

Plasticizer Contamination in Edible Vegetable Oil in a U.S. Retail Market

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ABSTRACT: With the wide application of plastics, the contamination of plasticizers migrating from plastic materials in the environment is becoming ubiquitous. The presence of phthalates, the major group of plasticizers, in edible items has gained increasingly more concern due to their endocrine disrupting property. In this study, 15 plasticizers in 21 edible vegetable oils purchased from a U.S. retail market were analyzed using gas chromatograph–mass spectrometry. Di(2-ethylhexyl) phthalate (DEHP) and diisobutyl phthalate (DiBP) were detected in all oil samples. Benzylbutyl phthalate (BzBP), dibutyl phthalate (DBP), and diethyl phthalate (DEP) were detected at a rate of 95.2, 90.5, and 90.5%, respectively. The detection rates for all other plasticizers ranged from 0 to 57.1%. The content of total plasticizers in oil samples was determined to be 210–7558 $\mu\text{g}/\text{kg}$, which was comparable to the content range in oil marketed in Italy. Although no significant difference ($p = 0.05$) in the total content of plasticizer was observed among oil species (soybean, canola, corn, and olive), the wider range and higher average of total content of plasticizers in olive oil than other oil species indicated the inconsistency of plasticizer contamination in olive oil and a possible priority for quality monitoring. No significant difference ($p = 0.05$) in the total content of plasticizers was found among glass-bottle ($n = 4$), plastic-bottle ($n = 14$), and metal-can ($n = 3$) packaging, implying that oil packaging is not the major cause of plasticizer contamination. The daily intake amount of plasticizers contained in edible oil on this U.S. retail market constituted only a minimum percentage of reference dose established by US EPA, thus no obvious toxicological effect might be caused. However, the fact that DEHP content in two olive oils exceeded relevant special migration limits (SMLs) of Europe and China might need attention.

KEYWORDS: plasticizer, phthalate, edible vegetable oil, contamination, US retail market, gas chromatograph–mass spectrometry

INTRODUCTION

Because of excellent properties in durability, electric isolation, strength, sealing, extendibility, and workability, plastics have been continuously expanding in application since their discovery and are now widely used in almost all areas of human life.¹ In the past half century, the global production of plastics has grown from about 1.5 million tons in 1950 to 260 million tons in 2007.² In the United States, the plastics industry gained a \$16.2 billion trade surplus in 2010 and is growing 2.3% per year. With the coming of new uses and the increase of existing applications, the plastics market is expected to continue increasing in the coming decades. Currently, plastics has exceeded paper, metal, and glass in commodity packaging, becoming the most popular packaging material.³ A large variety of merchandise, especially edibles such as drink, cooking oil, vegetable, fruits, meat, and microwave food, are transported to the consumers' end in plastic packaging for the purpose of extending shelf life and safeguarding from natural agents.⁴ However, while directly contacting edible items in production, storage, and packaging, migrating components from plastic materials may greatly affect the quality of food.

For the purposes of performance improvement and processing convenience, plastic polymer is normally mixed with various additives. These additives may include colorants, antioxidants, lubricants, flame retardants, antimicrobial agents, and stabilizers. Besides them, plasticizers are a group of additives in plastic materials, especially in polyvinylchloride

(PVC), to soften resin for processing.^{1,5,6} Some synthetic polymer materials are found to contain as high as 40% of plasticizers. Because they are not chemically bound to polymer chains, plasticizers may migrate from plastic materials into the contacting medium, causing contamination.^{4,7} Although the use of other replacement plasticizers, such as adipates, citrates, and acelates, is increasing, phthalates are still the major plasticizer group used in polymer materials today.^{4,8} It was estimated that the worldwide production of phthalates exceeds 3.5 million tons per year, of which 90% are used as plasticizers.⁹ With the wide application of plastic materials in human life, phthalates become ubiquitous in the environment^{7,10–12} and packed edible items.^{5,8,13} Because of their toxicological properties and potential hazard to human health, phthalates have received increasingly more attention from both the scientific and public communities.

Phthalates are normally classified as endocrine-disrupting compounds (EDCs) for their ability of interfering with the endocrine system.¹⁴ After exposure, phthalates in the human body are rapidly metabolized and excreted in urine and feces.^{15–17} Urinary monoester phthalates as the major metabolites and the biologically active toxicants are usually

