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Monitoring Phthalates in Table and Fortified Wines by Headspace Solid-Phase Microextraction Combined with Gas Chromatography–Mass Spectrometry Analysis

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ABSTRACT: Phthalates are a class of endocrine disruptors extensively used in plastic production as plasticizers, and as a result, they can be found in foods as a result of their migration ability. The occurrence of phthalates was monitored in 20 Portuguese wines using a simple, reliable, and environmentally friendly analytical method, headspace solid-phase microextraction combined with gas chromatography–mass spectrometry. Satisfactory figures of merit of method, linearity (correlation coefficient of ≥0.992), recovery (80.3–107.6%), precision (relative standard deviation of <13%), and limits of detection (0.03–0.11 μg/L) and quantification (0.09–0.36 μg/L) were achieved. Dibutyl phthalate and di-*n*-octyl phthalate were found in measurable quantities in table and fortified wines. The obtained results revealed that these wines do not represent any concern for human exposure, because their concentrations were lower than the tolerable daily intakes established by the European Food Safety Authority.

KEYWORDS: *phthalates, wines, HS–SPME/GC–MS, risk exposure*

1. INTRODUCTION

Phthalates are toxic to variable degrees depending upon their chemical structure and their capacity to migrate within organisms.[1] They can be found in numerous products, including foods, medical devices, electronic and informatic equipment, children's toys, clothes, certain pharmaceuticals, and also cosmetics as enhancers of adhesive qualities and improve the toughness of varnish or even the penetration of active ingredients, becoming ubiquitous environmental contaminants.[2,3] Numerous studies have described that exposure to phthalates is correlated with an earlier onset of puberty, male and female infertility, deformities in the male reproductive system, detrimental changes to sperm motility and mobility, and certain types of cancers, with possibly many more health problems as well.[1,4−6] Because wines may easily contact different kinds of materials containing these contaminants, including vats, pipes, tanks, and hoses coated with epoxy resin, and as a result of its better solubility in solutions with a high ethanol content, its kinetic diffusion from material-containing phthalates to wines is promoted.[1] In this sense, wines represent a health concern after long periods of maturation, storage/aging, and transportation.[1,7,8] To guarantee human health, limits on the amounts of substances able to migrate into the food were established on materials applied for food packaging.[9] These limits are designed as specific migration limits (SMLs) and are determined in milligrams of constituent per kilogram of food.[10] Moreover, tolerable daily intakes (TDIs) for numerous phthalates were detailed by the European Food Safety Authority (EFSA) in 2005 (Table 1).[11]

Concern related to the health risks and ubiquitous occurrence of phthalates encourages the development of sensitive and reproducible analytical tools that permit their detection and quantification at trace amounts in environmental,[12] biological,[13] and food[10,14,15] samples. To quantify a low concentration of phthalates in alcoholic beverages, headspace solid-phase microextraction (HS–SPME) using polydimethylsiloxane/divinylbenzene (PDMS/DVB), carbowax/divinylbenzene (CW/DVB), polyacrylate (PA),[4] and calix[6]arene[8] fibers has been suggested to concentrate phthalates prior to gas chromatography–mass spectrometry (GC–MS) analysis. SPME offered several benefits, such as shortening the sample preparation and increasing reliability, selectivity, and sensitivity when compared to conventional extraction procedures, such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), and dispersive liquid–liquid microextraction (DLLME). On the other hand, GC–MS is the suitable analytical tool used for phthalate detection, because of its sensitivity and chromatographic resolution for these compounds.[4,5,8,14,16]

The goal of the current study is to monitor the occurrence and establish the profiles of four phthalates, dibutyl phthalate (DBP), butyl benzyl phthalate (BBP), di-*n*-octyl phthalate (DOP), and 2,2,4,4-tetrabromodiphenyl ether (BDE), in 20 samples of table and fortified wines, through a simple, reliable, and environmentally friendly analytical method, HS–SPME/GC–MS. As far as we known, this is the first research reporting
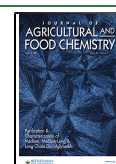
Table 1. Physicochemical Parameters of the Investigated Phthalates[a]

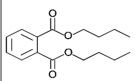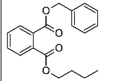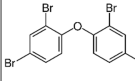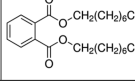| Phthalates	Formula	MW	Water solubility 25 °C (mg/L)	log K_{ow}	Vapor pressure (25 °C, Pa)	LD$_{50}$ (g/kg mouse)	TDI (mg/kg)
DBP		222.24	9.9	2.42	4.73×10^{-3}	8 - 10	0.01
BBP		312.36	2.7	4.73	2.49×10^{-3}	2	0.50
BDE		485.79	1.1×10^{-2}	6.0	2.52×10^{-3}	> 0.5	-
DOP		390.56	9.4×10^{-3}	8.06	0.07	13	0.15

[a]MW, molecular weight; log K_{ow}, log of the octanol−water partition coefficients; LD$_{50}$, median lethal dose; and TDI, tolerable daily intake by the EFSA.

