

*Acta Alimentaria*, Vol. 45 (1), pp. 141–148 (2016)

DOI: 10.1556/066.2016.45.1.17

## DETERMINATION OF PHTHALATES IN PLUM SPIRIT AND THEIR OCCURRENCE DURING PLUM SPIRIT PRODUCTION

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(Received: 26 April 2015; accepted: 12 May 2015)

Phthalates are ubiquitous environmental contaminants that, due to their lipophilicity, migrate more readily into beverages with higher ethanol content. The aim of this work was to study the occurrence of phthalates in samples during the plum spirit production and in the final product, plum spirit manufactured by registered producers from five European countries, using gas chromatography-mass spectrometry (GC-MS). A decreasing trend of mean values was observed for diethyl phthalate (DEP), di-*iso*-butyl phthalate (DiBP), and di-*n*-butyl phthalate (DBP) as the distillation process went on. Levels of benzyl-butyl phthalate (BBP) and di-(2-ethylhexyl) phthalate (DEHP) increased in the distillation phase compared to concentrations in the preceding phases. In commercial plum spirits, DEHP and DiBP were detected in the highest concentrations. Results also indicated that a moderate daily consumption of plum spirit does not pose a health risk regarding the Tolerable Daily Intake of BBP, DEHP, and DBP.

**Keywords:** phthalates, plum spirit, gas chromatography-mass spectrometry, distillation

Exposure to phthalates can occur via ingestion of food/beverage contaminated with plasticizers (HEUDORF et al., 2007). Due to their toxicity, safety margins of exposure were set for the most relevant phthalates in food and beverages. World Health Organization (WHO) has set the maximum allowable level of di-(2-ethylhexyl) phthalate (DEHP) in drinking water at 8 µg l<sup>-1</sup> (WHO, 2005). Slovakian legislation defines the maximum allowable amount of phthalates as the sum of DEHP and di-*n*-butyl phthalate (DBP) (2 mg kg<sup>-1</sup>) in fruit, leafy vegetables, flour, and alcoholic beverages (MINISTERSTVO PŮDOHOSPODÁRSTVA SLOVENSKEJ REPUBLIKY A MINISTERSTVO ZDRAVOTNÍCTVA SLOVENSKEJ REPUBLIKY, 2004). European Food Safety Authority (EFSA) established the Tolerable Daily Intake (TDI) values for benzyl-butyl phthalate (BBP), DBP, and DEHP as 500, 10, and 50 µg kg<sup>-1</sup> body weight (bw), respectively (EFSA, 2005a, b, c).

Spirits, which includes all distilled beverages, constitute 50.1% of all alcoholic beverages consumed worldwide, 15% in Croatia (WHO, 2014). Due to the widespread presence of phthalates, the production of plum spirit carry a risk of phthalate contamination during the

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harvesting (plastic pipes and collecting bags), manufacturing (in tubing and pumps that connect different production phases), and packaging process (JURICA et al., 2013; CHATONNET et al., 2014). Thus, exposure to phthalates from spirits in general population could not be neglected and should be monitored. Plum spirit is a distilled alcoholic beverage traditionally made in countries of Central and Eastern Europe. In Croatia, in 2012, 165 000 l (0.04 l per capita (adults 15+ years)) of plum spirit was sold legally on the domestic market and approximately 72% (415 000 l) of the legally produced plum spirit is exported (CADP, 2013).

The main objectives of this study were to determine the concentrations of different phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), DBP, di-*iso*-butyl phthalate (DiBP), BBP, DEHP, and di-*n*-octyl phthalate (DOP)) during the five stages of Croatian plum spirit manufacturing process and concentrations of phthalates in the final product – plum spirit manufactured by registered producers from Croatia, Slovenia, Austria, Serbia, and Bosnia and Herzegovina; and to assess the risk of phthalate intake for the population consuming legally produced plum spirit. To the best of our knowledge, this is the first paper to report the DMP, DEP, DBP, DiBP, BBP, DEHP, and DOP levels in plum spirits of registered producers from multiple countries.