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Table 1. Oil Species, Producing Site, Packaging Material, Packing Size, and Expiration Date

no.	species	producing site	packaging material	packing size (oz)	expiration date
1	safflower oil	domestic	transparent polycarbonate bottle	32	Mar 01, 2014
2	grapeseed oil	imported	glass bottle with polyethylene lining lid	17	Feb 23, 2014
3	sunflower oil	imported	glass bottle with polyethylene lining lid	17	Feb 23, 2014
4	soybean oil	domestic	transparent polycarbonate bottle	48	Feb 28, 2014
5	soybean oil	domestic	transparent polycarbonate bottle	16	Nov 12, 2014
6	canola oil	imported	transparent polycarbonate bottle	16	Aug 14, 2014
7	peanut oil	domestic	transparent polycarbonate bottle	24	Dec 01, 2014
8	corn oil	domestic	transparent polycarbonate bottle	40	Dec 08, 2014
9	corn oil	domestic	yellow opaque polyethylene bottle	64	Dec 05, 2014
10	olive oil	imported	transparent polycarbonate bottle	17	Sep 02, 2014
11	olive oil	imported	glass bottle with polyethylene lining lid	17	Aug 13, 2014
12	olive oil	imported	transparent polycarbonate bottle	8.5	May 29, 2014
13	olive oil	imported	glass bottle with polyethylene lining lid	17	Oct 31, 2014
14	olive oil	imported	transparent polycarbonate bottle	17	Jun 20, 2014
15	soybean oil	unknown	transparent polycarbonate bottle	48	May 30, 2014
16	canola oil	domestic	Metal tin with polyethylene nozzle	5	Jul 02, 2013
17	canola oil	domestic	Metal tin with polyethylene nozzle	6	Sep 25, 2014
18	soybean oil	domestic	Metal tin with polyethylene nozzle	6	Apr 17, 2014
19	soybean oil	unknown	transparent polycarbonate bottle	16	Nov 27, 2013
20	corn oil	unknown	transparent polycarbonate bottle	16	Oct 17, 2013
21	canola oil	unknown	transparent polycarbonate bottle	16	Nov 29, 2013

taken as biomarkers in assessing the exposure to phthalates from multiple sources via different routes.¹⁸ Phthalates may inhibit the development of the immature male reproduction tract. In animal tests, decreased sperm production and testosterone levels were observed with male mice/rats after prenatal exposure to di(2-ethylhexyl) phthalate (DEHP).^{19,20} In human endocrine effect studies, a significant inverse association was identified between the concentration of monobutyl phthalate, the metabolite of dibutyl phthalate (DBP), in mother's breast milk and 3-month-old male offspring's free testosterone levels.²¹ In addition, phthalates may induce female prematurity. A study in Puerto Rico among 41 young girls (6 months to 8 years old) indicated a possible association between premature thelarche and the concentration of DEHP and/or its monoester in serum.²²

Phthalates may enter into human's body via ingestion, inhalation, and dermal routes as well as parenteral exposure from phthalate-containing medical devices. Among them, ingestion of food is believed to be the primary exposure route.¹⁸ Because phthalates are hardly water- and predominantly fat-soluble, phthalate contamination in oily food has become a serious concern.^{23–28} The occurrence of phthalates in edible vegetable oils has been reported recently.^{25,29–36} However, almost none of those reports came from the U.S. In this study, 14 phthalates and one adipate were analyzed in 21 edible vegetable oils purchased from a U.S. retail market. The objectives of this study include: (i) understanding the contamination level of plasticizers in edible vegetable oils on the US retail market, (ii) identifying primary plasticizer contaminants in oil, (iii) determining any significant difference in plasticizer contamination level among different species of oil and different packaging materials, and (iv) estimating toxicological effect of plasticizers in oil.

MATERIALS AND METHODS

Materials and Chemicals. All edible vegetable oils tested in this study were purchased from supermarkets in Dover, DE, in February 2013. At purchase, all oils were sealed in plastic, glass, or metal bottles/

cans. Information on oil species, producing site (domestic or imported), packaging material, packing size, and expiration date are listed in Table 1. Oil samples were stored in their original packages at room temperature in the laboratory until sample taking for analysis one week later.

HPLC grade acetonitrile and acetone were purchased from Fisher Scientific (Fair Lawn, NJ). To remove possible plasticizer contamination, all solvents were further purified before use by distillation using a full-glass set. Q-sep™ QuEChERS (quick, easy, cheap, effective, rugged, and safe) dSPE (dispersive solid phase extraction) 2 mL tubes each containing 150 mg of MgSO₄ and 50 mg of PSA (primary secondary amine) were purchased from Restek (Bellefonte, PA).

For plasticizer standards, dimethyl phthalate (99%, DMP), diethyl phthalate (99%, DEP), dipropyl phthalate (>99%, DPP), diallyl phthalate (98%, DAP), diisobutyl phthalate (>99%, DiBP), di-*n*-butyl phthalate (99%, DBP), dipentyl phthalate (97%, DPnP), di-*n*-heptyl phthalate (97%, DHpP), diphenyl phthalate (98%, DPhP), dinonyl phthalate (97%, DNP), and didodecyl phthalate (98%, DDP) were purchased from ACROS Organics (Fair Lawn, NJ). Dihexyl phthalate (>98%, DHxP), di(2-ethylhexyl) adipate (>98%, DEHA), and di(2-ethylhexyl) phthalate (>98%, DEHP) were purchased from TCI America (Portland, OR). Diethyl phthalate-*d*₄ (DEP-*d*₄) was purchased from AccuStandard (New Heaven, CT). Benzyl butyl phthalate (98%, BzBP) and di-*n*-octyl phthalate-*d*₄ (DOP-*d*₄) were purchased from Aldrich (Milwaukee, WI).

