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# A survey of phthalates in flavored olive oils from Turkey

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**SUMMARY.** Phthalates are organic contaminants that are used as plasticizers in many plastic food packaging materials. Because of their lipophilic character, oils are the primary source of human exposure to phthalates. In this study, the presence of five phthalate esters; diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) in olive oils flavored with different materials and marketed in Turkey in 2020 was determined. The samples included BBP, DINP, DIDP at lower concentrations than their LOQ. DEHP was the most abundant phthalate in all samples at varying concentrations between < LOQ to 1.81 mg/kg. The highest amount of DEHP was found in the oil sample flavored with bergamot (1.81 mg/kg). DBP was detected in 9 of the 25 samples at concentrations from 0.11 to 0.27 mg/kg.

***KEYWORDS***: *Contaminants; Diethylhexyl phthalate (DEHP); Flavored olive oil; Phthalates; Turkey.*

***RESUMEN. Estudios sobre ftalatos en aceites de oliva aromatizados de Turquía.*** Los ftalatos son contaminantes orgánicos que se utilizan como plastificantes en muchos materiales plásticos de envasado de alimentos. Debido a su carácter lipofílico, los aceites son la principal fuente de exposición humana a los ftalatos. En este estudio se determinó, la presencia de cinco ésteres ftalatos, ftalato de dietilhexilo (DEHP), ftalato de dibutilo (DBP), ftalato de butilbencilo (BBP), ftalato de diisononilo (DINP) y ftalato de diisodecilo (DIDP) en aceites de oliva aromatizados con diferentes materiales comercializados en Turquía en 2020. Las muestras incluían BBP, DINP, DIDP en concentraciones más bajas que su LOQ. El DEHP fue el ftalato más abundante en todas las muestras en concentraciones variables entre <LOQ y 1,81 mg/kg. La mayor cantidad de DEHP se encontró en la muestra de aceite con sabor a bergamota (1,81 mg/kg). Se detectó DBP en 9 de las 25 muestras en concentraciones de 0,11 a 0,27 mg/kg.

***PALABRAS CLAVE:*** *Aceite de oliva aromatizado; Contaminantes; Ftalato de dietilhexilo (DEHP); Ftalatos; Turquía.*

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## 1. INTRODUCTION

Virgin olive oil (VOO) is considered a high-value vegetable oil which possesses a characteristic aroma, taste and color. Olive oil is produced by only mechanical methods, thus there is no use of chemicals during its production. Besides, unlike the other vegetable oils, olive oil can be consumed directly without further refining (Fitó *et al.,* 2000). Virgin olive oil contains some minor compounds including tocopherols, sterols, carotenoids, squalene and phenolic compounds which have health-promoting attributes. A high consumption of virgin olive oil could reduce oxidative stress, prevent some cancer types, heart disease, and ageing (Nath and Nath 2000; Owen *et al.,* 2000). Barbagallo *et al.* (2017) reported that olive oil has many phytochemicals including phenolic components and secoiridoids that exert antioxidant activities.

Innovative and healthy products attract consumers' attention. One innovative product is flavored olive oils. Flavored olive oils are improved with healthy ingredients which make them preferable to consumers. Several materials are used to make flavored olive oils. These materials are essential oils, fruits, herbs, mushrooms, nuts, spices and vegetables (Sousa *et al.,* 2015). These innovative oils are generally produced via the maceration method. In this method, flavoring materials are mixed with virgin olive oil and the mixture is left for a certain time, enabling the increase in some constituents such as aroma compounds and phenolics which exhibit antioxidant and antimicrobial properties (Moldão-Martins *et al.,* 2004; Ayadi *et al.,* 2009; Baiano *et al.,* 2010; Jović *et al.,* 2018). Beside aroma, these flavoring materials may contribute to a longer shelf-life for virgin olive oils due to their preventive effect from oxidation. In addition, some constituents, especially phenolics, contribute to human health (Baiano *et al.,* 2010).

