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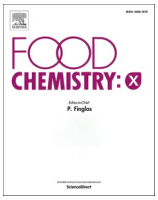

Commercial beers: A source of phthalates and di-ethylhexyl adipate

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A R T I C L E I N F O

Keywords:
Beer
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DLLME
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Chemical compounds studied in this article:
benzyl butyl phthalate (BBP) (CID 2347)
di-butyl phthalate (DBP) (CID 3026)
diethyl phthalate (DEP) (CID 6781)
di-ethylhexyl adipate (DEHA) (CID 7641)
di-ethylhexyl phthalate (DEHP) (CID 8343)
di-isobutyl phthalate (DIBP) (CID 6782)
dimethyl phthalate (DMP) (CID 8554)
dioctyl phthalate-d4 (DNOP-d4) (CID 16212237)

A B S T R A C T

Beer is one of the most consumed beverages worldwide. Different materials used along its production and packaging can result in human exposure to phthalates and adipates. The aim of this study was to assess simultaneously the levels of phthalates and di-ethylhexyl adipate (DEHA) in commercial beer samples (n = 66) with a method based on DLLME and detection with GC–MS/MS, and further evaluate human exposure. Six out of seven compounds studied were found in the beers analysed, with levels ranging from 1.77 to 205.40 μg/L. The most prevalent was DEHA at 205.40 μg/L, while dimethyl phthalate (DMP) was not present in any sample. Samples with 5–6 % alcohol, packed in aluminium cans and produced in an industrial environment presented the highest level of these contaminants. Despite low-risk exposure to phthalates and adipate with beer, it is important to remember the ubiquitous nature of these compounds, which can lead to cumulative exposure.

1. Introduction

Beer history dates as far back as 5000 BCE; there are several reports on the production of cereal-based fermented beverages all over the world in Ancient Egypt, Mesopotamia, and China (Cabras & Higgins, 2016; Grigg, 2004). Nowadays, there are about 80 different styles of beer, Pale Ale, Pilsner, Lager, or Stout, for example (Brewers of Europe, 2021). Beer is one of the most consumed alcoholic beverages in the world, with a growing tendency, mainly due to the already established beer markets in Western Europe and North America and converging consumption patterns in the rest of the world (Betancur, Motoki, Spence, & Velasco, 2020; Piron & Poelmans, 2016). In Europe, in 2019, 402 million hectolitres of beer were produced, and 369 million hectolitres were consumed (Brewers of Europe, 2021).

Packaging is the enclosure of products in a bag, box, cup, tray, tube, bottle, or other container to contain, protect and/or preserve said products. Beer is normally packed in glass bottles and aluminium cans with a plastic layer inside. The use of plastic in food packing has risen due to its low cost, versatility in size and shape production and thermoseal ability. Different types of plastic have been used in food packaging such as polyesters, polystyrene, polyamides, and polyvinyl chloride (PVC). PVC is widely used due to its high resistance to chemicals and stable electrical properties however it is heavy and stiff, requiring the addition of plasticizers to become more malleable (Marsh & Bugusu, 2007; Risch, 2009; Robertson, 2009; Shin & Selke, 2014). The most common plasticizers are phthalates (PEs) and adipates, both are classified as external plasticizers because they do not chemically bond with the plastic and can migrate to the packed product due to external factors such as temperature, pH alterations, radiation and contact with the content itself (Bocqué, Voirin, Lapinte, Caillol, & Robin, 2016).

The widespread use and consequent exposure of PEs are of great concern to human health. PEs exposure has been linked to several health issues, such as endocrine and reproductive disruption, infertility, altered foetal development, cardiotoxicity, hepatotoxicity, asthma, and allergies (Benjamin et al., 2017; Giuliani, Zuccarini, Cichelli, Khan, & Reale, 2020).

The migration of compounds from food packaging materials to food products has become one of the major sources of assumed food toxicity. There are several legislations in place to control the use of these compounds in order to protect consumers. In Regulation 10/11/EU, there is

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a list of certain PEs likely to contact with food and beverages, such as benzyl-butyl phthalate (BBP), di-butyl phthalate (DBP) and di-ethylhexyl phthalate (DEHP), that are considered toxic for reproduction, CMR (Carcinogenic, Mutagenic, Reprotoxic) category 1B in annex IV of Regulation EU No. 143/2011 EC, and states that these should be banned beginning of 1st January 2015 (Giuliani et al., 2020).

Literature on the evaluation of PE alcoholic beverages is scarce, though 9 recent studies that include some beer samples showed evidence of their presence (Aghaziarati, Yamini, & Shamsayei, 2020; Cariou et al., 2016; Carnol, Schummer, & Moris, 2017; Fierens et al., 2012; March & Cerdà, 2015; Rodríguez-Ramos, Socas-Rodríguez, Santana-Mayor, & Rodríguez-Delgado, 2020; Russo, Notardonato, Avino, & Cinelli, 2014; Vidal, Ibañez, & Escandar, 2016; Ye et al., 2009). In general, there is a lack of studies focused on the determination of PEs in beer samples with a sample pool large enough to draw assertive conclusions.

One of the main obstacles for a correct determination of residual levels of phthalates in food matrices is the high probability of cross contamination in the course of the different stages of the analytical process. This is due to the ubiquity of these analytes, which can be present in all types of plastic materials such as pipette tips, septa, inner linings of vial caps, etc. (González-Sálamo, Socas-Rodríguez, & Hernández-Borges, 2018; Haji Harunarashid, Lim, & Harunsani, 2017; Yang et al., 2015). In liquid samples, liquid–liquid extraction (LLE), with different lipophilic solvents such as *n*-hexane, cyclohexane and dichloromethane are commonly used (González-Sálamo et al., 2018; Jurica et al., 2016; Startin et al., 1987). Other very commonly technique is the Quick, easy, cheap, effective, rugged and safe (QuEChERS) that combines extraction and clean up in the same procedure (Cunha & Fernandes, 2020; Fasano, Cirillo, Esposito, & Lacorte, 2015; González-Sálamo et al., 2018; Rodríguez-Ramos et al., 2020). Nowadays there is a search for environmental friendly extraction techniques with less volume of organic solvents, such as dispersive liquid–liquid microextraction (DLLME) (Montevecchi, Masino, Zanasi, & Antonelli, 2017; Pérez-Outeiral, Millán, & Garcia-Arrona, 2016) or without any use of organic solvent such as solid-phase microextraction (SPME) (Moreira, André, & Cardeal, 2015; Ye et al., 2009). Usually the quantitative determination is achieved by GC(Cao, Zhao, & Dabeka, 2015; Cariou et al., 2016; Carrillo, Martínez, & Tena, 2008; David, Sandra, Tienpont, Vanwalleghem, & Ikonomou, 2003; Del Carlo et al., 2008; March & Cerdà, 2015; Russo et al., 2014; Sanchis, Yusà, & Coscollà, 2017; Wang et al., 2017; Yang et al., 2015), or liquid chromatography (LC) (González-Sálamo et al., 2018; Yang et al., 2015), both coupled to mass spectrometry (MS) detection.