Oil Extraction and Clean-Up. All glass vessels were heated at 300 °C for 2 h and then rinsed with acetone three times immediately before use. Solvent and oil sample transfer was performed using acetone-rinsed glass pipettes or glass syringes. All septa were rinsed with acetone three times immediately before use.

About 0.4 g of oil sample was accurately weighed into a 10 mL serum vial. Then 25 μL of internal standard acetone solution, which contained 5.00 mg/L DEP-*d*₄ and 12.4 mg/L DOP-*d*₄, were added into the vial. After 5.00 mL of acetonitrile were added into the vial, the vial was sealed using an aluminum crimp seal lined with a Teflon-coated silicone septum. The vial was then put on a reciprocating shaker and vigorously shaken for 10 min. After setting still for 1 h in dark, 1.00 mL of acetonitrile phase was transferred into a Q-sep QuEChERS dSPE tube, which was prewashed with 1.20 mL of acetonitrile each time three times. After hand-shaking for 5 min, the tube was centrifuged at 13000 rpm for 4 min. A portion of supernatant

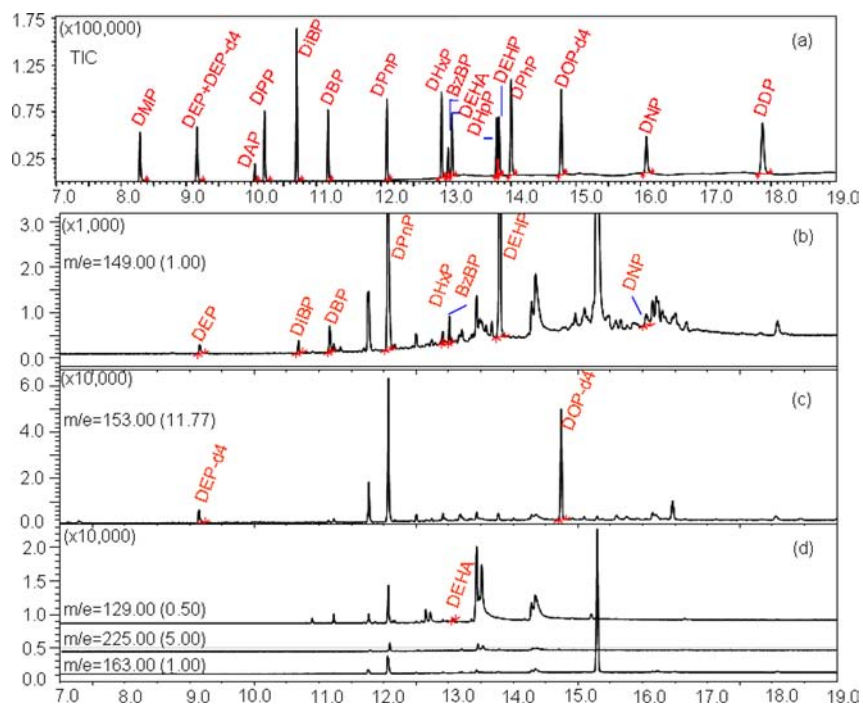


Figure 1. GC-MS TIC spectrum of plasticizer standards with each concentration at ~ 2 mg/L (a) and SIM spectrum of an oil sample extract at $m/e = 149$ (b), $m/e = 153$ (c), and $m/e = 129, 225$, and 163 (d).