Phthalates are synthesized by a double-esterification mechanism of 1,2-benzenecarboxylic acid, and they are reported to have branched, apolar and linear components (Notardonato *et al.,* 2018). The Environmental Protection Agency (EPA) classified phthalates as priority pollutants which endanger human health by carcinogenic effects, adverse reproductive effects, and by altering endocrine function (Cadogan, 2002; Rios *et al.,* 2010). Phthalates are used in different materials such as polyvinyl chloride, polyvinyl acetate and polyester materials (Rios *et al.,* 2010). In many previous studies associated with foods and phthalates, di (2-ethylhexyl) phthalate (DEHP), diisobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP) benzylbutyl phthalate (BBP) were determined to be the most commonly identified phthalates (Tsumura *et al.,* 2002; Jarošová *et al.,* 2006; Fierens *et al.,* 2012).

Phthalates have higher solubility in vegetable oils than in water, which makes them a risky food product. Therefore, the determination of the presence and amount of phthalates in vegetable oils is very important for human health and confidence (Cavaliere *et al.,* 2008). Several studies have been conducted on the presence of phthalates in olive oil. DEHP, DnBP and BBP were identified in extra virgin olive oils, olive oils and olive pomace oils from the Italian market. Olive pomace oils contained higher concentration of phthalates, while lower amounts were detected in extra virgin oils (Cavaliere *et al.,* 2008). DiBP, BBP and DEHP were determined as the most abundant phthalates in Sicilian virgin olive oils, while DiNP and DiDP were the most abundant in Molise olive oils (Mo Dugo *et al.,* 2011). In another study by Nanni *et al.* (2011), the researchers demonstrated that DEHP and DINP were found at the highest levels among phthalates in extra virgin olive oils in Italy.

According to the legislation in this field, five phthalates are listed due to their potential health effects on humans including dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), the sum of diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP) with their specific migration limits as 0.3, 30, 1.5 and 9 mg/kg, respectively (EFSA 2005a; 2005b, 2005c, 2005d, 2005e; European Commission 2011). To the best of our knowledge, there is no information on phthalates in flavored olive oils. The aim of this work is to evaluate the presence of phthalates in flavored olive oils sold in Turkish markets, and to determine whether phthalate concentrations depend on the type of flavoring materials. The results will be useful for future studies regarding specific phthalate exposure from flavored olive oils.

## 2. MATERİALS AND METHODS

### 2.1. Sample collection

A total of 25 flavored olive oil samples contained in glass bottles were purchased from a local firm in Turkey and analyzed in October 2020. Each sample was stored at -20 ℃ until analyses. Olive oils were flavored with Chili Black Pepper, Aniseed, Bergamot, Rosemary, Tee, Bay, Basil, Thyme, Black Pepper, Lemon, Mandarin, Orange, Garlic, Sumac, Green Pepper, Chili Pepper, Gentian and Mix (rosemary, black pepper, garlic, sun-dried tomato). The details of the flavoring material for olive oil and their codings are listed in Table 1.

**Table 1.** Codes and names of flavored olive oils

| Codes | Flavoring material |
|---|---|
| FOO1 | Chili Black Pepper |
| FOO2 | Aniseed |
| FOO3 | Bergamot |
| FOO4 | Rosemary |
| FOO5 | Tee |
| FOO6 | Bay |
| FOO7 | Basil |
| FOO8 | Thyme |
| FOO9 | Black Pepper |
| FOO10 | Lemon |
| FOO11 | Mandarin |
| FOO12 | Orange |
| FOO13 | Garlic |
| FOO14 | Sumac |
| FOO15 | Green Pepper |
| FOO16 | Basil |
| FOO17 | Chili Pepper |
| FOO18 | Gentian |
| FOO19 | Gentian |
| FOO20 | Lemon |
| FOO21 | Rosemary |
| FOO22 | Thyme |
| FOO23 | Mix (rosemary, black pepper, garlic, sun-dried tomato) |
| FOO24 | Basil |
| FOO25 | Rosemary |

## 2.2. Reagents and materials

Benzyl butyl phthalate (BBP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), 2,6-Di-tert-butyl-4-methylphenol (BHT), and dibutyl phthalate (DBP) as phthalate standards were purchased from Dr. Ehrenstorfer GmbH (Germany). The purities of all standards were 99% except for DINP (98%). Acetonitrile and *n*-hexane were obtained from Sigma – Aldrich (Milwaukee, WI, USA) at HPLC grade.