## 1. Materials and methods

### 1.1. Chemicals

Standards of DMP, DEP, DBP, DiBP, BBP, DEHP, and DOP, all of  $\geq 98\%$  purity were purchased from Supelco (Bellefonte, USA). Dichloromethane and hexane (HPLC grade) were obtained from Merck (Darmstadt, Germany) and anhydrous sodium sulphate (analytical grade) from Kemika (Zagreb, Croatia).

### 1.2. Sampling

Random samples of plum mash and plum distillate (N=67) were taken at a plum distillate production facility of a registered Croatian plum spirit producer during each of the five stages of the technological process: admission (N=26), pureeing (N=12), fermentation (N=6), transfer tank (N=8), and distillation (N=15). The samples were stored in glass and plastic containers and frozen at  $-18\text{ }^{\circ}\text{C}$  until analysis. The results were expressed as  $\mu\text{g}$  of phthalate per kg of plums. The sampling was carried out on a single batch of plums imported from two suppliers from surrounding countries and taken into the process of making the spirit. The sampling was done according to the modified standard protocol described by the Commission Regulation (EC) No 333/2007 (EC, 2007).

The commercially available plum spirits manufactured by eight different producers were purchased at several various supermarkets. Twenty (N=20) samples were taken altogether; two from each of the seven brands (three Croatian, one Austrian, one brand from Bosnia and Herzegovina, one Serbian, and one Slovenian; one sample per batch) and six samples from different batches of plum spirit produced by one registered Croatian producer using the process described in this study. These plum spirits, packed in labelled glass bottles with twist-off caps with documentary stamps, were analysed immediately upon purchase. The results of analysis were expressed as  $\mu\text{g}$  of phthalate per l of spirit.

### 1.3. GC-MS analysis

A 2 ml of plum mash, plum distillates, or plum spirit sample was extracted at pH 4–5 with 3 ml of dichloromethane in a 10-ml glass tube. After 10 min of mixing using Ultra-Turrax (IKA-Werke GmbH & Co. KG, Germany) at 2500 r.p.m., the mixture was left to settle until the layers separated. The water layer was discarded and 2 g of anhydrous sodium sulphate was added to the organic extract to remove the residual water. An aliquot of 1  $\mu\text{l}$  of extract was injected into the GC-MS system. Blank samples were prepared in the same way as samples, using 2 ml of deionised water instead of spirit. The blank concentration of each phthalate was subtracted from the concentration found in the sample.

Analysis was performed on a Thermo Scientific gas chromatograph-mass spectrometer Trace 1300-ITQ 700 (Milan, Italy). One  $\mu\text{l}$  of extract was injected in splitless mode at 280 °C. Analytes were separated onto a DB-5 capillary column (30 m $\times$ 0.25 mm, 0.25  $\mu\text{m}$  film thickness, Agilent Technologies Inc., Santa Clara, USA). Helium flow rate was 1 ml min<sup>-1</sup>. The GC oven temperature was set at 40 °C for 5 min, raised to 280 °C at 10 °C min<sup>-1</sup>, and held for 21 min at 280 °C. The temperature of the transfer line and the ion source was 250 °C. The MS was operated in electron ionization mode (70 eV). Selected ion monitoring was used for quantification ( $m/z$  163 for DMP and  $m/z$  149 for DEP, DBP, DiBP, BBP, DEHP, and DOP). Limit of detection (LOD) and limit of quantification (LOQ) ranged from 1.17  $\mu\text{g l}^{-1}$  (DMP) to 4.30  $\mu\text{g l}^{-1}$  (DOP) and from 3.90  $\mu\text{g l}^{-1}$  (DMP) to 14.32  $\mu\text{g l}^{-1}$  (DOP). Analytical recoveries ranged from 92.3% (DMP) to 98.6% (DEHP).