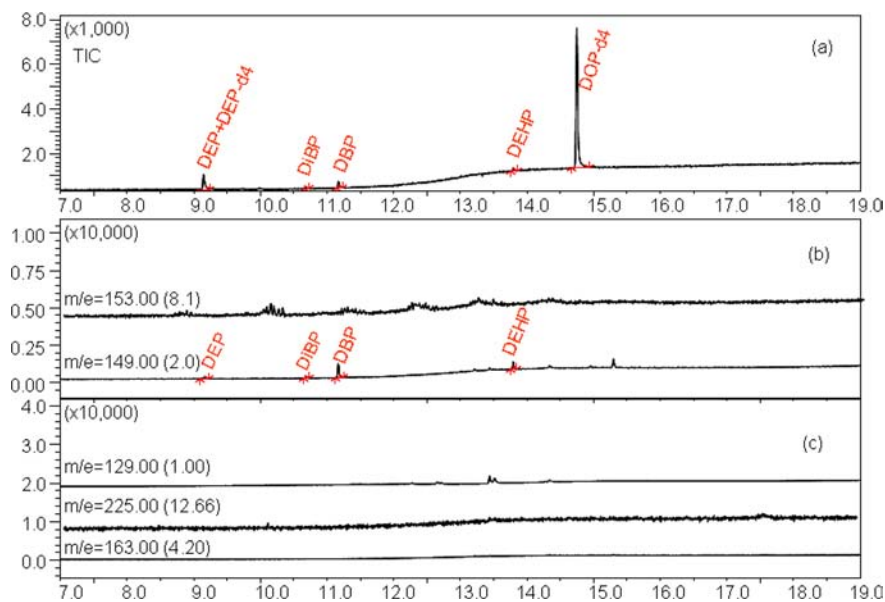


Figure 2. GC-MS TIC spectrum of acetonitrile (a) and an SIM spectrum of the third time rinse of dSPE tube at $m/e = 149$ and 153 (b), and $m/e = 129, 225$, and 163 (c).

was transferred into a GC-vial for gas chromatograph–mass spectrometry (GC-MS) analysis.

Triplicates were performed for each oil sample.

Method-Validation Sample Preparation. To check linearity range, different amounts of mixed standards were spiked into 0.400 g oil samples in serum vials with the spiking concentration of each standard at approximately 2×10^1 , 4×10^1 , 2×10^2 , 1×10^3 , and 1×10^4 $\mu\text{g}/\text{kg}$. To evaluate extraction recovery, mixed standards were spiked into 0.400 g oil sample in four replicates at two spiking concentrations, i.e., each standard at approximately 5×10^1 and 1×10^3 $\mu\text{g}/\text{kg}$, respectively, as well as into blank vials in triplicates. Another set of triplicates of the oil sample without standard-spiking

was also prepared for extraction. The recovery of each spiked standard was calculated based on eq 1.

$$\text{recovery}(\%) = \frac{A_1 - A_0}{A_b} \times 100\% \quad (1)$$

where A_0 , A_1 , and A_b are the peak area of each individual standard in the nonspiked, spiked oil sample, and the spiked blank, respectively.

Method precision is evaluated through the relative standard deviation (RSD) of recovery of those spiked replicates. The limit of detection (LOD) and quantification (LOQ) were estimated based on the signal-to-noise ratio of 3:1 and 10:1, respectively.^{25,27}

GC-MS Analysis and Quantification. The instrumental analysis was performed on a Shimadzu QP2010S GC-MS equipped with an

Table 2. Method Validation Parameters

plasticizer	linearity range ($\mu\text{g}/\text{kg}$)	spiked at $5 \times 10^1 \mu\text{g}/\text{kg}$		spiked at $1 \times 10^3 \mu\text{g}/\text{kg}$		LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)
		recovery (%)	RSD (%)	recovery (%)	RSD (%)		
DMP	1.9×10^1 – 9.3×10^3	99.8	5.2	101.3	6.6	2.3	7.6
DEP	1.8×10^1 – 8.9×10^3	100.6	9.1	100.2	7.2	2.8	9.3
DAP	3.4×10^1 – 8.6×10^3	99.6	6.3	100.5	5.8	7.5	25.1
DPP	1.8×10^1 – 9.2×10^3	98.9	7.9	97.7	4.7	1.8	5.9
DiBP	1.8×10^1 – 8.7×10^3	96.8	5.2	97.5	7.1	1.6	5.2
DBP	1.6×10^1 – 8.0×10^3	93.7	6.4	94.5	9.8	1.4	4.8
DPnP	1.7×10^1 – 8.3×10^3	89.1	6.5	88.4	5.9	1.5	4.9
DHxP	1.2×10^1 – 6.1×10^3	82.4	6.7	82.7	5.1	2.3	7.7
BzBP	3.3×10^1 – 8.3×10^3	80.4	8.4	80.8	6.7	6.7	22.2
DEHA	2.6×10^1 – 6.6×10^3	83.3	5.4	82.8	6.5	6.8	22.5
DHpP	1.6×10^1 – 8.2×10^3	72.6	5.0	71.5	6.8	2.9	9.5
DEHP	1.8×10^1 – 8.8×10^3	69.4	10.2	71.1	7.3	5.4	17.9
DPhP	1.6×10^1 – 8.1×10^3	69.5	6.9	68.8	5.2	3.9	13.2
DNP	1.6×10^1 – 8.1×10^3	65.4	6.0	66.1	6.8	4.0	13.2
DDP	1.9×10^1 – 9.3×10^3	61.3	7.1	60.9	5.2	5.2	17.3

autosampler. A Shimadzu SHRXI-SMS column (30 m \times 0.25 mm \times 0.25 μm) was used for separation. The carrier gas was helium, and the linear velocity was set at 31.2 cm/s. The temperature of injector was set at 250 $^\circ\text{C}$, and the splitless injection was chosen with a sampling time of 1 min. Postsampling split ratio was 1:5. The column temperature began from 80 $^\circ\text{C}$ and was held for 3 min. Then the column temperature was increased to 300 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$ and kept for 6 min. The temperature for interface and ion source was set at 260 and 220 $^\circ\text{C}$, respectively. The electron impact energy was 70 eV. To minimize plasticizer contamination introduced in GC-MS analysis, the injector needle-rinsing vials were sealed with silicone septum caps instead of plastic diffusion caps. Additionally, the rinse solvent was replaced every 12 h.

