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Development of Rapid Determination of 18 Phthalate Esters in Edible Vegetable Oils by Gas Chromatography Tandem Mass Spectrometry

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ABSTRACT: A simultaneous and fast determination of 18 phthalic acid esters (PAEs) in edible vegetable oils was developed. After solvent extraction, the PAEs in the oil sample were further cleaned up by solid-phase extraction. After concentration, the extract was directly injected into gas chromatography tandem mass spectrometry (GC−MS/MS) in positive-ion electron impact (EI) mode. Method quantification limits of 18 PAEs were between 0.01 and 0.1 mg/kg. Quantitative recoveries ranging from 63.9 to 115.3% were obtained by analysis of spiked oil. The relative standard deviations were less than 15% (n = 6). The method could potentially overcome the interference from large amounts of lipids and pigment. It was applied to real sample and shown to be a rapid and reliable alternative for determination and confirmation of PAEs in routine analysis.

KEYWORDS: *Phthalic acid esters, phthalates, vegetable oil, multi-residue analysis, solid-phase extraction, gas chromatography tandem mass spectrometry*

INTRODUCTION

Phthalic acid esters (PAEs), known as phthalates, are widely used as industrial production in food packaging and medical devices.[1] Consequently, the ubiquitous contamination of PAEs has become another important source in foods, in addition to migration from packaging materials. Many papers have been published and found that some phthalates and/or their metabolites are suspected as human cancer-causing agents and endocrine disruptors.[2−4] It was shown that the most frequently used ester, di-2-ethylhexyl phthalate (DEHP), became a ubiquitous pollutant in the environment and, particularly, in foods.[5] For example, DEHP, as cloudy agents, was used unlawfully in drinks and caused a severe food security crisis in Chinese Taiwan on May 24, 2011. Consequently, these PAEs are not allowed to be used as food additives by the Ministry of Health, and plasticizers have become a focus control for the national regulatory authorities.

Because phthalates are lipophilic, they tend to distribute mostly in fatty foods. Therefore, a sensitive and accurate method for analysis of phthalates in vegetable oil and, in general, in fatty matrices is needed and very important for the health of consumers.

Extraction and cleanup are the most challenging parts for PAE analysis in food, especially in fatty food samples. Solvent or liquid−liquid extraction is the most frequently used method because of its convenient and effective properties.[6] As for cleanup steps, gel permeation chromatography (GPC) is currently applied.[7,8] However, GPC often consumes large volumes of hazardous organic solvent. Several studies applied solid-phase extraction (SPE) for quantification of low levels of PAEs.[9−11] This procedure significantly reduces the consumption of organic solvents.

The gas chromatography mass spectroscopy (GC−MS) method is almost the routine detection method for phthalates,[12−14] and it has been selected as national standards for the determination of PAEs in food of China;[15] however, its limit of detection (LOD) is not high enough for fat-containing samples. Similar to other hazardous compounds, the development direction of PAE analysis in food is always a simple pretreatment and stronger resolution and sensitivity for instrumental analysis. In addition, it should be suitable for complex samples. The aim of the present study was to develop a simple, rapid, and reliable method to determine phthalates in vegetable oil. After solvent extraction, SPE was used as an effective cleanup method before gas chromatography tandem mass spectroscopy (GC−MS/MS). The whole method could be completed within 1 h, and it can also overcome the interference from lipids and pigments, which increase sensitivity to some extent.

MATERIALS AND METHODS

Instrument. The GC−MS/MS analysis was performed on an Agilent 7890A-Quattro micro GC−MS/MS with a CTC PAL auto sampler. Ultrasonic wave purger KQ-600E (Kunshan Ultrasonic Instrument Company, Ltd.), centrifuge TDL-5-A (Shanghai Anting), and SPE equipment (Waters, Milford, MA) were used for extraction and cleanup. The silica/PAS glass SPE columns (1 g/6 mL) were provided by Hang Zhou Fu Yu Technology Service Company, Ltd.