## 2.3. Preparation of standards

1000 mg/L of stock standard solutions were prepared by dissolving appropriate amounts of the phthalates in hexane. DBP, DEHP, DINP, DIDP, and BBP from stock solution were transferred to 50-mL volumetric flasks at concentrations of 50 µL, 250 µL, 1500 µL, 1500 µL and 2500 µL, respectively. The intermediate standard mixtures (ISM) were then prepared by diluting with *n*-hexane. 40, 80, 160, 320, 480, 640 µL of the ISM for each phthalate were then transferred to 10-mL glass tubes, and 1 g of reference vegetable oil and 10 µL of BHT (1000 ppm) were added. Then the mixture was centrifuged at 2500 rpm at 10-15 °C immediately after vortexing for 2 minutes.

## 2.4. Sample preparation

### *2.4.1. Quality assurance and quality control of phthalate analyses*

Only glass laboratory equipment was used for sample preparation in order to prevent cross contamination. The sample flow line was purged with *n*-hexane to avoid potential contamination with samples. A special sample preparation laboratory was used for analysis of phthalates.

### *2.4.2. Oil sample preparation*

The previous method reported by Ierapetritis *et al.* (2014) was followed with some modifications. 1 g oil was weighed into 10-mL glass tubes, and 10 µL of the internal standard (BHT) were added. Approximately 10 mL of acetonitrile were used for the completion of the final volume. The samples were centrifuged at 2500 rpm for 10 min after a primary vortexing. The upper phase was transferred to another glassware and left to dry under nitrogen at 40 °C until a final 1 mL remained at the bottom. The extract was then injected into the GC-MS after a settling time of 1 hour.

## 2.5. Chromatographic analysis by GC–MS

GC–MS was operated in the electron impact mode because ionization by this technique is the most sensitive and reproducible. A 6890GC-5973MS (Agilent Technologies, Palo Alto, CA, USA) device was used. The colum used was a HP-5MS capillary column (30 m × 0.25 mm I. D. × 0.25 µm film thick-

Table 2. GC parameters and conditions

| Parameter | Condition |
|---|---|
| Carrier gas | Helium (purity: ≥ 99.999%) |
| Flow rate (mL/min) | 1.0 mL/min |
| Injection volume (μL) / mode | 1 μL / splitless |
| Injection temperature (°C) | 280 |
| Temperature program | 80 °C for 1 min, Ramp to 280 °C, rate: 15 °C/min. Hold at 280 °C for 15 min |
| Electron impact energy (eV) | 70 |
| Source temperature (°C) | 230 |

Table 3. CAS identifiers, retention times, SIM ions and time windows for the phthalates and internal standard (BHT) in SIM mode

| Compounds | CAS # | Retention time (min) | SIM ion (m/z) | Time window (min) |
|---|---|---|---|---|
| BHT (IS) | 128-37-0 | 8.099 | 205[1], 145, 177, 220 | 4.00-10.00 |
| DBP | 84-74-2 | 11.354 | 223[1], 150, 205 | 10.00-12.50 |
| BBP | 85-68-7 | 13.748 | 238[1], 91, 150, 206 | 12.50-14.00 |
| DINP | 68515-48-0 | 16.074 | 307[1], 150, 167 | 15.50-18.00 |
| DIDP | 68515-49-1 | 17.909 | 293[1], 150, 167 | 15.50-18.00 |
| DEHP | 117-81-7 | 14.750 | 279[a], 150, 167 | 14.00-15.50 |

[1] Quantitative ion; DEHP: diethylhexyl phthalate; DBP: dibutyl phthalate; BBP: butyl benzyl phthalate; DINP: diisononyl phthalate; DIDP: diisodecyl phthalate; BHT: 2,6-Di-tert-butyl-4-methylphenol

ness). All other operation conditions are listed in Table 2. Selected-ion monitoring (SIM) was used for data acquisition. The compound names, CAS identifiers, retention times, SIM ions and time windows of the five phthalates and internal standard (BHT) are shown in Table 3.