Quantification was conducted in selected ion monitoring (SIM) mode based on peak area using the internal calibration method. For the internal standards, i.e., DEP- d_4 and DOP- d_4 , the ion monitored for quantification was $m/e = 153$. For DMP and DPhP, the ion monitored for quantification was $m/e = 163$ and 225, respectively. For all other phthalates, the ion monitored for quantification was $m/e = 149$. For DEHA, the monitored peak was $m/e = 129$. Ions monitored for confirmation include $m/e = 104, 112, 181, \text{ and } 207$. The total ion current (TIC) spectrum of plasticizer standards is shown in Figure 1a, and the SIM spectra of an oil sample extract are shown in Figure 1b–d.

The analysis of plasticizers is a challenge due to the ubiquity of plastic parts and devices in laboratory. Blank problems may greatly affect the trace analysis of plasticizers, especially DBP and DEHP.³⁷ Blank test was performed for distilled acetonitrile, the extraction solvent used for this study. The GC-MS TIC spectrum for acetonitrile was shown Figure 2a. Minimum contamination of DEP, DiBP, DBP, and DEHP was observed, and the concentration was quantified to be 0.9, 0.2, 1.0, and 1.1 $\mu\text{g}/\text{L}$, respectively, based on peak area ratio of ion at $m/e = 149$ to that of internal standards at $m/e = 153$. Blank test for the third time acetonitrile rinsate (nonspiked) from the dSPE tube was also performed. The GC-MS SIM spectra of the rinsate at $m/e = 149$ and 153 are shown in Figure 2b, and those at $m/e = 129, 225, \text{ and } 163$ are shown in Figure 2c. Minimum contamination of DEP, DiBP, DBP, and DEHP was observed, and the concentration was 1.0, 0.2, 1.3, and 1.2 $\mu\text{g}/\text{L}$, respectively, demonstrating very similar to the residues in distilled acetonitrile.

The concentration of each plasticizer in the oil sample was calculated using the following equation:

$$C(\mu\text{g}/\text{kg}) = \frac{[C_0(\mu\text{g}/\text{L}) - C_b(\mu\text{g}/\text{L})] \times 0.00500(\text{L})}{W(\text{kg})} \quad (2)$$

where C_0 , C_b , and C are the concentration of each plasticizer in extracts, solvent, and oil, respectively; W is the mass of oil or plastic pieces.

Statistic Analysis. For the determination of significant difference between two groups of data, Student's t test was used.

RESULTS AND DISCUSSION

Method Validation Parameters. The obtained values of method validation parameters are listed Table 2. A wide linearity ranging from low 10^1 to high $10^3 \mu\text{g}/\text{kg}$ was observed for each plasticizer. The values of linear regression coefficient, r , were all greater than 0.999 (data not shown). The recovery ranged from 101.3 to 60.9%, gradually decreasing with the increase of molecular weight of the plasticizer. The recoveries of the method used in this study appeared comparable to those recoveries reported in other studies.^{25,33,36} As the indicating parameter of reproducibility of the method, RSD values at the two spiking concentration levels were all below 10.5%, demonstrating the high precision of this method. LOD and LOQ of this study ranged from 1.4 and 4.8 $\mu\text{g}/\text{kg}$, respectively, for DBP to 7.5 and 25.1 $\mu\text{g}/\text{kg}$, respectively, for DAP. These values are also comparable to those reported in other studies.^{33,36}

Plasticizer Contamination in Oil Samples. Plasticizer concentrations in each oil sample are listed in Table 3. Among the monitored 14 phthalates and one adipate, DEHP and DiBP were detected in all oil samples, being the most frequently detected phthalate plasticizers. On the contrary, DPhD and DDP were not detected in any oil samples. The second most frequently detected phthalate plasticizer was BzBP, with a detection rate at 95.2%, followed by DBP and DEP both at 90.5%, DPnP at 57.1%, DAP at 52.4%, and DEHA at 47.6%. The detection rates for all other plasticizers in this study were all below 15%. High frequencies of detection of DEP, DEHP, DiBP, and DBP in oil were also observed in other studies.^{25,26,36} However, the BzBP was either excluded or not detected in oil studies from China.^{25,35,38} On the contrary, a high detection rate of BzBP, as well as DEHP and DiBP, in olive oil was reported in Italy.³⁴ The inconsistency of BzBP detection rate in oil among studies implied that the application of plasticizers may vary greatly among geographic regions.