Table 2. Parameters for Calibration, LOD, and LOQ for Phthalates Using the HS−SPME/GC−MS Method[a]

RT (min)	phthalate	linear range (μg/L)	ethanol (%)	equation	R^2	LOD (μg/L)	LOQ (μg/L)	ME (%)
13.18	DBP	0.5−60	12	$y = 143383x + 384213$	0.998	0.03	0.09	12
			18	$y = 59140x + 158287$	0.997	0.04	0.11	18
22.55	BBP	1−60	12	$y = 6866.5x + 40473$	0.994	0.07	0.24	15
			18	$y = 41956x + 354949$	0.993	0.08	0.27	20
26.49	BDE	1−60	12	$y = 24218x + 43752$	0.996	0.05	0.16	28
			18	$y = 6284.7x + 178462$	0.992	0.11	0.36	34
27.77	DOP	1−60	12	$y = 73346x + 240909$	0.996	0.06	0.19	35
			18	$y = 31881x + 253828$	0.995	0.07	0.22	42

[a]RT, retention time; R^2, regression coefficient; LOD, limit of detection; LOQ, limit of quantification; and ME, matrix effect.

the quantification of phthalates in table and fortified wines, produced on Madeira Island. The analytical tool was validated in terms of linearity, selectivity, precision (intra- and interday), recovery, and limits of detection (LOD) and quantification (LOQ) with different ethanol contents to mimic table (12%, v/v) and fortified (18%, v/v) wines. The risk of human exposure via drinking the investigated wines was assessed on the basis of TDI values established by the EFSA.

2. MATERIALS AND METHODS

2.1. Standards and Materials. Dibutyl phthalate (DBP, 99%), benzyl butyl phthalate (BBP, 98%), di-*n*-octyl phthalate (DOP, ≥98%), and 2,2,4,4-tetrabromodiphenyl ether (BDE, 97%) were purchased from Sigma-Aldrich Química S.A. (Spain). Tartaric acid (foodstuff grade), ethanol (99.8%), and sodium hydroxide (NaOH, ≥98%) were obtained from Riedel-de-Haën (Madrid, Spain), whereas sodium chloride (NaCl, 99.5%) was supplied by Panreac (Barcelona, Spain), and He (GC carrier gas) of purity 5.0 was supplied from Air Liquide, Portugal. Ultrapure water (H$_2$O) was obtained from a Milli-Q Plus system (18 MΩ/cm, Millipore, Bedford, MA, U.S.A.). The digital stirring plate (Cimarec) was purchased from Thermo Scientific (Waltham, MA, U.S.A.), while the SPME holder for manual sampling together with 65 μm PDMS/DVB fiber was obtained from Supelco (Bellefonte, PA, U.S.A.). The PDMS/DVB fiber was day-to-day conditioned on the basis of the endorsements of the manufacturer to avoid carryover among sets of analyses.

2.2. Samples. Table wines (9 red wines and 1 white wine, 12%, v/v) were collected from local producers. These wines were obtained from eight *Vitis vinifera* L. grapes, namely, two red, Tinta Negra and Bastardo, four white called noble varieties, Malvasia, Bual, Verdelho, and Sercial, and other recommended white varieties (Terrantez and Malvazia Roxa). The grapes were detached from the stalks, crushed,

and conserved in a stainless-steel vat. The must was removed from the solid parts and transferred to other stainless-steel vats. Then, a sulfating agent (20 g of SO$_2$/100 kg of must) and *Saccharomyces cerevisae* (20 g/100 kg) were added to must prior to the fermentation step. The fermentation process were considered finished when the sum of glucose and fructose was lower than 2 g/L. According to the suppliers, these wines were stored in vats for periods of time ranging from 4 to 6 months.

A total of 10 monovarietal Madeira wines from three white *V. vinifera* L. grapes (Bual, Malvasia, and Sercial), aged from 3 to 20 years old (Y) and matured in oak casks, were analyzed in the current study. On the basis of the age, the wines can be classified as vintage (a precise year of aged in casks, after 17 years) and blended (B, a mean aging time of 3, 5, 10, or 15 years) wines. These wines were aged in American oak casks (processed by a lighter toasting). The addition of natural grape spirit is added to finish the fermentation process to attain an ethanol content of 18−19% (v/v) and a precise sugar content. The samples were supplied by a Madeira wine producer, Henriques & Henriques, Vinhos, S.A.