Reagents and Chemicals. All solvents were high-performance liquid chromatography (HPLC)-grade and purchased from J.T.Baker (Phillipsburg, NJ). The standard mixture solution dissolved in isooctane containing 1000 mg/L methyl phthalate (DMP), diethyl phthalate (DEP), diisobuthyl phthalate (DIBP), di-*n*-buthyl phthalate (DBP), bis-2-methoxyethylphthalate (BMEP), bis(4-methyl-2-pentyl) phthalate (BMPP), bis-2-ethoxyethyl phthalate (BEEP), di-*n*-pentyl

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Table 1. Parameters for the Mass Spectrometric Detection of Analytes, Including Ions and Collision Energy (CE), Retention Time (RT), LOD (S/N = 10), and Linearity (R^2)

number	PAEs	ion pair for identification	ion pair for quantification	CE (eV)	RT (min)	linear equation	R^2	LOD(mg/kg)
1	DMP	163 > 135	163 > 77	20	8.06	$y = 6.28x + 0.054$	0.9956	0.01
2	DEP	149 > 121	149 > 93	15	8.92	$y = 5.11x + 0.059$	0.9961	0.01
3	DIBP	149 > 121	149 > 93	15	10.79	$y = 5.43x + 0.063$	0.9975	0.01
4	DBP	149 > 121	149 > 93	15	11.61	$y = 6.28x + 0.048$	0.9985	0.01
5	BMEP	149 > 121	149 > 93	15	11.94	$y = 0.334x - 0.015$	0.9964	0.06
6	BMPP	149 > 121	149 > 93	15	12.66	$y = 2.67x - 0.025$	0.9986	0.04
7	BEEP	149 > 121	149 > 93	10	13.07	$y = 0.62x - 0.027$	0.9989	0.05
8	DPP	149 > 121	149 > 93	15	13.53	$y = 6.03x - 0.13$	0.9979	0.02
9	DHXP	149 > 121	149 > 93	10	15.82	$y = 2.05x - 0.083$	0.9994	0.04
10	BBP	149 > 121	149 > 93	15	15.97	$y = 1.73x - 0.057$	0.9990	0.05
11	HEP	149 > 121	149 > 93	15	17.09	$y = 2.48x - 0.057$	0.9995	0.04
12	BnBP	149 > 121	149 > 93	15	17.45	$y = 0.516x - 0.024$	0.9956	0.06
13	DCHP	149 > 121	149 > 93	15	18.17	$y = 2.72x - 0.05$	0.9989	0.04
14	DEHP	149 > 121	149 > 93	15	18.34	$y = 2.71x + 0.025$	0.9990	0.01
15	DOP	149 > 121	149 > 93	15	20.95	$y = 2.70x - 0.109$	0.9964	0.05
16	DINP		293		22.09	$y = 12981x + 467$	0.9929	0.1
17	DIDP		307		23.73	$y = 9121x + 550$	0.9931	0.1
18	DNP	149 > 121	149 > 93	15	23.53	$y = 1.65x - 0.047$	0.9917	0.06
IS	D4-DEHP	153 > 97	153 > 125	15	18.30			