### 2.6. Validation parameters

The validation procedure was performed according to the specifications established in the Turkish “TS EN 14372:2004; Child use and care articles – Cutlery and feeding utensils- Safety requirements and tests”.

The method validation was performed by assessing recovery, linearity, specificity, and precision of peak areas. Limit of detection (LOD) and limit of quantification (LOQ) were determined by multiplying the signal from experimental noise ratios by 3 and 10 times, respectively. Calibration curves prepared from internal standards were used for evaluating the data. The linearities in all cases were satisfactory with correlation coefficients higher than 0.990. The method performance parameters are given in Table 4. The average recoveries of the five phthalates were between 87 and 100% and the RSD values were less than 20% for overall concentrations.

## 3. RESULTS AND DISCUSSION

Table 5 shows the phthalates found in flavored olive oils. In all analyzed samples BBP, DINP and DIDP were less than LOQ. In previous reports, DBP, BBP, DINP and DIDP were identified in olive oil samples marketed in Italy (Fusari and Rovellini, 2009; Mo Dugo *et al.,* 2011).

The current study revealed that DEHP was present in 18 of 25 samples with a mean value ranging from < LOQ to 1.81±0.025 mg/kg. The oil sample flavored with bergamot (FOO3) included the highest level of DEHP at 1.81±0.025 mg/kg, followed by the oil flavored with mandarin (FOO11, 1.61±0.045 mg/kg). In this study, the concentration of DEHP in 5 of the 25 samples was past the substance migration limit (SML) (1.5 mg/kg) as determined by EU 2011/10 for this phthalate. In a study by Mo Dugo *et al.* (2011), the DEHP levels in all olive oil samples from 3 years were detected with mean values of 1.171, 1.935 and

**Table 4.** Method performance parameters (n=3)

| Phthalates | Linear Equation | $R^2$ | LOD (mg/kg) | LOQ (mg/kg) | RSD (%) | Recovery (%) |
|---|---|---|---|---|---|---|
| DBP | y =1300x-1593 | 0.95 | 0.06 | 0.09 | 11 | 94 |
| BBP | y=4137x-4493 | 0.97 | 1.97 | 2.28 | 10 | 87 |
| DINP | y=6823x-8488 | 0.95 | 1.37 | 1.75 | 4 | 100 |
| DIDP | y=6849x-8525 | 0.95 | 1.20 | 1.40 | 3 | 92 |
| DEHP | y=3006x-3097 | 0.99 | 0.10 | 0.23 | 7 | 87 |

DEHP: diethylhexyl phthalate; DBP: dibutyl phthalate; BBP: butyl benzyl phthalate; DINP: diisononyl phthalate; DIDP: diisodecyl phthalate; LOD: limit of detection; LOQ: limit of quantification.

**Table 5.** Concentrations of phthalate esters in flavored olive oils (mg /kg) (n=2)

| Sample | DBP | BBP | DEHP | DINP | DIDP |
|---|---|---|---|---|---|
| FOO1 | 0.27±0.015 | <LOQ | 1.16±0.035 | <LOQ | <LOQ |
| FOO2 | <LOQ | <LOQ | 0.85±0.03 | <LOQ | <LOQ |
| FOO3 | <LOQ | <LOQ | 1.81±0.025 | <LOQ | <LOQ |
| FOO4 | <LOQ | <LOQ | 1.59±0.065 | <LOQ | <LOQ |
| FOO5 | 0.13±0.02 | <LOQ | 0.92±0.03 | <LOQ | <LOQ |
| FOO6 | <LOQ | <LOQ | 1.29±0.035 | <LOQ | <LOQ |
| FOO7 | <LOQ | <LOQ | 1.34±0.01 | <LOQ | <LOQ |
| FOO8 | <LOQ | <LOQ | 1.45±0.03 | <LOQ | <LOQ |
| FOO9 | 0.25±0.025 | <LOQ | 1.59±0.03 | <LOQ | <LOQ |
| FOO10 | 0.19±0.01 | <LOQ | 1.23±0.01 | <LOQ | <LOQ |
| FOO11 | 0.25±0.025 | <LOQ | 1.61±0.045 | <LOQ | <LOQ |
| FOO12 | 0.12±0.01 | <LOQ | 1.41±0.045 | <LOQ | <LOQ |
| FOO13 | <LOQ | <LOQ | 1.51±0.145 | <LOQ | <LOQ |
| FOO14 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO15 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO16 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO17 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO18 | <LOQ | <LOQ | 0.68±0 | <LOQ | <LOQ |
| FOO19 | <LOQ | <LOQ | 0.53±0.02 | <LOQ | <LOQ |
| FOO20 | 0.11±0.01 | <LOQ | 0.81±0.015 | <LOQ | <LOQ |
| FOO21 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO22 | 0.14±0.01 | <LOQ | 0.59±0.03 | <LOQ | <LOQ |
| FOO23 | 0.18±0.015 | <LOQ | 0.4±0.02 | <LOQ | <LOQ |
| FOO24 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |
| FOO25 | <LOQ | <LOQ | <LOQ | <LOQ | <LOQ |