2.3. Standard Solutions. Phthalate ethanolic standard solutions (500 μg/L) were prepared, labeled, and stored at −20 °C. The standard solutions, used to built the calibration curve (three individually replicates at each concentration was analyzed), were prepared by dilution of the stock solution in ethanol. Seven different model wines (total volume of 25 mL) were attained by dissolving 4.4 g/L tartaric acid in different ethanol contents (12 or 18%, v/v), adjusted to pH 3.3 with NaOH (1 M), and fortified with 100 μL of ethanolic standard solution from phthalates (see Table 2 for the concentration range).

2.4. HS−SPME Procedure. The HS−SPME conditions was adopted from a previous study optimized and validated for wines.[4] For each HS−SPME extraction, an aliquot of 2 mL of sample and 0.2 g of NaCl was put into a 4 mL glass vial. The vial was capped with a

Teflon [polytetrafluoroethylene (PTFE)] septum and placed in a thermostatic bath regulated to 80.0 ± 0.1 °C. The PDMS/DVB fiber was inserted into the headspace for 30 min under constant agitation (400 rpm). Then, the fiber was taken from the vial and placed into the GC injection port. The samples were analyzed in triplicate. Before the first extraction of the day, a fiber blank (10 min on the injection port at 250 °C) was performed for conditioning and ensuring the absence of carryover.

2.5. Gas Chromatography–Quadrupole Mass Spectrometry (GC–qMS) Conditions. After HS–SPME extraction, the fiber was placed into the injection port of an Agilent Technologies 6890N network gas chromatograph system (Palo Alto, CA, U.S.A.) for thermal desorption of phthalates at 250 °C for 6 min. The GC was equipped with a HP-5 fused silica capillary column (60 m × 0.25 mm inner diameter × 0.25 μm film thickness, SGE, Dortmund, Germany) and interfaced with an Agilent 5975 quadrupole inert mass selective detector. The oven program was initiated at 120 °C (hold for 3 min), then increased at a rate of 10 °C/min to 190 °C, then kept for 4 min, increased at a rate of 3 °C/min to 240 °C, and held for 20 min, in a total GC run time of 50.67 min. The column flow was constant at 1.0 mL/min using He of purity 5.0. The injection port functioned in the splitless mode and was held at 250 °C. For the 5975 MS system, the temperatures used were 270, 150, and 230 °C for the operating temperatures of the transfer line, quadrupole, and ionization source, respectively. Data acquisition was carried out in the scan mode (m/z 30–300) with electron ionization at an energy of 70 eV and ionization current of 10 μA. Phthalate identification was performed by manual interpretation by comparison of spectra and corresponding against the Agilent MS ChemStation software, equipped with a NIST05 mass spectral library with a similarity threshold higher than 80%, as well as the standards. The assays were performed in triplicate, and the results were presented by the mean ± standard deviation (SD).

2.6. Method Validation. The method validation was carried out agreeing with the European Union SANCO/12495/2011 guidelines.[17] The analytical performance was assessed in terms of linearity, selectivity, sensitivity (LOD and LOQ), precision (inter-/intraday), recovery (as a measure of trueness), and matrix effect. The method linearity was measured in the concentration range reported in Table 2 based on the average GC peak areas versus concentrations and correlation coefficients (R^2) for each phthalate analyzed. The non-existent interfering peaks at a retention time (RT) of the phthalates under study allow for the determination of the method selectivity, through the direct injection of an aliquot of the blank extract. The sensitivity of the method was assessed through LOD and LOQ, which were determined by 3 and 10 times the ratio of SD of the calibration curve interception and the slope of the regression curve, respectively. Three different phthalate concentrations (Table 3) within of the concentration range were used to evaluate the method precision. Seven replicates ($n = 7$) were carried out on the same day to determine intraday precision (repeatability), whereas for the interday precision (reproducibility), five replicates ($n = 5$) were analyzed in 6 consecutive days (a total of $n = 30$). The results were presented as relative standard deviation (% RSD). The recovery (accuracy) was performed as precision through the spiking of table and fortified wines at three concentration levels (Table 3). The slopes attained in calibration curves of phthalates in the sample and solvent-based matrix were compared to assess the matrix effect, with the calibration curves for both matrixes being prepared in a similar way.