Besides the detection rate, the total content of plasticizers in oil is a much more important parameter reflecting the

Table 3. Plasticizer Concentrations in Oil Samples (Mean \pm STD, $\mu\text{g}/\text{kg}$)^a

no.	DMP	DEP	DAP	DPP	DiBP	DBP	DPnP	DHxP	BzBP	DEHA	DHPP	DEHP	DPhP	DNP	DDP	total
1	nd	146 \pm 24	nd	nd	72.2 \pm 12	59.8 \pm 17	nd	nd	97.2 \pm 9	nd	nd	206 \pm 19	nd	nd	nd	581
2	nd	175 \pm 20	nd	nd	44.3 \pm 2	22.2 \pm 9	876 \pm 7	60.2 \pm 3	119 \pm 2	nd	59.8 \pm 7	550 \pm 6	nd	nd	nd	1906
3	nd	159 \pm 21	nd	nd	69.8 \pm 19	54.0 \pm 5	45.8 \pm 4	nd	112 \pm 7	nd	bq	192 \pm 46	nd	nd	nd	635
4	nd	180 \pm 16	nd	nd	46.2 \pm 10	43.1 \pm 9	nd	nd	117 \pm 12	nd	nd	209 \pm 36	nd	nd	nd	596
5	nd	172 \pm 9	nd	nd	67.4 \pm 9	55.8 \pm 15	44.3 \pm 5	nd	118 \pm 27	nd	nd	65.8 \pm 7	nd	nd	nd	523
6	nd	167 \pm 19	nd	nd	77.0 \pm 12	38.5 \pm 9	48.7 \pm 4	nd	100 \pm 2	nd	nd	125 \pm 16	nd	nd	nd	556
7	nd	174 \pm 14	nd	nd	45.3 \pm 7	23.7 \pm 10	51.1 \pm 1	nd	135 \pm 17	nd	nd	554 \pm 58	nd	nd	nd	983
8	nd	23.7 \pm 3	nd	nd	219 \pm 15	69.4 \pm 12	60.5 \pm 3	nd	nd	nd	nd	148 \pm 3	nd	nd	nd	521
9	nd	124 \pm 11	126 \pm 5	nd	105 \pm 15	68.8 \pm 7	76.7 \pm 5	nd	104 \pm 10	nd	nd	67.7 \pm 8	nd	nd	nd	672
10	nd	151 \pm 12	115 \pm 13	nd	82.6 \pm 16	53.4 \pm 7	51.8 \pm 4	nd	135 \pm 8	nd	nd	860 \pm 82	nd	nd	nd	1449
11	21.6 \pm 4	71.2 \pm 10	49.6 \pm 5	14.0 \pm 6	90.0 \pm 11	79.5 \pm 17	556 \pm 9	14.4 \pm 4	67.3 \pm 10	30.1 \pm 9	nd	469 \pm 54	nd	30.5 \pm 9	nd	1493
12	22.8 \pm 1	59.7 \pm 5	71.0 \pm 8	nd	112 \pm 17	65.7 \pm 15	73.9 \pm 5	nd	75.8 \pm 12	269 \pm 15	nd	2279 \pm 167	nd	82.5 \pm 14	nd	3112
13	nd	52.7 \pm 12	65.2 \pm 14	bq	56.8 \pm 11	33.3 \pm 6	24.6 \pm 1	nd	21.8 \pm 5	nd	nd	70.5 \pm 16	nd	nd	nd	326
14	nd	52.7 \pm 4	59.1 \pm 11	nd	96.4 \pm 9	92.6 \pm 12	512 \pm 25	40.1 \pm 7	252 \pm 8	236 \pm 27	nd	6166 \pm 122	nd	50.4 \pm 14	nd	7558
15	nd	24.8 \pm 11	61.9 \pm 15	nd	32.3 \pm 9	nd	nd	nd	40.2 \pm 6	55.9 \pm 7	nd	258 \pm 18	nd	nd	nd	473
16	nd	nd	nd	34.9 \pm 2	32.7 \pm 6	nd	nd	nd	43.4 \pm 12	25.4 \pm 3	nd	159 \pm 19	nd	nd	nd	295
17	nd	58.4 \pm 7	305 \pm 25	nd	77.8 \pm 12	95.8 \pm 7	nd	nd	31.8 \pm 9	44.8 \pm 6	nd	121 \pm 13	nd	nd	nd	734
18	nd	72.0 \pm 12	306 \pm 27	nd	91.8 \pm 10	49.0 \pm 4	nd	nd	107 \pm 11	48.0 \pm 4	nd	547 \pm 20	nd	nd	nd	1221
19	nd	bq	nd	20.0 \pm 7	70.3 \pm 4	18.3 \pm 2	nd	nd	40.9 \pm 6	bq	nd	50.3 \pm 7	nd	nd	nd	210
20	nd	46.5 \pm 7	78.1 \pm 10	nd	57.5 \pm 12	30.4 \pm 9	nd	nd	35.8 \pm 9	bq	nd	261 \pm 18	nd	nd	nd	516
21	nd	43.5 \pm 10	61.3 \pm 5	nd	55.4 \pm 9	16.7 \pm 7	nd	nd	38.7 \pm 4	bq	nd	96.7 \pm 8	nd	nd	nd	319

