitute a ~~dangerous~~ risk to the consumers.

Keywords: Plasticizers; Bisphenol A; spices and aromatic herbs; GC/MS; EDI

EXPERIMENTAL

Chemicals

All analytical standards, with certified purity > 99%, of Di-methyladipate (DMA), di-ethyladipate (DEA), di-methylphthalate (DMP), di-ethylphthalate (DEP), di-(2-methylpropyl)adipate (DiBA), di-n-butyladipate (DBA), di-propylphthalate (DPrP), benzylbenzoate (BB), di-(2-methylpropyl)phthalate (DiBP), di-butylphthalate (DBP), bisphenol A (BPA), benzylbutylphthalate (BBP), di-(2-ethylhexyl)adipate (DEHA), di-n-heptylphthalate (DiHepP), di-cyclo-hexylphthalate (DcHexP), di-(2-ethylhexyl)phthalate (DEHP), di-phenylphthalate (DPhP), di-(2-ethylhexyl)terephthalate (DEHT) and di-(2-ethylhexyl)sebacate (DEHS), were purchased from Aldrich Chemical (Chicago, Il, USA). Internal standards DBP-d4 and DEHP-d4 were bought from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). Individual stock solutions and work solutions were make in ethyl acetate.

n-hexane (99%), Na_2SO_4 (anhydrous, ACS grade), and the pre-packaged 15 ml centrifuge tube with 900 mg $MgSO_4$ and 150 mg PSA (an ethylenediamine-N-propyl phas) were bought from Sigma–Aldrich (Steinheim, Germany).

During samples preparation and analysis, laboratory glassware heated at 400 °C for at least 4 h were used (Fankhauser and Grob, 2007) always, while laboratory gloves were never used.

Samples

In this work, 144 samples of aromatic herbs, oregon (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*), fennel (*Foeniculum vulgare*), laurel (*Laurus nobilis*), mint (*Mentha piperita*), verbena (*Verbena officinalis*) and spices, black peper (*Piper nigrum*), coriander (*Coriandrum sativum*), carvi (*Carum carvi*) were investigated

(Table 1S). All samples, produced in Mediterranean areas, were dried, not packed and cut into small pieces.

Each sample, purchased in local markets of Algeria (n=26), Tunisia (n=65) and Sicily (Italy) (n=53), was cleaned of debris, ground by a mortar and finally stored until to analysis.

All the samples, suitably homogenized, were subjected to extraction in the solid phase (SPE) according to Cao et al. (2015). After centrifugation, all extracts, filtered through Na_2SO_4 and concentrated, were availables for GC-MS analysis. A procedural blank, prepared by SPE without sample, using the solvents and reagents only, was included with each samples set for quality assurance.

Instrumentation

The analyses were performed on a Shimadzu GC-MS 2010 QP-2010 equipped with a quadrupole mass spectrometer (Shimadzu Italia, Milan, Italy).

Analytes were separated on a Supelco SPB-5MS (5 % polydiphenylsiloxane, 95 % polydimethylsiloxane) capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness).

The experimental condition were: initial oven temperature temperature 60 °C, from 60 to 190 °C at 8 °C/min (5 min hold), from 190 to 240 °C at 8 °C/min (5 min hold), from 240 to 315 °C at 8 °C/min ; carrier gas helium (99.999% purity) at constant rate of 30 cm/sec; transfer line temperature 280 °C; injector temperature 250 °C. Injections were performed with a 60 s splitless injector, then at a split ratio of 1:15; the injection volume was 1 µL.

The acquisition was performed in full scan EI mode (with ionization energy and emission current at 70 eV and 250 µA, respectively) from 40 to 400 amu and in Single Ion Monitoring (SIM) using three characteristic mass fragments for each target analytes.

GC-MS method validation

For the analytical method validation, linearity, sensitivity, repeatability, extraction interferences, recovery and precision have been evaluated. The calibration curves were constructed with standard prepared in ethyl acetate and in aromatic herb extract (Italian thyme), previously analyzed. Each solution was injected five times each. All peak areas of target ions were normalized against the peak area of characteristic fragment (m/z 153) of DBP-d4 and DEHP-d4 (in relation to retention time of each compound), and used in quantitative analyses. The matrix effect was evaluated according to Di Bella et al. (2014). From the values obtained, it was observed that the interferences were negligible for any compound under analysis. Because of this the quantifications were performed by the calibration curves obtained from the standard in ethyl acetate. The linearity was determined by the R^2 coefficient: among 19 compounds under analysis, R^2 was between 0.9853 and 0.9988. The limits of detection (LOD) and of quantifications (LOQ) were calculated from the RSD% of six replicate injection at the lowest detectable concentration (with a signal-to-noise ratio < 3) for each compound, as: LOD ($mg \cdot Kg^{-1}$) = 3 x RSD% x concentration ($mg \cdot Kg^{-1}$) and LOQ ($mg \cdot Kg^{-1}$) = 10 x RSD% x concentration ($mg \cdot Kg^{-1}$). LOD and LOQ values were between 0.005 and 1.303 $mg \cdot Kg^{-1}$, and 0.017 and 3.013 $mg \cdot Kg^{-1}$, respectively.

For repeatability, relative standard deviations (RSD %) of peak area measurements, performed six times, were quantified at the lowest detectable concentration of each analyte.

The RSD% values determined were very good: always better than 5.43 %.

For recovery test of all analytes, appropriate known amounts of compound standards were added to an Italian thyme sample previously analyzed. The fortified sample was pre-treated following the protocol already described, after 24 hours. Recovery values, calculated on the average of three replicate analyses, were between 83 and 110 %.

Table 1S. Samples information

Item	Scientific name	Number of sample			Group
		Tunisia	Italy	Algeria	
Black pepper	*Piper nigrum L.*	6	0	0	Spice
Caraway	*Carum carvi L.*	5	4	4	Spice
Coriander	*Coriandrum sativum L.*	5	4	5	Spice
Fennel	*Foeniculum vulgare L.*	6	5	5	Aromatic herb
Laurel	*Laurus nobilis L.*	9	9	7	Aromatic herb
Mint	*Mentha piperita L.*	9	7	0	Aromatic herb
Oregano	*Origanum vulgare L.*	8	9	0	Aromatic herb
Rosemary	*Rosmarinus officinalis L.*	6	5	0	Aromatic herb
Thyme	*Thymus vulgaris L.*	6	6	0	Aromatic herb
Verbena	*Verbena officinalis L.*	5	4	5	Aromatic herb

References

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