3. RESULTS AND DISCUSSION

The phthalates chosen for the present study represent the most regularly detected contaminants in food-related products.[14] The main apprehension related to phthalate analysis is the risk of contamination, which might create the outcome of a false-positive result and overestimated concentration. The contamination sources can arise from any step of the analytical method: sampling, sample preparation, and chromatographic analysis.[15] Therefore, with the purpose to avoid contamination risks, all glassware material was washed with ethanol followed

Table 3. Recovery and Precision of Phthalates in Table (12%, v/v) and Fortified (18%, v/v) Wines at Three Spiked Levels[a]

RT (min)	phthalate	spiked level (μg/L)	% REC ± SD	precision (% RSD) intraday	precision (% RSD) interday
		12% (v/v) Ethanol			
13.18	DBP	0.5	92.9 ± 3.25	4.75	7.53
		30	93.6 ± 2.74	3.39	5.46
		60	105.3 ± 9.53	1.25	2.92
22.55	BBP	1	99.5 ± 5.97	9.62	8.83
		30	100.3 ± 3.01	6.38	7.98
		60	97.8 ± 2.85	3.21	4.84
26.49	BDE	1	96.9 ± 5.75	10.4	11.8
		30	80.4 ± 3.22	2.85	9.46
		60	89.9 ± 3.50	0.87	4.88
27.77	DOP	1	100.2 ± 8.02	1.62	8.92
		30	98.4 ± 7.84	2.68	7.96
		60	95.3 ± 2.86	0.92	1.42
		18% (v/v) Ethanol			
13.18	DBP	0.5	104.7 ± 4.19	4.51	8.82
		30	99.8 ± 3.99	3.54	8.47
		60	101.5 ± 4.11	1.28	4.61
22.55	BBP	1	105.1 ± 3.20	7.87	10.1
		30	98.2 ± 7.84	7.31	7.53
		60	106.4 ± 2.77	3.25	1.72
26.49	BDE	1	80.3 ± 6.03	10.9	12.2
		30	106.1 ± 5.37	4.69	6.56
		60	99.9 ± 4.99	3.51	5.67
27.77	DOP	1	98.7 ± 6.88	3.56	10.3
		30	107.6 ± 2.91	2.74	9.63
		60	97.7 ± 4.84	3.24	10.2

[a]RT, retention time; % REC, recovery percentage; and SD, standard deviation.

by H_2O and heated at 300 °C for 2 h prior to use. After that, all materials were introduced in desiccators containing aluminum oxide until HS–SPME/GC–MS analysis. Moreover, to screen the incidence of phthalates in the GC–MS system, a blank of equipment and direct injections of ethanol were performed. None of the target phthalates were detected.

3.1. Method Validation. The performance of the HS–SPME/GC–MS analytical method was evaluated for selectivity, linearity, precision (intra- and interday), accuracy (percent recovery), and sensitivity (LOD and LOQ), as designated in section 2.6. Additionally, the analytical method performance was carried out for different alcohol contents to mimic table (12%, v/v) and fortified (18%, v/v) wines (Tables 2 and 3). Figure 1 shows a typical HS–SPME/GC–MS chromatogram of phthalates in spiked fortified wine at 30 μg/L, and it is possible observe that the separation was concluded in less than 28 min.

The linearity of the method was assessed through calibration curves that were fit using least squares linear regression analysis. The obtained correlation coefficient (R^2) was higher than 0.992, with residuals not exceeding ±10%, which indicates the method linearity over the whole range of the investigated concentration. Nevertheless, it should be pointed out that R^2 is higher in the calibration curves with 12% (v/v) ethanol.
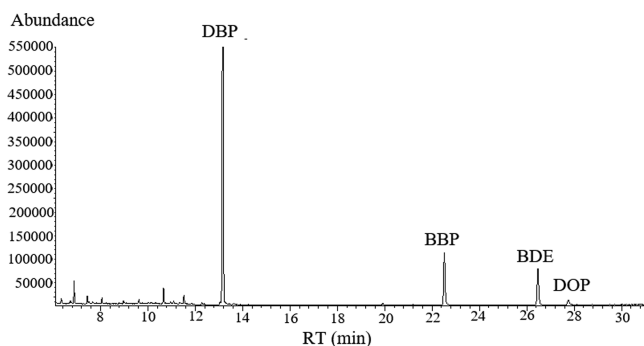
Figure 1. HS−SPME/GC−MS chromatogram of phthalates in spiked fortified wine (30 μg/L) using the PDMS/DVB fiber at 80 °C for 30 min. Abbreviations: DBP, dibutyl phthalate; BBP, benzyl butyl phthalate; DOP, di-n-octyl phthalate; and BDE, 2,2,4,4-tetrabromo-diphenyl ether.