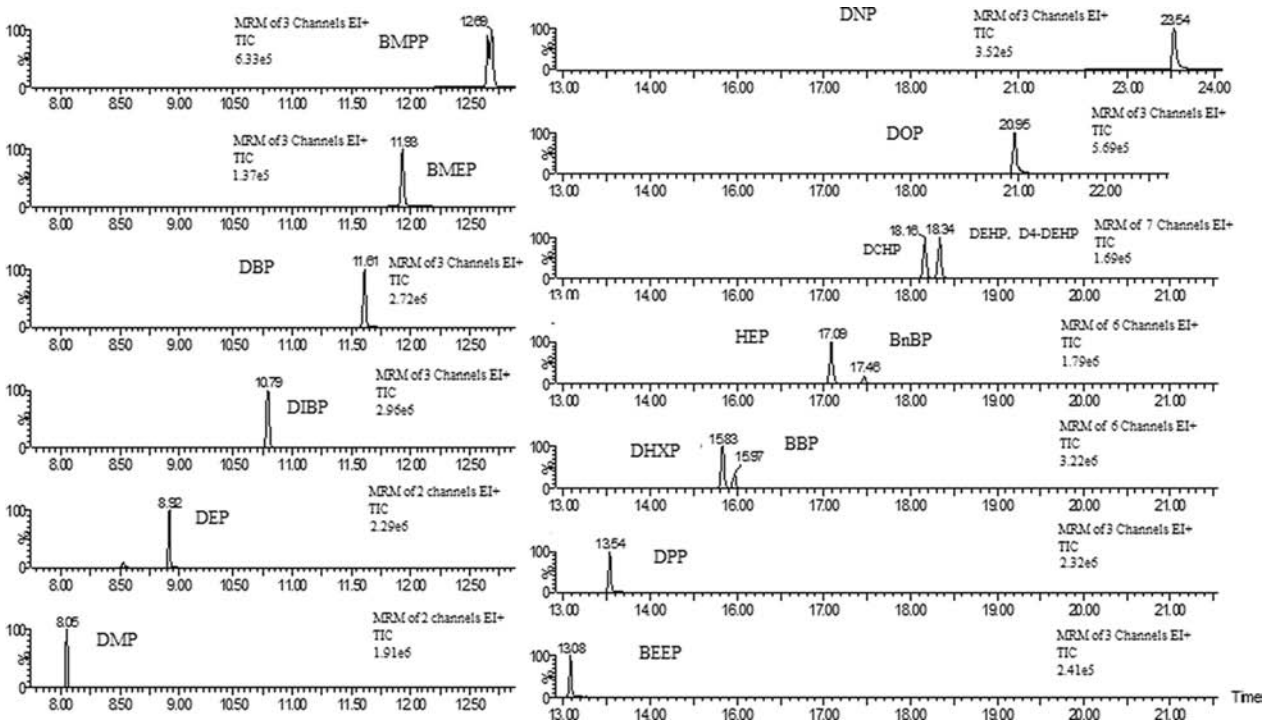

Figure 1. Gas chromatograms of PAEs for a sample of vegetable oil spiked with 1 mg/kg in EI−MS/MS mode.

phthalate (DPP), di-*n*-hexyl phthalate (DHXP), benzyl buthyl phthalate (BBP), hexyl-2-ethylhexyl phthalate (HEP), bis-2-buthoxyethyl phthalate (DBEP), dicyclohexyl phthalate (DCHP), bis-2-ethylhexyl phthalate (DEHP), di-*n*-octyl phthalate (DOP), and di-*n*-nonyl phthalate (DNP) were purchased from Chem Service (West Chester, PA). The D4-DEHP standard (0.2 mg/mL), as an internal standard, was also from Chem Service (West Chester, PA). Diisononyl phthalate (DINP, 3.1 mg/mL) and diisodecyl phthalate (DIDP, 2 mg/mL) were also from Chem Service (West Chester, PA).

Sample Preparation. In total, 31 vegetable oil samples were purchased from the local supermarket of Shijiazhuang in the Hebei province of China from October to November in 2011. All samples were stored in a ventilated and dry place under room temperature. The oil sample (0.40 g) was weighed into a 10 mL glass centrifuge tube accurately, together with 100 μL of internal standard solution (10 μg/mL) and 4 mL of acetonitrile. After ultrasonic extraction for 5 min and centrifugation for 5 min at 4000 rad/min, 2 mL of supernatant was purified by passing through the silica/PAS column, which was pre-rinsed by 5 mL of dichloromethane and 5 mL of acetonitrile, at a flow rate of 1 mL/min. Then, the analytes were eluted using 5 mL of acetonitrile. Finally, the eluate was concentrated to dryness under nitrogen gas at 40 °C and redissolved in 1 mL of hexane before GC−MS/MS analysis.

GC−MS/MS Analysis. Analytes were separated using a DB-5 ms capillary column (30 m × 0.25 mm × 0.25 μm film thickness) (Agilent, Santa Clara, CA). The primary oven temperature was

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Table 2. Results of PAEs in Contaminated Samples of Vegetable Oil (mg/kg)[a]

compound	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6
DBP	0.30	0.25	*b*	*b*	*b*	*b*
DEHP	0.25	1.10	0.41	0.40	0.37	0.38
DINP	*b*	1.40	0.31	*b*	0.22	*b*

[a]Values in mg/kg. Results of other PAEs (DMP, DEP, DIBP, BMEP, BMPP, BEEP, DPP, DHXP, BBP, HEP, BnBP, DCHP, DOP, DIDP, and DNP) were <LOD. [b]Values below LOD.