The main objective of this work was to contribute for the assessment of six phthalates (DMP, DEP, DIBP, DBP, BBP, and DEHP) and one adipate (DEHA) in beers (n = 66) commercialized in Portugal from different origins (industrial or craft products), with different alcoholic contents and different types of packaging, through the application of an environmental-friendly analytical method based on dispersive liquid–liquid microextraction (DLLME) coupled to gas chromatography with tandem mass spectrometry (GC–MS/MS). Furthermore, the impact on consumers' health coming from the presence in beers of this kind of contaminants was evaluated.

2. Materials and methods

2.1. Standards and reagents

Analytical standards of dimethyl phthalate (DMP), diethyl phthalate (DEP), di-isobutyl phthalate (DIBP), di-butyl phthalate (DBP), benzyl butyl phthalate (BBP), di-ethylhexyl adipate (DEHA), di-ethylhexyl phthalate (DEHP), and internal standard dioctyl phthalate-d4 (DNOP-d4), all with standard purity of ≥99%, were obtained from Supelco/Sigma-Aldrich (St. Louis, MO, USA). The working solutions at 10 μgL^{-1} and 100 μgL^{-1} were prepared in ethanol (EtOH), HPLC grade, and kept refrigerated (~4 °C) until the analysis. Hexane was used as extraction solvent, and methanol (MeOH) HPLC grade, and purchased from Sigma-Aldrich (St. Louis, MO, USA).

MeOH, EtOH and hexane HPLC grade solvents were tested for the presence of phthalates and MeOH was found to have the least concentration. Therefore, it was selected as the washing solvent and blank solution. The same batch of solvent was used throughout the experiment.

Due to the ubiquitous nature of phthalates, all materials other than pipette tips were glassware. The glassware was carefully washed and previously rinsed with EtOH, and MeOH before use, also, calcinated when possible. The plastic pipette tips were left overnight in EtOH at 70 °C, rinsed with EtOH and dried before use. All vial caps had a layer of aluminium foil to avoid phthalate contamination.

2.2. Sampling

Beers (n = 66) of different brands (n = 50), composition, alcohol content (0–8,5 %) and packaging (aluminium can (C), glass bottle (B), pressurized (P)) were randomly purchased in several local supermarkets in Porto, Portugal (Supplemental Table 1). The samples were kept refrigerated, at 4 °C, until time of analysis.

2.3. Extraction procedure

A DLLME extraction based in a method developed by Caldeirão et al. (Caldeirão, Fernandes, da Silva Oliveira, Godoy, & Cunha, 2021) for herbal-based soft drinks was used. Briefly, a sample volume of 10 mL was first degasified by sonication for 15 min and added to a glass centrifuge tube. Then, samples were spiked with 50 μg/L of IS (DNOP-d4), 300 μL of *n*-hexane were added to the alcoholic samples, while to the non-alcoholic samples was added 200 μL of *n*-hexane (extractor solvent) and 100 μL of ethanol (dispersive solvent). The tube was capped with a layer of aluminium foil, vortexed for 30 s and centrifuged for 5 min at 1000 g. The resulting organic extract, around 200 μL, was transferred to an insert, placed inside an injection vial caped with aluminium foil and a volume of 1 μL was injected into the GC–MS/MS system.

2.4. GC–MS/MS conditions

An Agilent 7890B gas chromatograph equipped with an Agilent 7693A auto-sampler (Agilent, Little Falls, DE, USA), and electronically controlled split/splitless injection port, coupled with a 7000C triple quadrupole mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA) with electron ionization (EI) chamber, was used for PE and DEHA analysis.

GC separation was achieved on a Phenomenex ZB-35HT Inferno TM column (30 m × 0.25 mm × 0.25 μm film thickness (Phenomenex, USA). The oven temperature started at 90 °C, was held for 1 min, then increased to 300 °C at a rate of 20 °C min^{-1} and held for 5 min, The total run time was 16.5 min. Ultrahigh-purity helium (99.999%; Gasin, Portugal) was used as carrier gas at a rate of 1.0 mL min^{-1}. The injector was maintained at 300 °C in pulsed splitless mode (0.5 min purge-off, 35 psi), and 1.0 μL of the extract was injected. A Merlin Microseal TM septum (Agilent) was used to prevent silicone rubber contamination on analysis due to septum degradation through repeated injections. The triple-quadrupole MS was operated in multiple reaction monitoring (MRM) mode, detecting three transitions per analyte (Supplemental Table 2). The electron energy was 70 eV and the temperatures of the transfer line, ion source, and quadrupole were 300, 230, and 150 °C, respectively. Helium was used as quenching gas (2.25 mL min^{-1}) and nitrogen as collision gas (1.5 mL min^{-1}). System control and data acquisition were performed in MassHunter® software.

2.5. Risk assessment

The risk assessment of phthalates and adipate was performed by determination of the Hazard Quotient (HQ), with the comparison of the estimated probable daily intake (PDI) for each compound with the available reference value (TDI), if HQ < 1 then the exposure risk is considered to be within safe limits (EFSA, 2019; EFSA, 2012; Brewers of Europe, 2021; SCF, 2000).

$$HQ = \frac{PDI}{TDI}$$

The PDI (μg/kg/bw/day) value was obtained considered a daily consumption of 0.145 L/day of beer, which is equivalent to the 53 L/year consumption estimation in Portugal of an average adult (70 kg).

$$PDI = \frac{Ci \times Cm}{BWi}$$

Ci – estimated average dose of beer intake per day (L/day).

Cm – average concentration of phthalates-adipate in beer samples (μg(L).

BWi – body weight of an average adult (70 kg).

2.6. Statistical analysis

The analysis was carried out with SPSS for Windows 29.0 (SPSS Corporation, Chicago, IL). Kolmogorov-Smirnov test was used to verify parametric or nonparametric characteristic of data. To evaluate the difference between samples a nonparametric test (Kruskal-Wallis Test) was chosen due to sample size, variable number, and non normal distribution. Statistical significance was assumed if a null hypothesis could be rejected at $p < 0.05$.

3. Results and discussion

3.1. Method performance

To evaluate the matrix effect, the slopes of calibration curves obtained from solvent (EtOH) and from the matrix (standards added to blank beer samples) were compared.

When analysing food samples there are usually high matrix effects observed, that negatively affect the quantification of the target compounds. The percentage of matrix effects was calculated for each compound tested, by the ratio of the slopes of the calibration curves in the matrix (beer sample) and in solvent (EtOH) multiplied by 100, in order to obtain the percentage of suppression or enhancement (Eq. (1)) (Caldeirão et al., 2021).

$$Matrix\ effect\ (\%) = \frac{m(CC\ matrix)}{m(CC\ EtOH)}\ x\ 100 \quad (1)$$

m(CC matrix) - slope of matrix-matched calibration curve.m(CC EtOH) - slope of solvent calibration curve.