The LOD and LOQ values for 12% ethanol ranged from 0.03 μg/L (DBP) to 0.07 μg/L (BBP) and from 0.09 μg/L (DBP) to 0.24 μg/L (BBP), whereas the LOD and LOQ values for 18% ethanol ranged from 0.04 μg/L (DBP) to 0.11 μg/L (BDE) and from 0.11 μg/L (DBP) to 0.36 μg/L (BDE), respectively. It is possible to observe that better LODs and LOQs were achieved for 12% (v/v) ethanol. Concerning DBP, the LOD and LOQ obtained were lower than the default specific migration limit (SML) of 0.30 mg/kg recognized by international regulation.[18,19]

With regard to the precision, the intraday precision for 12 and 18% (v/v) ethanol content ranged from 0.87 to 10.4% and from 1.28 to 10.9%, while the interday precision ranged from 1.42 to 11.8% and from 1.72 to 12.2%, respectively. The mean recovery of phthalates ranged from 80.4 to 105.3% for 12% (v/v) ethanol, while for 18% (v/v) ethanol, the mean recovery of phthalates ranged from 80.3 to 107.6%. According to the literature, a quantitative method should be validated as being capable of providing mean recoveries from 70 to 120% and precision with % RSD values lower than 20%. Similar precision and recoveries for phthalates (Table 4) were obtained in alcoholic beverages using SPME/GC−MS.[4,8,14]

The results illustrated in Tables 2 and 3 demonstrate that the analytical method performance is remarkably influenced by the ethanol content, because lower R^2 and higher LOD and LOQ values were observed for 18% (v/v) ethanol. This is in accordance with preceding studies that reported that the presence of a high concentration of ethanol interferes with target analyte extraction.[20,21] According to Russo et al.,[22] the recovery of phthalate esters decreases when the ethanol content increases, with the recovery ranging from 78 to 105%, from 71 to 95%, and from 28 to 92% for 13, 17, and 20% (v/v) ethanol, respectively. These authors verify that an ethanol content below 20% (v/v) guarantees good recoveries for phthalates under study.

Finally, the matrix effects were evaluated, because this parameter can affect the determination of analytes at trace amounts. A value below ±20% was classified as no matrix effects, because the difference is close to the repeatability data. Values ranging from ±20 and ±50% were classified as medium matrix effects, whereas when exceeding ±50%, a strong matrix effect was observed.[23] As observed in Table 2, BDE and DOP showed medium matrix effects, with this effect being more pronounced in wines with a higher ethanol content (18%, v/v, ethanol).

The analytical method established was related to other gas chromatography (GC) and liquid chromatography (LC) methods described in the literature for phthalate quantification in alcoholic beverages.[4,8,10,14−16,22,24−26] The sample volume, LOD, LOQ, and recovery (analytical performance) were used to demonstrate the advantages of the HS−SPME method validated here. Overall, the current analytical method proposed used the lower sample volume (only 2 mL) and displayed the identical or enhanced analytical performance than the mentioned methods, except for one researcher that used hollow fiber solid-phase microextraction (HF−SPME/GC−MS).[16] Furthermore, HS−SPME is an environmentally friendly and economical extraction procedure because it does not require solvents.

3.2. Quantification of Phthalates in Table and Fortified Wines. The validated analytical method was applied to monitor the concentration of phthalates in 10 table wines

Table 4. Comparison of Analytical Parameters from Several Studies Carried out for Phthalate Quantification in Alcoholic Beverages[a]

sample (mL)	extraction procedure	analytical method	LOD (μg/L)	LOQ (μg/L)	REC (%)	reference
wine (4)	HS−SPME	GC−MS	0.2−1.3	0.4−1.3	70−131	4
beer (5)	HS−SPME	GC−MS	0.003−3.43		86−109	8
beer (4)	HS−SPME	GC−MS	0.01−0.59	0.02−1.96	74−101	14
wine (1)	HF−SPME	GC−MS	0.01−0.03	0.02−0.10	68−115	16
wine (100)	MIP−SPE	LC−MS	0.03−0.20	0.09−0.68	74−98	24
beer (30)	MSPE	GC−MS/MS	0.01−2.75	0.02−9.15	79−122	25
wines (5)	DLLME	HPLC−DAD	1.5−2.2	5−7.3	92−105	15
wines (10)	UA−DLLME−SFO	GC−FID	0.64−2.82	1.93−8.47	75−98	10
wine (10)	USVADLLME	GC−MS	0.02−0.10	0.08−0.34	85−101	26
wine (100)	SPE	GC−MS	0.2−14	0.5−25	78−105	22
wine (2)	HS−SPME	GC−MS	0.03−0.11	0.09−0.36	93−108	this work

[a]Abbreviations: HS−SPME, headspace solid-phase microextraction; HF−SPME, hollow fiber solid-phase microextraction; MIP−SPE, molecular imprinted polymer solid-phase microextraction; MSPE, magnetic solid-phase extraction; DLLME, dispersive liquid−liquid microextraction; UA−DLLME−SFO, ultrasound-assisted dispersive liquid−liquid microextraction followed by solidification of a floating organic drop; USVADLLME, ultrasound-vortex-assisted dispersive liquid−liquid microextraction; SPE, solid-phase extraction; GC−MS, gas chromatography−mass spectrometry; LC−MS, liquid chromatography−mass spectrometry; GC−MS/MS, gas chromatography−tandem mass spectrometry; HPLC−DAD, high-performance liquid chromatography with a diode array detector; GC−FID, gas chromatography with a flame ionization detector; LOD, limit of detection; LOQ, limit of quantification; and REC (%), recovery percentage.