### 1.4. Statistical analysis

Statistical analyses were performed using Statistica9 for Windows (StatSoft, USA). Concentrations of the same phthalates in different sampling containers were compared by Mann-Whitney test. A  $P < 0.05$  was considered statistically significant.

## 2. Results and discussion

Seven different phthalates were monitored in samples (N=67) taken from five different phases in the plum spirit production process. DMP and DOP were below the LOD. DEP, DiBP, and DBP were detected in 32.8% while DEHP and BBP were detected in 26.9% and 7.5% of the samples, respectively (Table 1). A decreasing trend of mean values was observed for low-molecular weight and more volatile DEP, DBP, and DiBP as the process continued. Phthalates were present in the earliest phase, even before the beginning of the production process, presumably due to the plastic bags used during the plum picking and storing. At the admission and pureeing phase, DEHP was detected to a lesser extent (less than 20% of the samples). In the final phase of plum spirit production (distillation), mean concentrations of BBP and DEHP increased by 68.8 and 52.9%, respectively, compared to their concentration in the penultimate phase (transfer tank). The probable reason is that the plum distillate, as a more acidic medium, might have drawn out BBP and DEHP from the plastic and rubber components of the pumps or other equipment used in production. Such a stimulating effect from an acidic medium on phthalate migration from plastic has been described by BošNIR and co-workers (2007).

Table 1. Occurrence and phthalate levels ( $\mu\text{g kg}^{-1}$ ) in samples from different phases of the technological process of plum spirit production

PAEs	Phases of technological process												All phases (N=67)				
	Admission (N=26)			Pureeing (N=12)			Fermentation (N=6)			Transfer tank (N=8)			Distillation (N=15)			Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )
	Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Range (mean value) ( $\mu\text{g kg}^{-1}$ )	Positive samples (%)	Range (mean value) ( $\mu\text{g kg}^{-1}$ )			
DMP	0	ND	ND	0	ND	ND	0	ND	0	ND	0	ND	0	ND	0	ND	
DEP	38.5	ND-314 (44.0)	ND-335 (55.0)	33.3	ND-314 (44.0)	ND-116 (33.0)	50.0	ND-116 (33.0)	37.5	ND-136 (47.0)	13.3	ND-29.0 (2.00)	32.8	ND-335 (36.0)			
DiBP	38.5	ND-636 (56.0)	ND-679 (84.0)	33.3	ND-679 (84.0)	ND-88.0 (29.0)	50.0	ND-88.0 (29.0)	37.5	ND-101 (30.0)	13.3	ND-80.0 (7.00)	32.8	ND-679 (45.0)			
DBP	38.5	ND-1522 (84.0)	ND-197 (31.0)	33.3	ND-197 (31.0)	ND-57.0 (23.0)	50.0	ND-57.0 (23.0)	37.5	ND-60.0 (18.0)	6.67	ND-28.0 (2.00)	32.8	ND-1522 (43.0)			
BBP	7.69	ND-210 (10.0)	ND	0	ND	ND	0	ND	12.5	ND-128 (16.0)	13.3	ND-295 (27.0)	7.46	ND-295 (12.0)			
DEHP	19.2	ND-29.0 (3.00)	ND-76.0 (9.00)	16.7	ND-29.0 (3.00)	ND-32.0 (9.00)	50.0	ND-32.0 (9.00)	50.0	ND-43.0 (17.0)	26.7	ND-286 (26.0)	26.9	ND-286 (11.0)			
DOP	0	ND	ND	0	ND	ND	0	ND	0	ND	0	ND	0	ND			
$\Sigma$ PAEs	38.5	ND-1708 (196)	ND-1287 (179)	33.3	ND-1287 (179)	ND-279 (93.0)	50.0	ND-279 (93.0)	50.0	ND-409 (128)	26.7	ND-534 (64.0)	37.3	ND-1708 (146)			

PAEs: phthalic acid esters or phthalates; ND: not detected (&lt;LOD); N: number of samples

No significant difference in phthalate concentrations was observed for type of container, although glass containers displayed slightly lower concentrations. This study showed that the packaging materials (glass vs. plastic) had no evident influence, which is in accordance with studies by CARRILLO and co-workers (2008) and DEL CARLO and co-workers (2008), which tested the impact of packaging material on phthalate content in wine samples.