^a“nd” stands for “below LOD”, while “bq” stands for “below LOQ”, indicating a concentration higher than LOD, but lower than LOQ.

seriousness of contamination. The total content of plasticizers investigated in this study ranged from 210 to 7558 $\mu\text{g}/\text{kg}$. Except for four out of the five olive oil samples and the only grapeseed oil sample included in this study, the total plasticizers in all samples were not higher than 1221 $\mu\text{g}/\text{kg}$. The content for the four olive and the grapeseed oil samples ranged from 1449 to 7558 $\mu\text{g}/\text{kg}$. It should be pointed out that the content of total plasticizers in the remaining one olive oil sample was only 326 $\mu\text{g}/\text{kg}$. The total content in all oil samples seemed noticeably higher than that in oil on China market reported by Wang et al., which was from 70 to 317 $\mu\text{g}/\text{kg}$.³⁸ However, it should be noted that in the study of Wang et al., only five phthalates were analyzed in one of each sunflower, soybean, and peanut oil and two corn oil samples. Being consistent with this study, a high content of plasticizers was observed in olive oil in the study of Dugo et al.³⁴ in which DEHP content ranged from below LOD to 6249 $\mu\text{g}/\text{kg}$. In another study of phthalate contamination in vegetable oil in Italy, as high as 6500 $\mu\text{g}/\text{kg}$ diisononyl phthalate was detected.³³ If including other plasticizers analyzed, the total plasticizer content in those oil samples could be even higher. By the above comparison, the high contamination level of total plasticizers in oil in this U.S. market appeared to be similar to that in Italy.

Oil Species and Packaging Effect. The content of total plasticizers in oil samples assorted in oil species is shown in Figure 3. The average of total content in soybean, canola, corn,

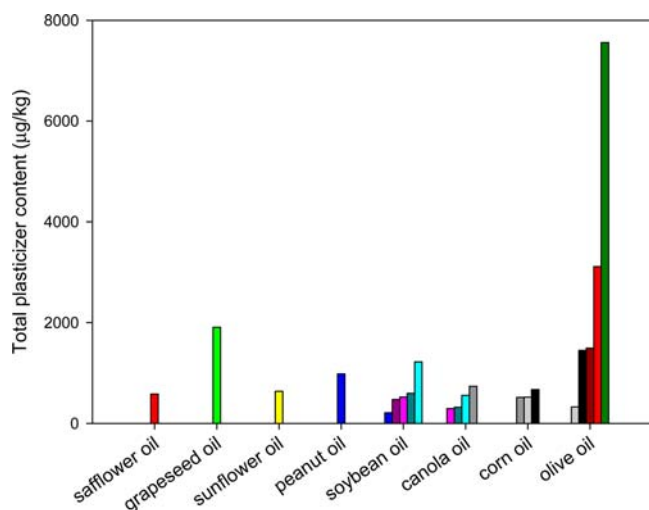


Figure 3. Total content of plasticizers in different species of oil.

and olive oil were 605, 476, 570, and 2788 $\mu\text{g}/\text{kg}$, respectively. No significant difference (at $p = 0.05$) was found between any two groups of the above four species of oil. No species of oil investigated in this study was more significantly contaminated by plasticizers than the other. However, the wide range and the high average of total content in olive oil samples were very impressive. The relative standard deviation of the total content of plasticizers in olive oil was 102%, while that in soybean, canola, and corn oil was 62, 44, and 16%, respectively. As indicated by the wide range and high average of olive oil, the contamination level of plasticizers in olive oil may vary greatly. Different technologies, processes, and storage used in olive oil production may be responsible for this inconsistency of contamination.³⁴ Compared with olive oil, soybean, canola, and corn oil samples in this study were demonstrated to have much lower average plasticizer content and narrower

distribution range. This phenomenon was also observed in oils on Italian retail market.³³ Although only one sample of grapeseed oil was included in this study, its relatively high content of total plasticizers (1906 $\mu\text{g}/\text{kg}$) indicated that grapeseed oil is an oil species which may be highly contaminated by plasticizers. Thus, olive and grapeseed oil might possess the priority in plasticizer contamination monitoring among edible oils in the U.S. retail market.

To find out the packaging effect on the content of plasticizers in olive oil, statistic analysis was performed among the five olive oil samples. No significant difference (at $p = 0.05$) in the total content of plasticizers was observed between glass bottle ($n = 2$) and plastic bottle ($n = 3$) packaging. Nevertheless, similar statistical analysis was also performed among all 21 oil samples. No significant difference (at $p = 0.05$) in the total content of plasticizers was observed between any two groups of glass bottle ($n = 4$), plastic bottle ($n = 14$), and metal can ($n = 3$) packaging. This finding was consistent with the result reported by Nanni et al.³³ This observance implied that oil packaging was not the major cause of plasticizer contamination. Instead, contamination from the process of production and storage may be the major source of plasticizer in oil. Attention may need to be paid to the possible migration of plasticizers from plastic parts, equipment, and containers used in oil production process.