Table 3. Recovery Results of 18 PAEs in Vegetable Oil by GC−MS/MS (n = 6)

		spiked 0.2 mg/kg		spiked 1 mg/kg		spiked 2 mg/kg	
number	PAEs	recovery (%)	RSDs (%)	recovery (%)	RSDs (%)	recovery (%)	RSDs (%)
1	DMP	63.9	9.4	69.1	11.3	72.3	10.6
2	DEP	69.6	8.4	68.7	12.5	66.2	11.1
3	DIBP	68.4	7.8	64.6	5.4	67.7	10.7
4	DBP	65.6	8.3	71.4	5.8	64.0	8.7
5	BMEP	76.7	5.9	85.1	7.3	102.3	5.9
6	BMPP	77.6	6.8	80.5	7.6	96.3	8.5
7	BEEP	79.8	6.2	87.5	8.7	88.7	7.3
8	DPP	101.3	5.4	83.2	6.3	88.6	6.1
9	DHXP	102.6	6.1	96.1	8.5	79.2	6.7
10	BBP	93.8	8.2	104.6	9.3	78.9	6.5
11	HEP	85.8	5.8	96.3	11.8	87.2	6.6
12	BnBP	86.5	5.9	99.1	12.6	89.0	10.7
13	DCHP	79.5	8.8	93.5	12.1	87.1	9.9
14	DEHP	102.2	6.4	86.0	9.4	103.2	9.1
15	DOP	93.2	6.6	89.7	7.5	115.3	6.7
16	DINP	91.6	7.2	87.8	9.1	111.6	8.9
17	DIDP	81.3	6.5	76.9	8.4	99.3	9.0
18	DNP	81.6	7.2	88.0	11.0	97.4	5.6

programmed from 65 °C (1 min) to 220 °C at 20 °C/min, then raised to 290 °C at a rate of 5 °C/min, and held for 3 min, with a post-run temperature of 290 °C for 2 min. The injection port was 260 °C. Helium was used at a constant flow of 1 mL/min.

The MS operating conditions were as follows: ion source and transfer line temperatures were 230 and 250 °C, respectively. The electron energy was 70 eV; the resolution was in units; and the emission current was 250 μA. The MS/MS method development was performed in two steps. The first step was performed in full-scan mode in the 50−500 amu scan range, and the second step was in MS/MS mode. Figure 3 shows the chromatogram of 18 PAEs in full-scan mode. All of the MS/MS spectra have been obtained by selecting the base peak (m/z 163 for DMP, m/z 153 for D4-DEHP, and m/z 149 for all of the other analytes) as the precursor ion. Electron impact (EI)−MS/MS spectra of all PAEs, except DMP, exhibit three product ions (m/z 121, 93, and 65).

Table 1 lists the parameters and collision energy of parent ions and the quantification and identification ions for PAEs.

RESULTS AND DISCUSSION

MS/MS Acquisition. Upon data acquisition, confirmation criteria for the identification and quantification of PAEs include the following: retention time for all m/z monitored for a given analyte should maximize simultaneously ±1 s, and integration of ion chromatograms was performed with the Masslynx Software using ICIS peak detection with an ion ratio confirmation parameter. For all PAEs, except DMP and internal standard (IS), m/z 93 was considered as quantification ions and m/z 121 was considered as identification ions. For DINP and DIDP, acquisition was carried out in single ion monitoring (SIM) mode and m/z 293 and 307 were selected for quantification because of their specificity, respectively.

Method Performance. Cross-contamination from glassware, environment, solvents, and samples is a common problem in all PAE analyses; thus, special care was taken with glassware, vials, and caps before use. All glassware used was soaked in acetone for at least 30 min, then rinsed with hexane, and dried at 200 °C for 2 h. Method blank samples were performed every 10 samples. Also, the chromatographic system was initially and regularly checked for contamination of phthalates by running blank injections. To evaluate the matrix effect, a five-point calibration curve was constructed using free matrix extract spiked with standards. It was shown in Table 1 that good linearity was obtained in the selected concentration range (0.1, 0.25, 0.5, 0.75, and 1 μg/mL) with D4-DEHP at 1 μg/mL.

Accuracy was estimated through recovery experiments by spiking blank sample (n = 6). The experiments were conducted at high (2 mg/kg), intermediate (1 mg/kg), and low (0.2 mg/kg) levels of the 18 PAEs, respectively. Figure 1 shows the TIC chromatogram of 16 PAEs in spiked blank sample by MS/MS analysis in multiple reaction monitoring (MRM) mode. The result in Table 3 indicated that the recoveries ranged from 63.9 to 115.3% and the relative standard deviations (RSDs; n = 6) ranged from 5.4 to 13.2%.