Table 5. Concentration (μg/L $\pm$ SD) and Occurrence of Phthalates in Table and Fortified Wines[a]

wine	phthalate (μg/L) $\pm$ SD				
	DBP	BBP	BDE	DOP	total (μg/L)
			Table Wines		
red wine 1	1.77 $\pm$ 0.12	-	1.03 $\pm$ 0.01	1.65 $\pm$ 0.05	4.45
red wine 2	20.8 $\pm$ 0.21	-	1.31 $\pm$ 0.06	1.06 $\pm$ 0.03	23.2
red wine 3	6.07 $\pm$ 0.42	-	-	1.66 $\pm$ 0.11	7.73
red wine 4	5.03 $\pm$ 0.10	-	-	1.34 $\pm$ 0.05	6.37
red wine 5	7.17 $\pm$ 0.23	-	-	1.02 $\pm$ 0.02	8.19
red wine 6	8.77 $\pm$ 0.35	-	-	2.20 $\pm$ 0.26	11.0
red wine 7	10.6 $\pm$ 1.06	-	-	2.01 $\pm$ 0.12	12.6
red wine 8	2.71 $\pm$ 0.08	-	-	2.02 $\pm$ 0.25	4.73
red wine 9	0.71 $\pm$ 0.02	-	-	2.11 $\pm$ 0.14	2.82
white wine	1.04 $\pm$ 0.01	-	-	1.58 $\pm$ 0.05	2.62
average	6.47 $\pm$ 0.26	-	1.17 $\pm$ 0.04	1.67 $\pm$ 0.11	8.37
FO (%)	100	-	10	100	
			Fortified Wines (Years Old)		
FW (3Y) a	4.16 $\pm$ 0.33	-	-	2.10 $\pm$ 0.06	6.26
FW1 (5Y) a	5.41 $\pm$ 0.23	-	-	1.97 $\pm$ 0.12	7.38
FW2 (3Y) b	5.27 $\pm$ 0.66	-	-	1.99 $\pm$ 0.04	7.17
FW3 (5Y) b	9.81 $\pm$ 0.12	-	-	1.30 $\pm$ 0.07	11.1
FW4 (10Y) b	14.6 $\pm$ 0.11	-	-	2.12 $\pm$ 0.20	16.7
FW5 (20Y) b	23.2 $\pm$ 1.05	-	-	2.43 $\pm$ 0.13	25.6
FW6 (3Y) c	1.66 $\pm$ 0.05	-	-	2.44 $\pm$ 0.06	4.10
FW7 (5Y) c	3.29 $\pm$ 0.23	-	-	-	3.29
FW8 (10Y) c	6.01 $\pm$ 0.10	-	-	-	6.01
FW9 (15Y) c	9.23 $\pm$ 0.22	-	-	1.91 $\pm$ 0.09	11.1
average	8.26 $\pm$ 0.31	-	-	2.03 $\pm$ 0.08	9.87
FO (%)	100	-	-	80	

[a]-, not detected; FO, frequency of occurrence. The lowercase letters indicate the fortified wines from the same *V. vinifera* L. grapes.

and 10 fortified wines. Table 5 shows the phthalate concentration, average, and frequency of occurrence (% FO) found in all wines under study. As observed, DBP was quantified in all table and fortified wines (FO = 100%), with the concentration ranging from 0.71 to 20.8 μg/L, while DOP was found in 100 and 80% of tables and fortified wines, respectively. BBP was not found in any of the analyzed samples. The determined concentrations varied significantly between samples and wine types, indicating that there were many different sources of contamination. Tanks and hoses coated with epoxy resin might be the two main sources of contamination. In addition, as presented in Table 5, the concentration of phthalates in older fortified wines (10−20 years, with an average of 13.4 $\pm$ 0.37 μg/L) was significantly (p < 0.05) higher (2.7 times) compared to younger wines (3−5 years, with an average of 4.93 $\pm$ 0.27 μg/L). The obtained results indicated that the phthalate concentration depends upon the wine quality as well as the aging process, with the choice of the raw material being a critical condition. Nevertheless, it is difficult to specify the strict origins of the phthalates, which might be a curious subject for future studies. With the TDI established by the EFSA for DBP taken into account (Table 1), the daily intake of DBP for adults drinking wine was predictable through the highest concentrations of DBP (23.2 μg/L) determined in the current study. Considering that a 60 kg adult drinks 100 mL of wine, the extreme DBP consumption determined is 2.32 μg, which is lower than the TDI (0.60 mg or 600 μg). DOP was detected in all table wines and ranged from 1.02 to 2.20 μg/L, while in fortified wines, it was detected in 8 of 10 samples, in a concentration range from 1.30 to 2.44 μg/L. In addition, BDE

was found in a much lower concentration, ranging from 1.01 to 1.31 μg/L, and low FO (10%). The results suggested that the wine samples under study do not represent any concern for human exposure.