All compounds show matrix suppression effects, resulting in an underestimation of the amount of analyte (Supplemental Fig. 1 and Supplemental Table 3), most with values ranging from 44% and 70%, except for DEHA with a value of 6% and DEHP of 10%. The results were similar to those reported by Fierens et al., 2012, which analysed the presence of phthalates in several food groups with a method based on LLE followed by GC-EI-MS and verified that beer samples were especially affected by matrix interferences (Fierens et al., 2012).

Due to the matrix effect, the linearity was determined using matrix-matched calibrators. These calibrators comprised ten concentration levels ranging from 1 μg/L to 200 μg/L and were subjected to the entire extraction process. The calibration curves were constructed by plotting the compound/IS ratio against the concentrations of the analytes. The results demonstrated good linearity within the tested concentrations, with determination coefficients (r^2) above 0.96 for all analytes (Table 1).

Intra-day and inter-day precision were determined at three different concentration levels (5 μg/L, 35 μg/L, and 85 μg/L) six replicates per level per day, in two different days for a period of two weeks. Intra-day %RSD (relative standard deviations) at the three concentration levels for all the target analytes were on average of 6%. The inter-day %RSD, at concentration of 35 μg/L, was on average of <8% for all analytes. Our intra-day RSD% results were better that those reported by Carnol et al. (2017) that determined average inter/intra-day %RSD 15.49/13.59 (HS-SPME, beer samples), and our inter-day values were better than those reported by Russo et al. (2014) which achieved inter and intra-day precision of %RSD 4.5/9.5 (SPE, beer samples). Still the authors used different extraction methodologies that may justify these differences.

The detection limits of the method were determined by successive analyses of sample extracts with decreasing amounts of the compounds until a 3:1 signal-to-noise ratio was reached (Table 1). The quantification limits were established as the lowest concentration assayed quantified with acceptable precision (<20%), which were the lowest calibration level of the calibration curve (Table 2). DIBP, DBP and BBP presented lower LOD and LOQ (0.3 μg/L and 1 μg/L, respectively) than DEHA and DEHP (0.6 μg/L and 2.0 μg/L), and DMP and DEP (1.5 μg/L and 5.0 μg/L). These results are somewhat similar to those achieved by Pérez-Outeiral et al. (2016) using an ultrasound-assisted DLLME/GC-FID for the analysis of PE in different liquid samples (water, wine, vinegar and sangria), with LOD and LOQ of 0.7–2.82 μg/L and 1.93 – 8.47 μg/L, respectively. However, Wang et al. (2017) obtained lower LOD and LOQ (0.003–0.570 μg/L and 0.01–1.86 μg/L, respectively) with a DLLME/GC–MS method used for analysis of PE in several beverages, such as mineral water, carbonated beverages, teas and liquors.

Table 2
Risk assessment evaluation.

	PDI	HQ	
DMP	0	–	
DEP	0.000842	–	
DIBP	0.002082	.	
DBP	0.001115	0.00002	**<1**
DEHA	0.013767	0.00005	**<1**
BBP	0.001245	0.00002	**<1**
DEHP	0.003085	0.00006	**<1**

Table 1
Method performance.

Phthalate	Linearity		LOD (μg/L)	LOQ (μg/L)	Intra-day precision % RSD			Inter-day precision % RSD
	CC slope	r^2			5 (μg/L)	35 (μg/L)	85 (μg/L)	35 μg/L
DMP	0.0008	0.983	1.5	5	10.43	17.41	20.4	11.48
DEP	0.0019	0.991	1.5	5	11.69	12.28	1.94	13.34
DIBP	0.0013	0.986	0.3	1	3.10	4.81	1.36	7.23
DBP	0.0014	0,982	0.3	1	2.37	5.61	1.77	2.54
DEHA	0.0001	0.964	0.6	2	1.72	2.79	2.49	3.28
BBP	0.0004	0.975	0.3	1	3.95	4.97	4.30	13.36
DEHP	0.0002	0,976	0.6	2	1.98	3.55	7.19	6.98

3.2. Occurrence of phthalates and di-ethylhexyl adipate in beer samples

The validated method was applied to extract and quantify six phthalates and di-ethylhexyl adipate in 66 beer samples. Thirty-two out of 66 samples presented levels above the LOQ for at least one compound. The most frequent analyte was DEHA, present in 24 samples, followed by DEHP in 11 samples. DMP was not found in any samples, possibly due to its more common use as a personal care product rather than an additive in plasticizers (Giuliani et al., 2020; Wang, Zhu, & Kannan, 2019). Among the positive samples (n = 20), the majority were contaminated with only one analyte. Seven samples showed the presence of two contaminants with the following combinations: DEHA/DEHP (n = 2), DBP/DEHP (n = 2), DEHA/DIBP (n = 1) and DEHA/BBP (n = 2). In four samples the co-occurrence of 3 analytes was found: DBP/DEHP/DEHA (n = 2), DIBP/DBP/DEHA (n = 1), DIBP/BBP/DEHA (n = 1). Lastly, one sample (S29) had the highest number of detected compounds (n = 5): DEP, DIBP, DBP, DEHA, and BBP (Fig. 1). Similar results were reported by Russo et al. (2014), with one sample of beer also contaminated with five plasticizers. In the literature, only nine works have reported the presence of phthalates in beer samples (Aghaziarati et al., 2020; Cariou et al., 2016; Carnol et al., 2017; Fierens et al., 2012; March & Cerdà, 2015; Rodríguez-Ramos et al., 2020; Russo et al., 2014; Vidal et al., 2016; Ye et al., 2009). The most frequent PEs reported are DBP, DEP, DEHP, DNOP, DIBP and BBP, which is in accordance with the result here obtained. However, the most detected analyte in this study was the adipate, DEHA, which is a common substitute of DEHP in the industry (Behairy, Abd El-Rahman, Aly, Fahmy, & Abd-Elhakim, 2021). To the best of our knowledge, no available papers have provided screening or evidence of this particular plasticizer in beers.

Among the samples analysed, the sample S44 presented the highest concentration of DEHA, 205.40 μg/L, which is unusual for a beer sample (Fig. 1). According with the literature, these high levels are only found in beverages with higher alcohol content such as wines and spirits beverages (Del Carlo et al., 2008; Fan, Liu, & Xie, 2014; Jurica et al., 2016).