Method reproducibility studies were performed by injecting three replicates of the same standard solution on 3 different days and on the same day. Both the intra- and interday precisions showed RSDs below 15%.

Comparison of MS/MS Acquisition to SIM Mode. The majority of methods for the determination of PAEs were usually carried out in SIM mode. However, GC−MS still could not avoid the interference of complex matrices. In this work, MS/MS was better at increasing the sensitivity by drastically

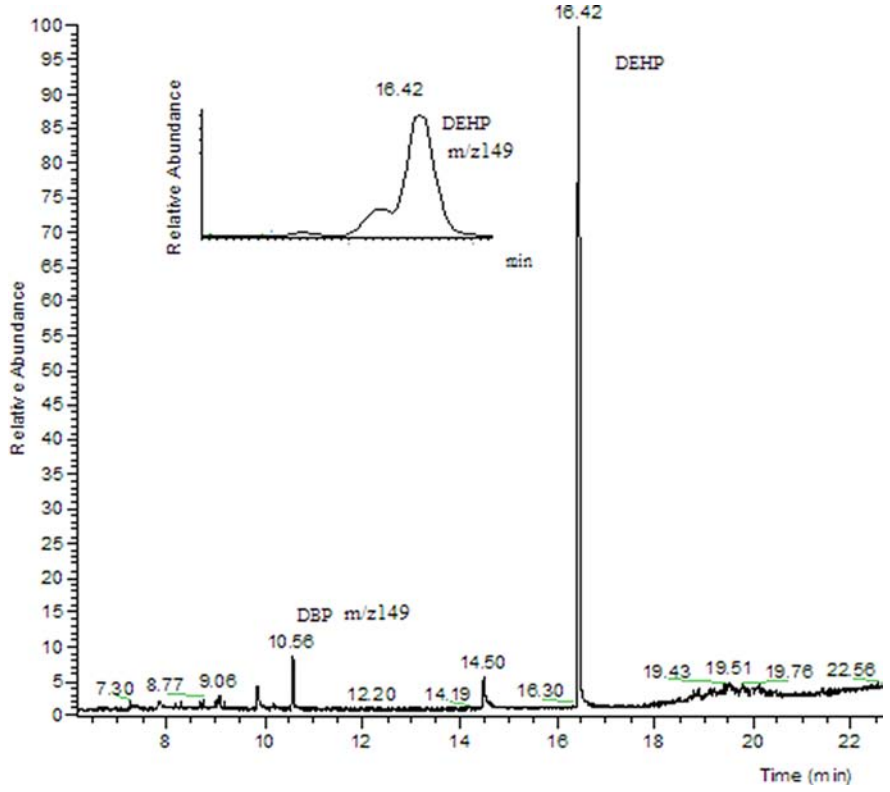

Figure 2. Chromatogram of DEHP applying GC−SIM−MS in a vegetable oil sample.