Phthalate concentrations determined in 20 glass bottled plum spirit samples from five different countries of Central and Eastern Europe are presented in Table 2. DMP and DOP were below the LOD in all of the analysed samples. The highest concentrations were found for DBP (mean 414.5  $\mu\text{g l}^{-1}$ ) and DEHP (mean 423.8  $\mu\text{g l}^{-1}$ ), which are the two most commonly identified phthalates in spirits (LEIBOWITZ et al., 1995, CHATONNET et al., 2014). The higher levels of DBP, BBP, and DEHP might have been caused by either contamination during production or the process of bottling or phthalate migration from the stoppers during storage. CHATONNET and co-workers (2014) reported similar results for DBP and DEHP concentrations in French grape spirit, while BBP levels were higher in our study. DBP (22–204  $\mu\text{g l}^{-1}$ ) and DEHP (128–492  $\mu\text{g l}^{-1}$ ) concentrations in vodka samples determined by LEIBOWITZ and co-workers (1995) were lower than concentrations measured in our study. Within this Alcohol Measures for Public Health Research Alliance (AMPHORA) project, which assessed the quality of illegally produced alcohol in a European-wide study, different phthalates (DMP, DEP, diallyl phthalate (DAP), DiBP, DBP, BBP, DEHP, diheptyl phthalate (DHP), and DOP) were measured, but not detected, in six samples of Croatian homemade spirits (pear, plum, and marc) (LACHENMEIER et al., 2011). The probable reason why phthalates were not detected in these spirits was low sensitivity of the analytical method used. The same phthalates were measured in 257 samples of alcoholic beverages in the study by LEITZ and co-workers (2009), in which only two samples contained DEP (used as denaturing agent for ethyl alcohol) in concentrations of 608  $\text{mg l}^{-1}$  and 210  $\text{mg l}^{-1}$ , while other phthalates were not detected. Phthalate concentrations in different wines are lower than those measured in spirits. CARRILLO and co-workers (2008) showed that DBP was the main phthalate found in wine (N=10), followed by DEHP and DEP, with total phthalate concentrations between 2.7 and 15  $\mu\text{g l}^{-1}$ . CINELLI and co-workers (2013) reported DEHP and DBP concentrations of up to 26.6 and 312.4  $\mu\text{g l}^{-1}$ , respectively, in commercial wines (N=11). In white and red wines (N=36) from Italy, DiBP, DBP, BBP, and DEHP were found at the average level of 45–115  $\mu\text{g l}^{-1}$  (DEL CARLO et al., 2008). Lower phthalate concentration in wines compared to spirits might be explained by the lower ethanol content in wine. Due to their lipophilicity, phthalates migrate more readily into beverages with higher ethanol content, like spirits (CHATONNET et al., 2014).

Table 2. Concentration of phthalates ( $\mu\text{g l}^{-1}$ ) in eight different brands of plum spirit (N=20)

Phthalates	Mean $\pm$ SD	Median (Range)
DMP	ND	ND
DEP	16.7 $\pm$ 15.3	7.8 (4.2–50.0)
DiBP	38.3 $\pm$ 13.9	38.8 (20.1–65.7)
DBP	414.5 $\pm$ 355.9	405.6 (25.2–822.0)
BBP	78.9 $\pm$ 39.7	79.0 (10.5–122.0)
DEHP	423.8 $\pm$ 524.6	282.0 (16.0–1638.0)
DOP	ND	ND