In screening studies of several foods including beers and wines such as Fierens et al. (2012), and Cao et al. (2015), respectively, the alcoholic samples have a lower concentration of phthalates, when compared to other foods as fish and fish products, condiments or oils and fats, probably due the low amount of fat in beers and the lipophilic nature of the PE (Serrano, Braun, Trasande, Dills, & Sathyanarayana, 2014). Also, when a comparison is made between different alcoholic beverages, usually the beverages with a higher alcohol content have a higher concentration of PEs, as is demonstrated in the study of Fan et al. (2014), with the spirits having a much higher alcohol content and a higher

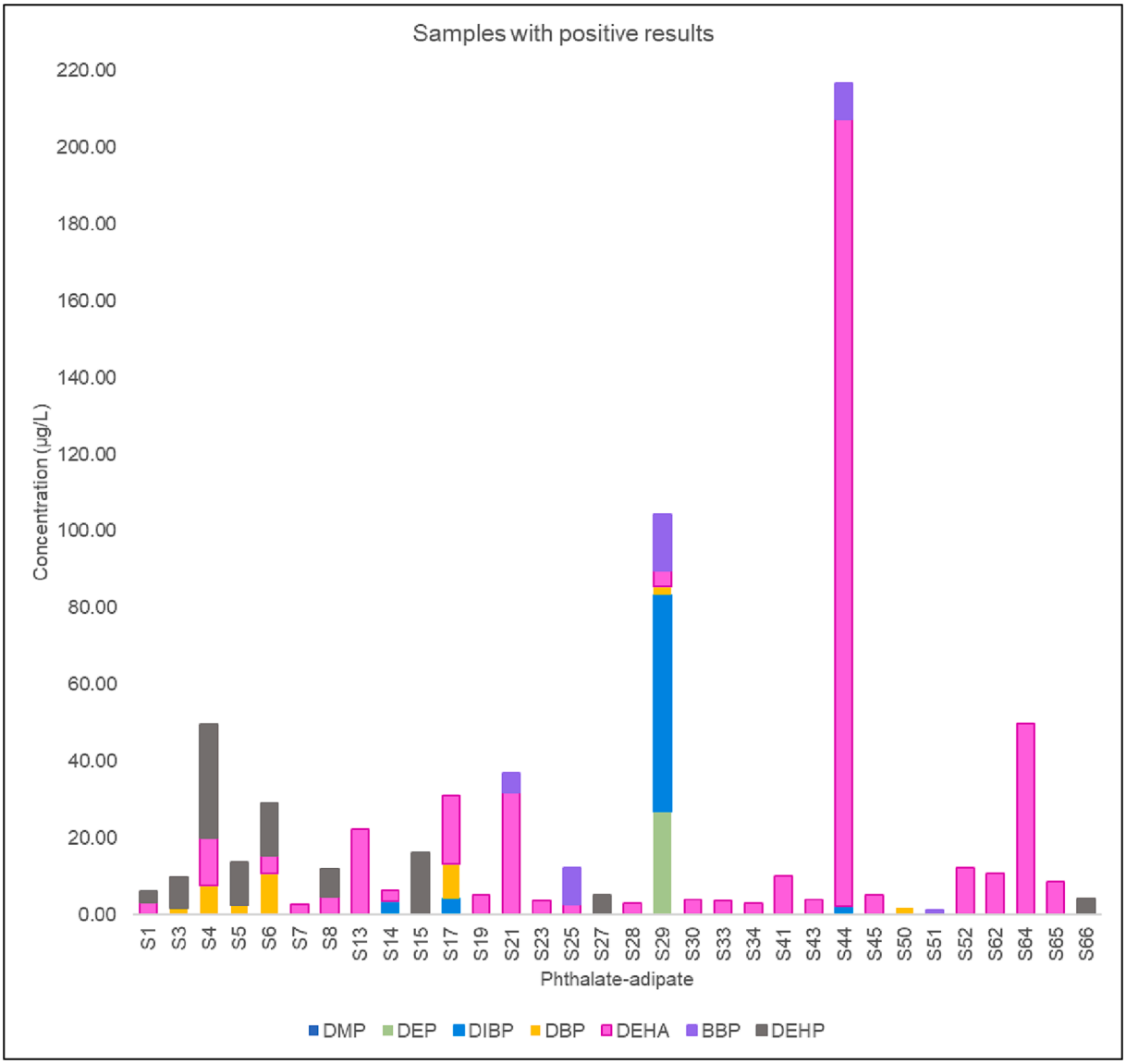

Fig. 1. Samples with detected phthalates concentrations above LOQ.

detection frequency of PEs than red wines. Also, Vidal et al. (2016), showed that beers have the lowest levels of PEs when compared with more alcoholic beverages such as cachaça, red and white wines. Aghaziarati et al. (2020) found that whisky samples have a higher level of PE than the beer samples. The alcohol content is a major factor in the migration of phthalates from package materials, tubbing and other equipment in the production process, probability due to their high solubility in ethanol (Grinbaum et al., 2019; Jurica et al., 2016).

In EU, DEHP and DEHA have a SML (specific migration limit) in food products of 1.5 mg/kg and 18 mg/kg respectively; here the samples studied were found to be well below these limits. Other PEs are not as strictly regulated, with a limit of 60 mg/kg (EFSA, 2019). Therefore, the levels of DEP, DIBP, and BBP that were detected in the samples were also significantly below the recommended limit.

3.3. Alcoholic vs. non-alcoholic samples

Among the 59 samples of alcoholic beer analysed 31 were contaminated, while from 7 non-alcoholic beers, only one contained phthalates. The average level of phthalates compared to adipate in alcoholic samples was 10.73 μg/L (Fig. 2a), which is higher than the phthalate levels reported by Ye et al. (2009) with DEHP – 5.04 μg/L and DBP – 2.66 μg/L or by Fierens et al. (2012) with DMP/DEP – 0.1 μg/L.

In the non-alcoholic positive sample, the PEs level was 3.74 μg/L (DEHA) (Fig. 2a). We could not find any studies on non-alcoholic beer. However assuming that both alcoholic and non-alcoholic beers are exposed to a similar concentration of PEs from plastic equipment and tubbing during production and packaging during storage, it is possible that the environment could be contamination source. Higher and more frequent levels of PE in alcoholic samples suggest that the presence of alcohol in beer samples may be an influencing factor in PEs migration during production and from the packages to the final product.

To assess whether different alcohol concentrations could result in varying levels of phthalate esters (PEs) in the alcoholic samples, the samples were categorized into four groups: (1) samples with a percentage of alcohol between 4 and 5 (n = 14); (2) samples with a percentage of alcohol between 5 and 6 (n = 29); (3) samples with a percentage of alcohol between 6 and 7 (n = 8); and (4) samples with a percentage of alcohol between 7 and 8.5 (n = 7), as seen in Fig. 2b. The results demonstrate that there is a higher average and diversity of PEs in the samples with a lower alcohol percentage (5–6 % alcohol), contrary to what could be expected. This suggests that other factors, as mentioned earlier, such as the manufacturing environment of the samples or the type of container material, may have a greater influence on the contamination of these types of samples. No statistically significant differences were observed between the samples in the different alcohol categories.

3.4. Types of packaging

Two main different types of packaging were studied: aluminium cans (n = 37) and glass bottles (n = 28). Additionally, one pressurized beer was analysed. Beer samples in aluminum cans exhibited higher average levels of total phthalates-adipate (13.48 μg/L) with five different analytes detected. In contrast, beer samples in glass bottles had lower average total levels (7.19 μg/L) but a higher number of different analytes detected (n = 6). The analytes present in both aluminum cans and glass bottles were DIBP, DBP, DEHA, BBP, and DEHP. Only one sample of pressurized beer was analyzed due to the similarity of the obtained results with the other packaging types. It was found to be contaminated with DEHP, and the low concentration may indicate that the contamination originated from the tubing used in the beer dispenser equipment rather than the beer storage recipient.