The total phthalate concentration ranged from 2.62 to 23.2 μg/L for table wines and from 3.29 to 25.6 μg/L for fortified wines. On average, the total phthalate concentration in fortified wines was higher, almost 1.2 times, than table wines, which confirms that the higher ethanol content promotes the phthalate migration. In addition, the difference in the total phthalate concentration could be related to different wine-making processes.[15] The results discovered that the validated analytical method is suitable for phthalate quantification in table and fortified wines.

HS−SPME/GC−MS represented a suitable routine practice because it is simple, economical, precise, accurate, and environmentally friendly. In addition, the concentration of phthalates in table and fortified wines was lower than the TDI established by the EFSA, which revealed that these samples did no represent any risk for human exposure through consumption.

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Notes

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■ REFERENCES

(1) Chatonnet, P.; Boutou, S.; Plana, A. Contamination of Wines and Spirits by Phthalates: Types of Contaminants Present, Contamination Sources and Means of Prevention. *Food Addit. Contam., Part A* **2014**, *31*, 1605−1615.

(2) Guo, Y.; Zhang, Z.; Liu, L.; Li, Y.; Ren, N.; Kannan, K. Occurrence and Profiles of Phthalates in Foodstuffs from China and Their Implications for Human Exposure. *J. Agric. Food Chem.* **2012**, *60*, 6913−6919.

(3) Swan, S. H. Environmental Phthalate Exposure in Relation to Reproductive Outcomes and Other Health Endpoints in Humans. *Environ. Res.* **2008**, *108*, 177−184.

(4) Carrillo, J. D.; Salazar, C.; Moreta, C.; Tena, M. T. Determination of Phthalates in Wine by Headspace Solid-Phase Microextraction Followed by Gas Chromatography-Mass Spectrometry: Fibre Comparison and Selection. *J. Chromatogr. A* **2007**, *1164*, 248−261.

(5) Montevecchi, G.; Masino, F.; Zanasi, L.; Antonelli, A. Determination of Phthalate Esters in Distillates by Ultrasound-Vortex-Assisted Dispersive Liquid-Liquid Micro-Extraction (US-VADLLME) Coupled with Gas Chromatography/Mass Spectrometry. *Food Chem.* **2017**, *221*, 1354−1360.

(6) Yost, E. E.; Euling, S. Y.; Weaver, J. A.; Beverly, B. E. J.; Keshava, N.; Mudipalli, A.; Arzuaga, X.; Blessinger, T.; Dishaw, L.; Hotchkiss, A.; Makris, S. L. Hazards of Diisobutyl Phthalate (DIBP) Exposure: A Systematic Review of Animal Toxicology Studies. *Environ. Int.* **2019**, *125*, 579−594.

(7) Benjamin, S.; Masai, E.; Kamimura, N.; Takahashi, K.; Anderson, R. C.; Faisal, P. A. Phthalates Impact Human Health: Epidemiological Evidences and Plausible Mechanism of Action. *J. Hazard. Mater.* **2017**, *340*, 360−383.

(8) Ye, C. W.; Gao, J.; Yang, C.; Liu, X. J.; Li, X. J.; Pan, S. Y. Development and Application of an SPME/GC Method for the Determination of Trace Phthalates in Beer Using a Calix[6]Arene Fiber. *Anal. Chim. Acta* **2009**, *641*, 64−74.

(9) European Union (EU).. Commission regulation (EU) No 10/ 2011 of 14 January 2011 on plastic materials and articles intended to into contact with food. *Off. J. Eur. Union* **2011**, *54*, 1 DOI: 10.3000/ 17252555.L_2011.012.eng.

(10) Pérez-Outeiral, J.; Millán, E.; Garcia-Arrona, R. Determination of Phthalates in Food Simulants and Liquid Samples Using Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction Followed by Solidification of Floating Organic Drop. *Food Control* **2016**, *62*, 171−177.

(11) Silano, V.; Barat Baviera, J. M.; Bolognesi, C.; Chesson, A.; Cocconcelli, P. S.; Crebelli, R.; Gott, D. M.; Grob, K.; Lampi, E.; Mortensen, A.; Riviere, G.; Steffensen, I.-L.; Tlustos, C.; Van Loveren, H.; Vernis, L.; Zorn, H.; Cravedi, J.-P.; Fortes, C.; Tavares Pocas, M. F.; Waalkens-Berendsen, I.; Wolfle, D.; Arcella, D.; Cascio, C.; Castoldi, A. F.; Volk, K.; Castle, L. Update of the risk assessment of di-butylphthalate (DBP), butyl-benzyl-phthalate (BBP), bis(2-ethylhexyl)phthalate (DEHP), di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) for use in food contact materials. *EFSA J.* **2019**, *17*, 5838−5923.