ND: not detected (<LOD); N: number of samples

Phthalate levels in alcoholic beverages have not yet been regulated. The EFSA established TDI values only for DBP, BBP, and DEHP (EFSA, 2005a, b, c) and these values were used in this study to estimate the drinking volume of plum spirit containing DBP, BBP, and DEHP that a 60-kg person could drink every day without apparent harmful effects potentially attributed to the intake of phthalates (Table 3). Assuming the worst-case scenario, the plum spirit sample with the highest DBP, BBP, and DEHP concentration was used for exposure calculation. The estimation showed that a daily intake of 0.73 l of plum spirit could be critical to human health regarding the exposure to DBP. Having this in mind, a daily consumption of one standard drink (0.03 l) does not pose a health risk. It should be pointed out that in cases of excessive drinking, the main health risk would arise from the high intake of ethanol. Our results also showed that the BBP, DEHP, and DBP intake for a 60-kg person drinking one 0.03-l glass of legally produced plum spirit, accounts for 0.01, 1.6, and 4.1% of the TDI for these phthalates, respectively. The main concern, however, is that, apart from legally produced plum spirit, people are also exposed to phthalates from homemade alcoholic beverages, which account for approx. 30% of all alcohol consumed (REHM et al., 2014).

Table 3. Exposure evaluation of plum spirit with regard to DBP, BBP, and DEHP concentration

Phthalates	Concentration in plum spirit ( $\mu\text{g l}^{-1}$ )	TDI ( $\mu\text{g kg}^{-1}$ bw/day)	Drinking volume for a 60-kg person to exceed TDI <sup>a</sup> ( $\text{l day}^{-1}$ )
DBP	822	10.0	0.73
BBP	122	500	246
DEHP	1638	50.0	1.83

TDI: Tolerable Daily Intake; <sup>a</sup>: Drinking volume (l)=[TDI ( $\mu\text{g kg}^{-1}$  bw/day)×60 kg]/mass concentration ( $\mu\text{g l}^{-1}$ )

It should be noted that the general population is exposed to phthalates from different sources (food, drink, dermal/inhalatory consumer products), so estimated exposure from spirits adds up the total intake amount. Also, human diet, as seen here for spirits, is contaminated with a mixture of different phthalates, all bearing its individual health risks.

### 3. Conclusions

This study quantified phthalates from the five stages of plum spirit production, as well as from marketed plum spirits from the countries in the region. Contamination of plum mash, distillate, and spirit with DEP, DiBP, and DBP may have arisen from storing plums in plastic bags during harvesting rather than from the technological process itself. The probable reason for the occurrence of BBP and DEHP in plum distillate was the drawing out of the phthalates from the plastic and rubber equipment in the technological process due to the more acidic nature of the distillate. Detection of phthalates in different natural spirits produced in controlled conditions suggested that monitoring of phthalates during production process is necessary, although phthalates determined in this study may not have significance when plum spirit is consumed in a moderate manner. Considering the widespread occurrence of phthalates in the environment and their detection in different alcoholic beverages, the monitoring of

phthalates should be the part of internal quality management and product safety control systems.

#### List of abbreviations

AMPHORA: Alcohol Measures for Public Health Research Alliance  
 BBP: benzyl-butyl phthalate  
 DAP: diallyl phthalate  
 DBP: di-*n*-butyl phthalate  
 DEHP: di-(2-ethylhexyl) phthalate  
 DEP: diethyl phthalate  
 DHP: diheptyl phthalate  
 DiBP: di-*iso*-butyl phthalate  
 DMP: dimethyl phthalate  
 DOP: di-*n*-octyl phthalate  
 EFSA: European Food Safety Authority  
 GC-MS: gas chromatography-mass spectrometry  
 LOD: limit of detection  
 LOQ: limit of quantification  
 ND: not detected  
 PAEs: phthalic acid esters or phthalates  
 TDI: Tolerable Daily Intake  
 WHO: World Health Organization

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This work has been supported by the Croatian Science Foundation under the project 8366

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