Among the phthalates analysed, DEHP is the only analyte present in all three types of packaging, aluminium cans, glass bottles and pressurized beer, which is reasonably explained by the widespread use of this specific phthalate in the plastic industry (Fig. 2c) (Frederiksen, Skakkebaek, & Andersson, 2007).

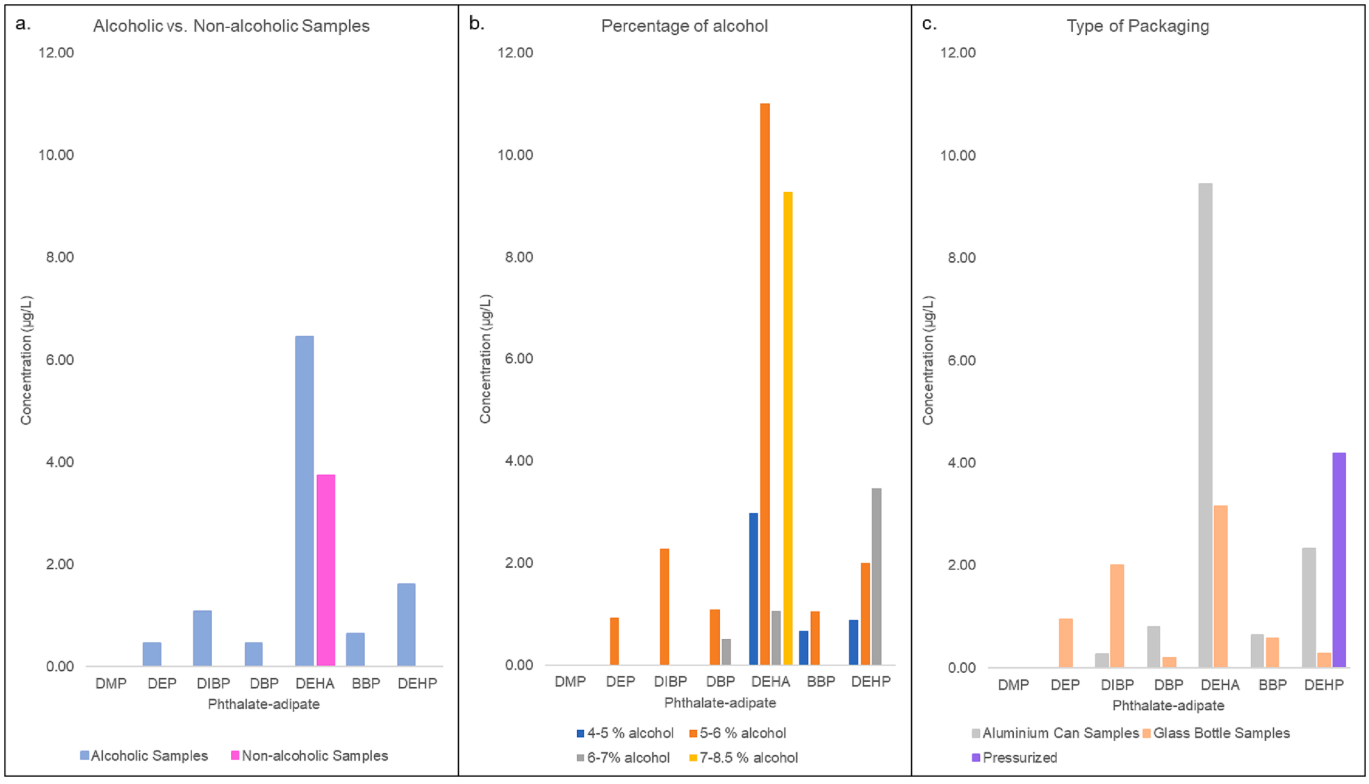

Fig. 2. a. Detection of phthalates-adipate in alcoholic and non-alcoholic commercial beer samples; **b.** Detection of phthalate-adipate in alcoholic samples with different alcohol percentage; **c.** Detection of phthalates-adipate in different types of packaging from commercial beer samples.

The results obtained with the packing are similar to those obtained by Carnol et al. (2017), which evaluated different PEs and one adipate in 15 samples of Luxembourgish beer (cans n = 3, glass bottles n = 10, and aluminium bottles n = 2) and not found statistical difference between packages. In the study of Rodríguez-Ramos et al. (2020) PEs were only detected in the beer samples in plastic and glass containers while aluminium containers did not present any PEs.

3.5. Origin of the samples

With a growth of the market and demand of craft beers, these types of samples were also included in our selection. Craft beer was considered any sample labelled as "craft", 53 beer samples were of industrial origin and 13 craft beers.

Four samples of craft origin were positive for DEHA and one sample was also positive for DEHP, with an average phthalate-adipate level of 5.8 μg/L. In 28 samples of industrial origin DEP, DIBP, DBP, DEHA, BBP and DEHP were detected (average level of 11.87 μg/L), as seen in Fig. 3a. The samples from industrial origin had a higher concentration of plasticizers contamination than the craft beer samples, probably due to the equipment used during processing. In an industrial environment, modern and sturdy equipment is necessary to maintain the necessary production quota of high volume for a long period of time, consequently plastic with its versatility, durability and low cost of production is a very common material. On the other hand, in craft beer production the materials used may have a lower plastic component. There are no studies on the presence of PEs in craft beers, therefore a comparison with other studies is not possible. No statistically significant differences were observed between the samples in the different production environment.

In other type of samples, such as wines, Del Carlo et al. (2008) considered the origin of the samples as a possible determinant factor in contamination. The authors found that some PEs were detected in all type of samples (commercial wines, private wines and experimental pilot plant samples) while other PEs, such as DBP and BBP, had a higher frequency of detection in commercial samples compared to the pilot plant samples. The differences were attributed to the production process since that only stainless-steel tanks and tubbing were used in the pilot plant.

3.6. Difference between commercial brands and off-brands

All samples from internationally and nationally recognized mass-produced brands were considered as commercial brands. On the other hand, off-brand beer samples were those produced on a smaller scale and associated with lesser-known brands.

In commercial brand samples, a higher diversity of plasticizers was detected – DEP, DIBP, DBP, DEHA, BBP and DEHP, in a wide variety of types of packaging – aluminium can, glass bottle and pressurized beer. As it is possible to see in Fig. 3b and 3c, there is a higher presence of analytes in glass bottles compared to off-brand samples, where the plasticizer content is higher in aluminum cans. On the other hand, in off-brand samples, where the production is smaller, the contamination seems to be mainly caused by the packing, as the lower diversity of analytes detected (DIBP, DBP, DEHA and DEHP) are concentrated mainly in samples packaged in aluminium cans. Alcohol content is also a factor, since the non-alcoholic samples were all negative in these off-brand samples. No statistically significant differences were observed between the samples in the different brand types.