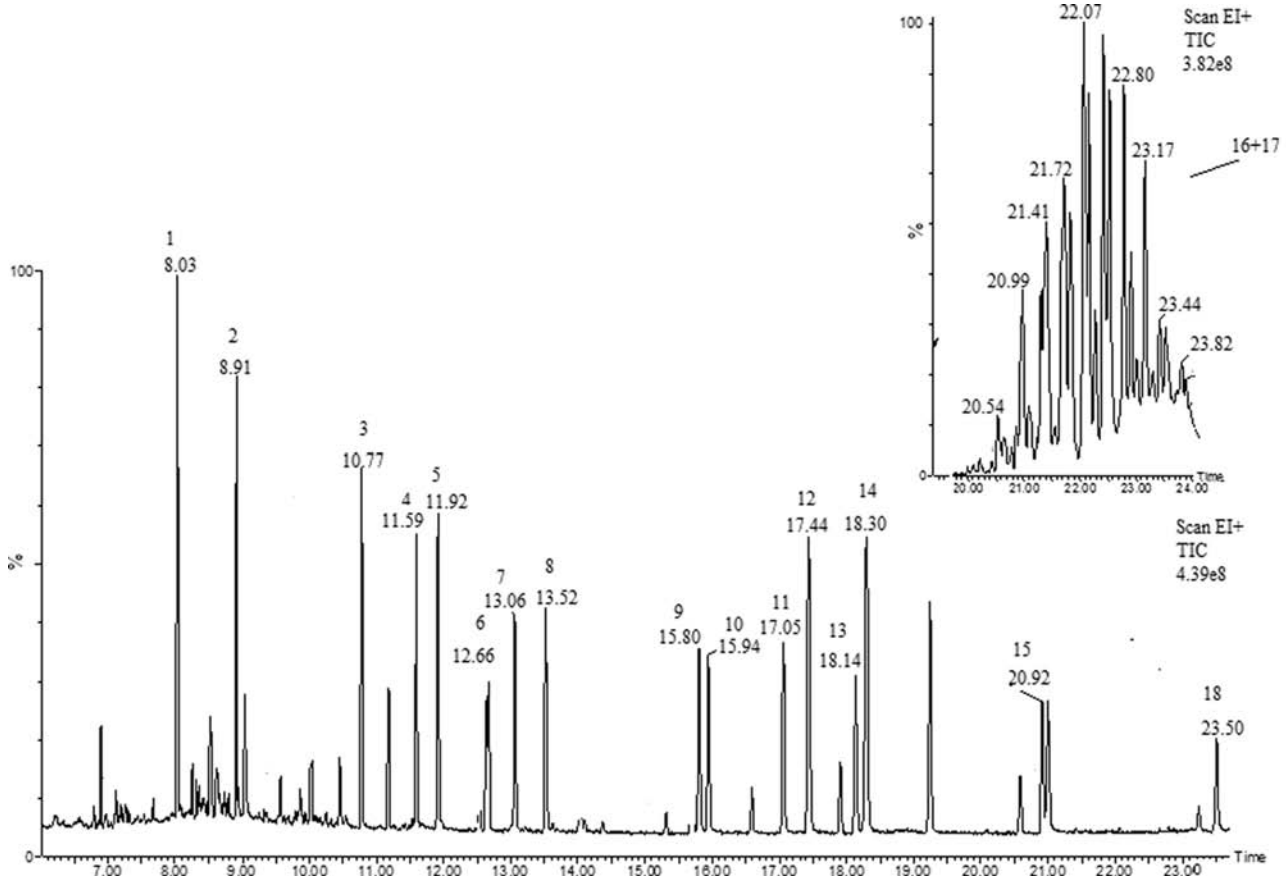

Figure 3. Chromatogram of PAEs in full-scan mode.

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reducing the background without loosing its specificity in analyte identification, which was also concluded by other works.[16−19] In our study, a comparison of chromatograms between GC−MS and GC−MS/MS in real samples was obtained. Figure 2 shows a representative example of the chromatographic profile obtained for a real sample by applying GC−MS in SIM mode. Obviously, quantification of DEHP showed interference when selecting m/z 149 as the quantification ion. Thus, it was confirmed that the MS/MS technique can minimize matrix interference and improve the signal/noise ratio.

Application to Real Samples. The developed method was applied to the analysis of 31 samples of vegetable oil. Because only few of the phthalates studied in this work may really be encountered in edible oils, namely, DBP, DEHP, and DINP, only those, if detected, are shown in real sample results. Analyses were performed in duplicate and, if any, levels in blank were subtracted. Results of detected PAEs in contaminated oil samples were shown in Table 2. The determined DBP concentrations corrected for the background ranged from the not detected level to 0.30 mg/kg. DEHP was found in six samples in a range from 0.25 to 1.1 mg/kg, and it does not exceed the substance migration limit (SML) (1.5 mg/kg). DIBP was found in three samples in a range of 0.22−1.40 mg/kg. DEHP and DINP were found to be the most widely detected PAEs. In fact, polyvinyl chloride (PVC) is the main plastic material in which phthalates are used. The most widely used phthalates in flexible PVC are DEHP and DINP. It may be an explanation why these two kinds of PAEs are the most frequently detected. According to the guidelines for phthalate residues in edible oils established by the Bundersverband NaturKost Naturwaren (BNN), for DEHP, the recommended value is 3 mg/kg, while for BBP, DINP, DIDP, and others, the recommended value is 5 mg/kg.[20] Samples with plasticizers at concentrations in the present study were within the prescribed limits.

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Notes

The authors declare no competing financial interest.

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