DEHP: diethylhexyl phthalate; DBP: dibutyl phthalate; BBP: butyl benzyl phthalate;
DINP: diisononyl phthalate; DIDP: diisodecyl phthalate; LOQ: limit of quantification.

0.445 mg/kg, respectively (Mo Dugo *et al.,* 2011). The DEHP concentrations were also lower than extra virgin olive oils in glass and in tinplate (Nanni *et al.,* 2011). No phthalates were detected in sample FOO14 (flavored with sumac), FOO15 (flavored with green pepper), FOO16 (flavored with mandarin basil), FOO17 (chili pepper), FOO21 (rosemary), FOO24 (basil) or FOO25 (rosemary).

As shown in Table 5, DBP was present in nine of 25 samples with a mean value ranging from < LOQ to 0.27±0.015 mg/kg. In a study by Sungur *et al.* (2015), DBP concentrations were 0.117-1.418 mg/kg for olive oils and 0.102-1.048 mg/kg for virgin olive oils. In our study, the concentration of DBP did not exceed the substance migration limit (SML) (0.3 mg/kg) as set by EU 2011/10. The DBP concentrations in virgin olive oils in the current study were similar to those reported by Sungur *et al.* (2015).

The results obtained showed that a relation could be found between phthalate concentrations and flavoring materials. In a survey work by Cao *et al.* (2015), the phthalate contents in different food stuffs were investigated and DEHP was detected at the highest levels in the herbs and spices samples in almost all food stuffs. In another study by Ning *et al.* (2017), the concentration of DEHP in raw green pepper ranged from 1.83 to 5.95 mg/kg, which exceeded the legislated limit by EU 2011/10. The findings in the previous reports are considered useful to explain the high DEHP concentrations in flavored olive oils with herbs as observed in our study. In our study, the DEHP concentrations were 1.81 mg/kg for olive oil flavored with bergamod, 1.59 mg/kg for olive oil flavored with rosemary, and 1.59 mg/kg for olive oil flavored with black pepper. In a study by Di Bella *et al.* (2018), in which phthalates were determined in Tunisian culinary spices and herbs, DEHP concentrations in spices and herbs including caraway, black pepper, mint, oregano, coriander, thyme, rosemary, fennel, verbena, and laurel were found to range from 0.6 to 1.18 µg/kg.

## 4. CONCLUSIONS

The results from our study regarding phthalates in flavored oils indicated that 6 of 25 virgin olive oil samples flavored with different materials included DEHP (1.45±0.03 – 1.81±0.025 mg/kg), and these concentrations exceeded the SML set by EU 2011/10 regulation (1.5 mg/kg). In this study, DBP concentrations in all analyzed samples did not exceed the migration limit (SML) (0.3 mg/kg) of EU 2011/10, while BBP, DINP and DIDP were found to be less than their LOQ in all analyzed samples. However, from a food safety perspective for phthalates, seven flavored olive oil samples contained no residue of phthalates. The findings of this study are useful for the flavored olive oil producers and oil industry and will be a guide for a phthalate-free flavored olive oil production.

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### Conflict of Interest

The authors declared that there are no conflicts of interest.

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