Samples of the same brand supposedly have similar production conditions and quality control standards, regardless of different alcohol content or packages. However, our results demonstrated that in most brands, the samples in aluminium can had higher levels of phthalates (Fig. 4). This may be explained by the production environment, where the contamination can be environmental or due to the processing equipment, also, commercial brand samples are produced at a much larger scale than off-brand samples, which means a more industrialized environment prone to several sources of phthalate-adipate contamination, such as air, tubbing and/or storage recipients; also, the

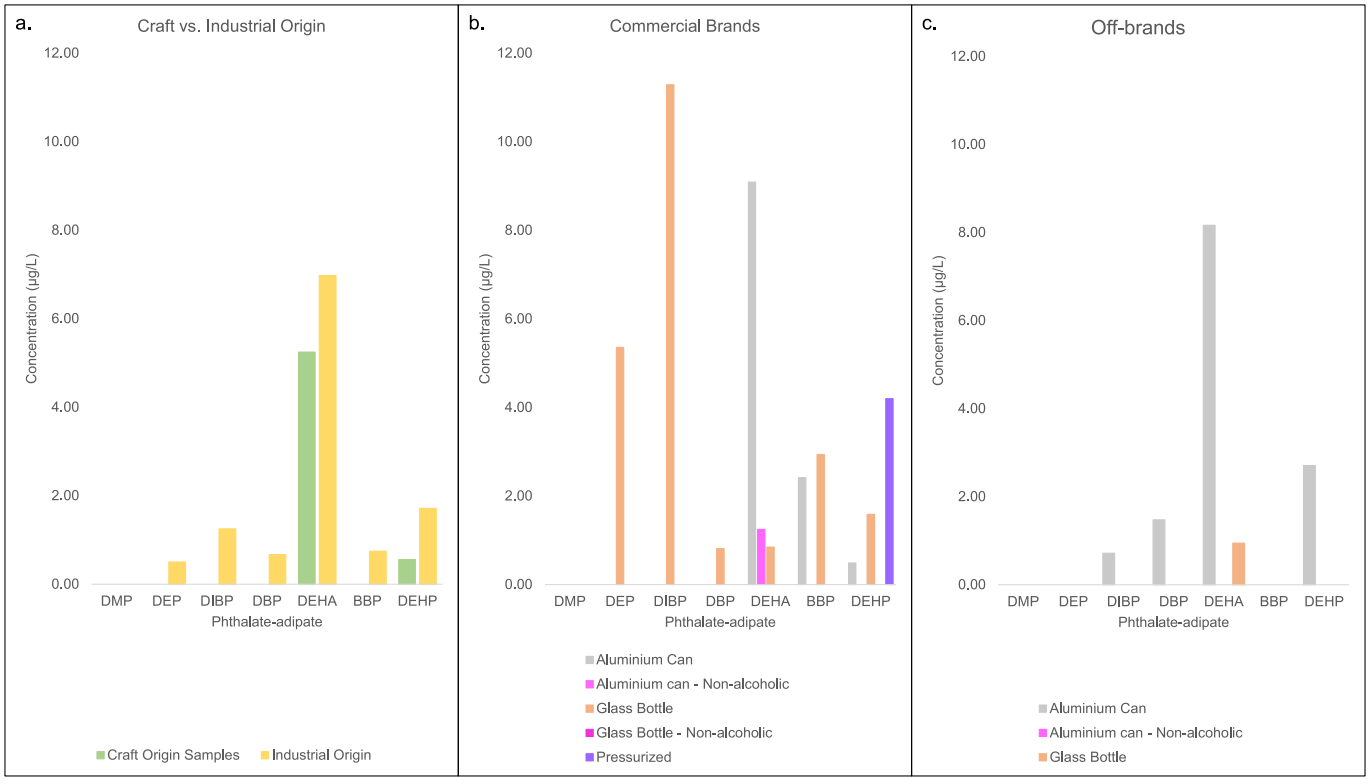

Fig. 3. a. Detection of phthalates-adipate in commercial beer samples of craft and industrial origin; **b.** Detection of phthalates-adipate in commercial brands beer samples; **c.** Detection of phthalates-adipate in off-brand beer samples.

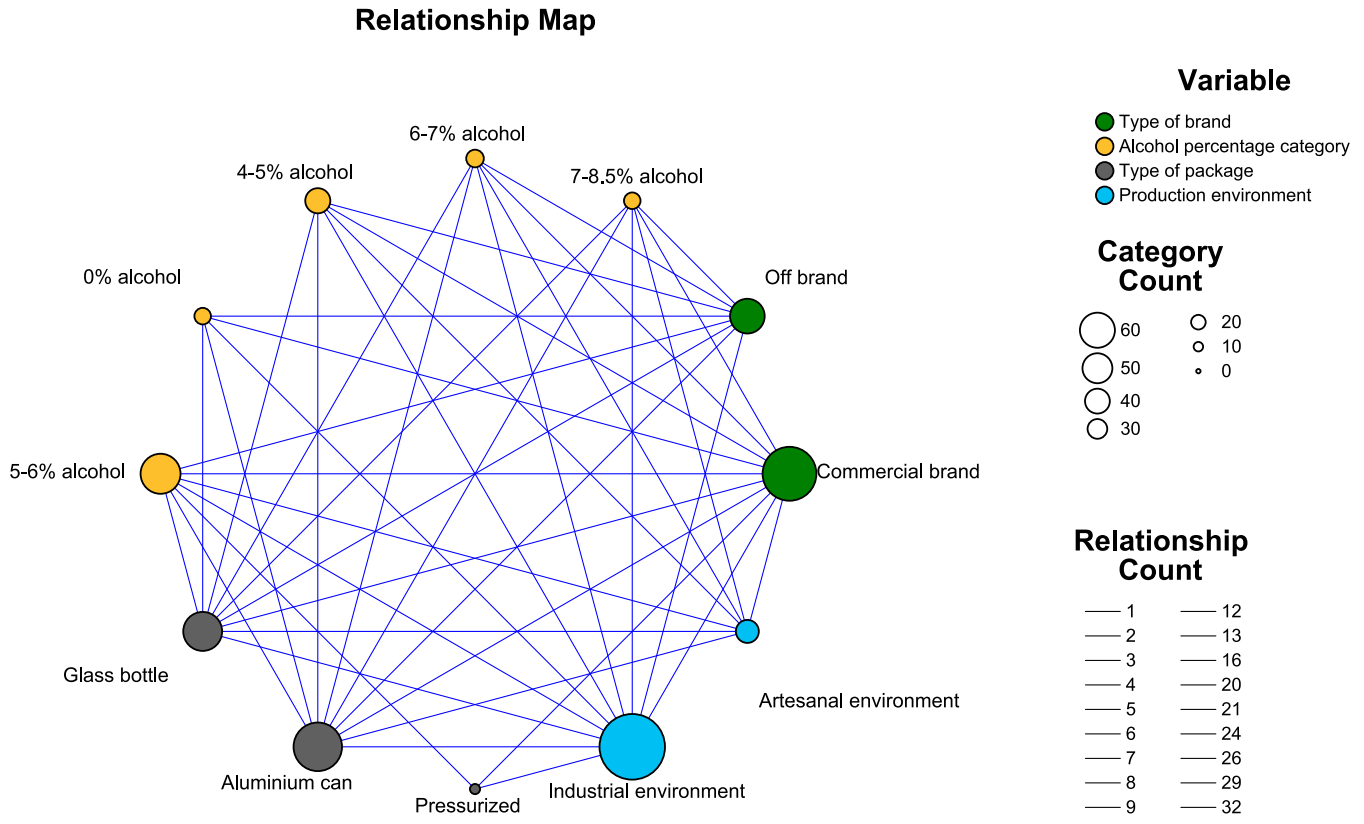

Fig. 4. Interrelation between sample characteristics and phthalate contamination.