Primary Contaminating Plasticizers. Among a group of samples, primary contaminants are determined normally based on their concentrations instead of the detection rate.^{7,26} However, the percentage of the each contaminant in the total amount of contaminants might be a more practical parameter for the determination of primary contaminants because the contribution of each contaminant in each sample is normalized. The box plot of the contribution of each plasticizer in the 21 oil samples is shown in Figure 4. On the basis of median and the

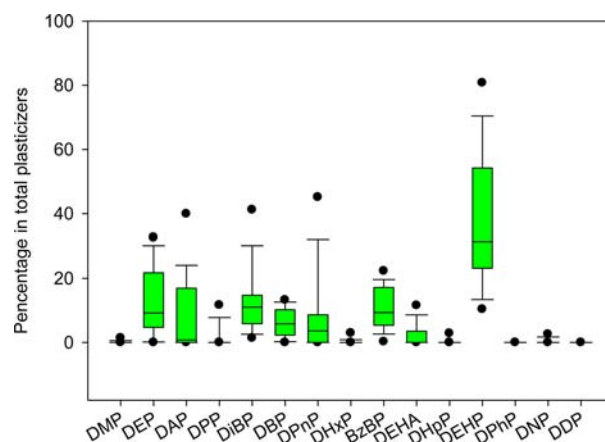


Figure 4. The contribution of each plasticizer in oil samples. The lower and the higher whiskers indicate the fifth and 95th percentiles, respectively. The lower and higher lines of the box indicate the 25th and 75th percentiles, respectively. The line inside of the box indicates the median and points appeared are outliers.

25th percentile line, the contribution of DEHP appeared much higher than other investigated plasticizers in all samples, becoming the most primary contaminating plasticizer in oil. In many other studies of oil and food, DEHP was also identified as the most primary phthalate plasticizer contaminant.^{26,29,34} This finding indicated that DEHP is still the most widely used plasticizer worldwide. Other primary contaminating plasticizers

identified in this study might include DiBP, BzBP, and DEP, with a median value of contribution at 11.0, 9.3, and 9.2%, respectively. Though DBP was detected at the same detection rate as DEP in this study, its median value of contribution was only 5.8%, demonstrating that it is less primary than DEP.

Toxicological Effect Estimation. Vegetable oil is an indispensable component in human being's regular diet. The contamination of plasticizers in edible oil may generate a hazard to human health.³⁴ On the basis of animal tests and possible toxicological effects on human health, The U.S. EPA has established reference dose (RfD) for chronic oral exposure. The RfD value for BzBP, DBP, DEHP, and DEP are 200, 100, 20, and 800 $\mu\text{g}/\text{kg}/\text{d}$, respectively.³⁹ DEHP is identified as the most dangerous phthalate, with the lowest Efd values among these four plasticizers. On the basis of RfD values, the daily allowed amount limit of BzBP, DBP, DEHP, and DEP for a 75 kg body weight adult is 15000, 7500, 1500, and 60000 μg , respectively. Assuming this adult takes 10 g edible oil daily, the intake amount of each of these four phthalate plasticizer from each of the oil sample investigated in this study was calculated (data not shown). The highest BzBP, DBP, DEHP, and DEP daily dose among all oil samples was 2.52, 0.926, 61.66, and 1.80 μg , respectively, which was 0.02, 0.01, 4.1, and 0.003% of the allowed limit, respectively. Obviously, the amount of plasticizers introduced into the human body merely through the intake of edible oil in this U.S. retail market is very low and might be not high enough to cause any toxicological effect. However, when taking the exposure to plasticizers from all diet components into consideration, its potential hazard to human beings, especially children, could not be recklessly neglected.

To control the contamination of edibles by migrating components from contacting materials, specific migration limits (SMLs) for DBP, BzBP, DEHP, and DEHA at 300, 30000, 1500, and 18000 $\mu\text{g}/\text{kg}$, respectively, have been established in both Europe and China.^{40,41} Similar limits were not yet established in the U.S. Assuming all plasticizers in oil were from migration during oil production processes, not from oil seed itself, when these SMLs were applied to evaluate the plasticizer contents obtained in this study, only DEHP content in solely two olive oils (i.e., samples 12 and 14) exceeded the limit by 0.52 and 3.11 times, respectively. To improve the quality of olive oil on the U.S. retail market, limits similar to SMLs might be needed imminently. For the side of olive oil producers, technologies and processes of olive oil production might need to be critically reviewed and strategies to prevent plasticizer contamination should be incorporated.

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Notes

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