(12) Abtahi, M.; Dobaradaran, S.; Torabbeigi, M.; Jorfi, S.; Gholamnia, R.; Koolivand, A.; Darabi, H.; Kavousi, A.; Saeedi, R. Health Risk of Phthalates in Water Environment: Occurrence in Water Resources, Bottled Water, and Tap Water, and Burden of Disease from Exposure through Drinking Water in Tehran, Iran. *Environ. Res.* **2019**, *173*, 469−479.

(13) Genuis, S. J.; Beesoon, S.; Lobo, R. A.; Birkholz, D. Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study. *Sci. World J.* **2012**, *2012*, 615068.

(14) Carnol, L.; Schummer, C.; Moris, G. Quantification of Six Phthalates and One Adipate in Luxembourgish Beer Using HS−SPME−GC/MS. *Food Anal. Methods* **2017**, *10*, 298−309.

(15) Fan, Y.; Liu, S.; Xie, Q. Rapid Determination of Phthalate Esters in Alcoholic Beverages by Conventional Ionic Liquid Dispersive Liquid-Liquid Microextraction Coupled with High Performance Liquid Chromatography. *Talanta* **2014**, *119*, 291−298.

(16) Li, J.; Su, Q.; Li, K. Y.; Sun, C. F.; Zhang, W. B. Rapid Analysis of Phthalates in Beverage and Alcoholic Samples by Multi-Walled Carbon Nanotubes/Silica Reinforced Hollow Fibre-Solid Phase Microextraction. *Food Chem.* **2013**, *141*, 3714−3720.

(17) European Union (EU). *Method Validation and Quality Control Procedures for Pesticides Residues Analysis in Food and Feed*; EU: Brussels, Belgium, 2007; SANCO/2007/3131.

(18) Wang, Y.; Zhu, H.; Kannan, K. A Review of Biomonitoring of Phthalate Exposures. *Toxics* **2019**, *7*, 21−49.

(19) Wang, W.; Leung, A. O. W.; Chu, L. H.; Wong, M. H. Phthalates Contamination in China: Status, Trends and Human Exposure-with an Emphasis on Oral Intake. *Environ. Pollut.* **2018**, *238*, 771−782.

(20) Câmara, J. S.; Alves, M. A.; Marques, J. C. Development of Headspace Solid-Phase Microextraction-Gas Chromatography−Mass Spectrometry Methodology for Analysis of Terpenoids in Madeira Wines. *Anal. Chim. Acta* **2006**, *555*, 191−200.

(21) Urruty, L.; Montury, M. Influence of Ethanol on Pesticide Extraction in Aqueous Solutions by Solid-Phase Microextraction. *J. Agric. Food Chem.* **1996**, *44*, 3871−3877.

(22) Russo, M. V.; Notardonato, I.; Cinelli, G.; Avino, P. Evaluation of an Analytical Method for Determining Phthalate Esters in Wine Samples by Solid-Phase Extraction and Gas Chromatography Coupled with Ion-Trap Mass Spectrometer Detector. *Anal. Bioanal. Chem.* **2012**, *402* (3), 1373−1381.

(23) Dias, J. V.; Nunes, M. da G. P.; Pizzutti, I. R.; Reichert, B.; Jung, A. A.; Cardoso, C. D. Simultaneous Determination of Pesticides and Mycotoxins in Wine by Direct Injection and Liquid Chromatography-Tandem Mass Spectrometry Analysis. *Food Chem.* **2019**, *293*, 83−91.

(24) Barciela-Alonso, M. C.; Otero-Lavandeira, N.; Bermejo-Barrera, P. Solid Phase Extraction Using Molecular Imprinted Polymers for Phthalate Determination in Water and Wine Samples by HPLC-ESI-MS. *Microchem. J.* **2017**, *132*, 233−237.

(25) Pang, Y.-H.; Yue, Q.; Huang, Y.-y.; Yang, C.; Shen, X.-F. Facile Magnetization of Covalent Organic Framework for Solid-Phase Extraction of 15 Phthalate Esters in Beverage Samples. *Talanta* **2020**, *206*, 120194.

(26) Cinelli, G.; Avino, P.; Notardonato, I.; Centola, A.; Russo, M. V. Rapid Analysis of Six Phthalate Esters in Wine by Ultrasound-Vortex-Assisted Dispersive Liquid−Liquid Micro-Extraction Coupled with Gas Chromatography−Flame Ionization Detector or Gas Chromatography−Ion Trap Mass Spectrometry. *Anal. Chim. Acta* **2013**, *769*, 72−78.