contamination may be sourced to the bottles in their production, transportation, and handling, before contact with the sample. It is of notice that, the only non-alcoholic beer sample with a positive result for the presence of phthalate-adipate is from a commercial brand, once again pointing to an environmental contamination, since there is no alcohol content to aid in the compounds migration to the sample. Notwithstanding, alcohol content is a major factor in phthalate contamination (Grinbaum et al., 2019), as all positive sample but one, are alcoholic. Still, it must be taken into consideration that these samples were from different batches with different production and expiration dates, which may also explain the different results.

For a more thorough analysis of the possible sources of contamination, it would be necessary to collect several samples from all the different stages of beer production, both on an industrial scale (larger and smaller) and at a craft/artesanal level. In addition, it would be important to know the composition of all plastic materials used in the production process, both of the equipment – tubing, tanks, and other pieces, and any protective gear used by the workers that may contact with the product. The packages should also be analysed because different brands may use packages produced by different companies that may have a different percentage of plasticizers integrated into their plastic recipients or coverings. All these factors may have a different contribution to phthalate-adipate contamination in the same type of food products, resulting in different contamination levels, and therefore should be evaluated.

3.7. Risk assessment

The risk assessment data reveals that beer is not a major exposure risk to phthalates and di-ethylhexyl adipate since HQ < 1. However, these contaminants are ubiquitous and have various exposure sources, thus humans can be exposed at higher levels.

4. Conclusions

The present work reports on the assessment of the presence of different plasticizers (six phthalates plus DEHA) in commercial beers. For determination a DLLME-GC–MS/MS based method was optimized and validated. The analytical method showed good linearity and precision, with low LOD and LOQ. The analysis of sixty-six samples of commercial beer samples with different types of packages (aluminium can, glass bottle and pressurized beer), different alcohol contents (alcoholic versus non-alcoholic) and different manufacture origins (craft and industrial origin), showed that DEHA was the most detected analyte, followed by DEHP, while DMP was not found in any sample. In all samples plasticizers levels found were below the recommended SML set by the EU. The presence of these compounds was observed to be dependent on the alcohol content, with higher average levels of plasticizers detected in samples packaged in aluminum cans compared to those in glass bottles or pressurized beer. Furthermore, samples from industrial production exhibited significantly higher total average levels and a wider range of contaminants compared to craft samples. This work demonstrated the presence of several different phthalates in beers albeit their health effects may not be severe due to the low levels found. The ubiquitous nature of these compounds means that we are susceptible to exposure from various sources leading to cumulative exposure.

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CRediT authorship contribution statement

Cheila Pereira: Methodology, Writing – original draft. **Sara C. Cunha:** Conceptualization; Supervision; Writing – review & editing. **José O. Fernandes:** Conceptualization; Supervision; Writing – review & editing..

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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References

Aghaziarati, M., Yamini, Y., & Shamsayei, M. (2020). An electrodeposited terephthalic acid-layered double hydroxide (Cu-Cr) nanosheet coating for in-tube solid-phase microextraction of phthalate esters. *Microchimica Acta, 187*(2), 118–127. https://doi.org/10.1007/s00604-019-4102-5

Behairy, A., Abd El-Rahman, G. I., Aly, S. S. H., Fahmy, E. M., & Abd-Elhakim, Y. M. (2021). Di(2-ethylhexyl) adipate plasticizer triggers hepatic, brain, and cardiac injury in rats: Mitigating effect of Peganum harmala oil. *Ecotoxicology and Environmental Safety, 208*, 1–13. https://doi.org/10.1016/j.ecoenv.2020.111620

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R. C., & Faisal, P. A. (2017). Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. *Journal of Hazardous Materials, 340*, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036

Betancur, M. I., Motoki, K., Spence, C., & Velasco, C. (2020). Factors influencing the choice of beer: A review. *Food Research International, 137*(109367), 1–19. https://doi.org/10.1016/j.foodres.2020.109367

Bocqué, M., Voirin, C., Lapinte, V., Caillol, S., & Robin, J.-J. (2016). Petro-based and bio-based plasticizers: Chemical structures to plasticizing properties. *Journal of Polymer Science Part A: Polymer Chemistry, 54*(1), 11–33. https://doi.org/10.1002/pola.27917

Brewers of Europe. (2021). Retrieved 25/08/2021 from https://brewersofeurope.org.

Cabras, I., & Higgins, D. M. (2016). Beer, brewing, and business history. *Business History, 58*(5), 609–624. https://doi.org/10.1080/00076791.2015.1122713

Caldeirão, L., Fernandes, J. O., da Silva Oliveira, W., Godoy, H. T., & Cunha, S. C. (2021). Phthalic acid esters and adipates in herbal-based soft drinks: An eco-friendly method. *Analytical and Bioanalytical Chemistry, 413*(11), 2903–2912. https://doi.org/10.1007/s00216-021-03219-5

Cao, X., Zhao, W., & Dabeka, R. (2015). Di-(2-ethylhexyl) adipate and 20 phthalates in composite food samples from the 2013 Canadian Total Diet Study. *Food Additives & Contaminants: Part A, 32*(11), 1893–1901. https://doi.org/10.1080/19440049.2015.1079742

Cariou, R., Larvor, F., Monteau, F., Marchand, P., Bichon, E., Dervilly-Pinel, G., … Le Bizec, B. (2016). Measurement of phthalates diesters in food using gas chromatography–tandem mass spectrometry. *Food Chemistry, 196*, 211–219. https://doi.org/10.1016/j.foodchem.2015.09.045

Carnol, L., Schummer, C., & Moris, G. (2017). Quantification of six phthalates and one adipate in Luxembourgish beer using HS-SPME-GC/MS. *Food Analytical Methods, 10*(2), 298–309.

Carrillo, J. D., Martínez, M. P., & Tena, M. T. (2008). Determination of phthalates in wine by headspace solid-phase microextraction followed by gas chromatography–mass spectrometry: Use of deuterated phthalates as internal standards. *Journal of Chromatography A, 1181*(1), 125–130. https://doi.org/10.1016/j.chroma.2007.12.053

Cunha, S. C., & Fernandes, J. O. (2020). Chapter 21 - Application in Food Analysis. In C. F. Poole (Ed.), *Liquid-Phase Extraction* (pp. 643–665). Elsevier. https://doi.org/10.1016/B978-0-12-816911-7.00021-9.

David, F., Sandra, P., Tienpont, B., Vanwalleghem, F., & Ikonomou, M. (2003). Analytical Methods Review. In C. A. Staples (Ed.), *Series Anthropogenic Compounds: Phtalate Esters* (pp. 9–56). Berlin Heidelberg: Springer. https://doi.org/10.1007/b11461.

Del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D., Mastrocola, D., & Cichelli, A. (2008). Determination of phthalate esters in wine using solid-phase extraction and gas chromatography–mass spectrometry. *Food Chemistry, 111*(3), 771–777. https://doi.org/10.1016/j.foodchem.2008.04.065

EFSA, Panel on Food Contact Materials, E., Aids, P., Silano, V., Barat Baviera, J. M., Bolognesi, C., Chesson, A., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., . . . Castle, L. (2019). 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 Journal, 17*(12), e05838. doi: 10.2903/j.efsa.2019.5838.

EFSA, C. S. (2012). Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. *EFSA Journal, 10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

Fan, Y., Liu, S., & Xie, Q. (2014). Rapid determination of phthalate esters in alcoholic beverages by conventional ionic liquid dispersive liquid–liquid microextraction coupled with high performance liquid chromatography. *Talanta, 119*, 291–298. https://doi.org/10.1016/j.talanta.2013.11.023

Fasano, E., Cirillo, T., Esposito, F., & Lacorte, S. (2015). Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry. *LWT - Food Science and Technology, 64*(2), 1015–1021. https://doi.org/10.1016/j.lwt.2015.06.066

Fierens, T., Servaes, K., Van Holderbeke, M., Geerts, L., De Henauw, S., Sioen, I., & Vanermen, G. (2012). Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology, 50*(7), 2575–2583. https://doi.org/10.1016/j.fct.2012.04.029

Frederiksen, H., Skakkebaek, N. E., & Andersson, A.-M. (2007). Metabolism of phthalates in humans. *Molecular Nutrition & Food Research, 51*(7), 899–911. https://doi.org/10.1002/mnfr.200600243

Giuliani, A., Zuccarini, M., Cichelli, A., Khan, H., & Reale, M. (2020). Critical review on the presence of phthalates in food and evidence of their biological impact. *International Journal of Environmental Research and Public Health, 17*(16), 5655. https://www.mdpi.com/1660-4601/17/16/5655.

González-Sálamo, J., Socas-Rodríguez, B., & Hernández-Borges, J. (2018). Analytical methods for the determination of phthalates in food. *Current Opinion in Food Science, 22*, 122–136. https://doi.org/10.1016/j.cofs.2018.03.002

Grigg, D. (2004). Wine, spirits and beer: World patterns of consumption. *Geography, 89*(2), 99–110. http://www.jstor.org/stable/40573955.

Grinbaum, M., Camponovo, A., Desseigne, J.-M., Poupault, P., Meisterman, E., Chatelet, B., … Lempereur, V. (2019). Phthalates: Potential sources and control measures. *BIO Web of Conferences, 12*(04008), 1–7. https://doi.org/10.1051/bioconf/20191204008

Haji Harunarashid, N. Z. I., Lim, L. H., & Harunsani, M. H. (2017). Phthalate sample preparation methods and analysis in food and food packaging: A review. *Food Analytical Methods, 10*(12), 3790–3814. https://doi.org/10.1007/s12161-017-0938-7

Jurica, K., Karačonji, I. B., Lasić, D., Lušić, D. V., Jurica, S. A., & Lušić, D. (2016). Determination of phthalates in plum spirit and their occurrence during plum spirit production. *Acta Alimentaria, 45*(1), 141–148. https://doi.org/10.1556/066.2016.45.1.17

March, J. G., & Cerdà, V. (2015). An innovative arrangement for in-vial membrane-assisted liquid-liquid microextraction: Application to the determination of esters of phthalic acid in alcoholic beverages by gas chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry, 407*(14), 4213–4217. https://doi.org/10.1007/s00216-015-8646-x

Marsh, K., & Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of Food Science, 72*(3), R39–R55.

Montevecchi, G., Masino, F., Zanasi, L., & Antonelli, A. (2017). Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry. *Food Chemistry, 221*, 1354–1360. https://doi.org/10.1016/j.foodchem.2016.11.019

Moreira, M. A., André, L. C., & Cardeal, Z.d. L. (2015). Analysis of plasticiser migration to meat roasted in plastic bags by SPME–GC/MS. *Food Chemistry, 178*, 195–200. https://doi.org/10.1016/j.foodchem.2015.01.078

Pérez-Outeiral, J., Millán, E., & Garcia-Arrona, R. (2016). 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, 62*, 171–177. https://doi.org/10.1016/j.foodcont.2015.10.016

Piron, E., & Poelmans, E. (2016). Beer, the Preferred Alcoholic Drink of All? Changes in the Global and National Beer Consumption Since 1960 and Convergence and Trends Since the 1990s. In I. Cabras, D. Higgins, & D. Preece (Eds.), *Brewing, Beer and Pubs: A Global Perspective* (pp. 205–227). Palgrave Macmillan UK. https://doi.org/10.1057/9781137466181_11.

Risch, S. J. (2009). Food packaging history and innovations. *Journal of Agricultural and Food Chemistry, 57*(18), 8089–8092. https://doi.org/10.1021/jf900040r

Robertson, G. L. (2009). *Food packaging and shelf life: A practical guide*. CRC Press.

Rodríguez-Ramos, R., Socas-Rodríguez, B., Santana-Mayor, Á., & Rodríguez-Delgado, M.Á. (2020). A simple, fast and easy methodology for the monitoring of plastic migrants in alcoholic and non-alcoholic beverages using the QuEChERS

method prior to gas chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 412*(7), 1551–1561. https://doi.org/10.1007/s00216-019-02382-0

Russo, M. V., Notardonato, I., Avino, P., & Cinelli, G. (2014). Determination of phthalate esters at trace levels in light alcoholic drinks and soft drinks by XAD-2 adsorbent and gas chromatography coupled with ion trap-mass spectrometry detection [10.1039/C4AY00926F]. *Analytical Methods, 6*(17), 7030–7037. https://doi.org/10.1039/C4AY00926F

Sanchis, Y., Yusà, V., & Coscollà, C. (2017). Analytical strategies for organic food packaging contaminants. *Journal of Chromatography A, 1490*, 22–46. https://doi.org/10.1016/j.chroma.2017.01.076

SCF. (2000). Opinion of the Scientific Committee on Food on a survey on dietary intake of the food contact material di(2-ethylhexyl)adipate (DEHA). Expressed on 19th October 2000.

Serrano, S. E., Braun, J., Trasande, L., Dills, R., & Sathyanarayana, S. (2014). Phthalates and diet: A review of the food monitoring and epidemiology data. *Environmental Health, 13*(1), 13–43. https://doi.org/10.1186/1476-069X-13-43

Shin, J., & Selke, S. E. (2014). 11-food packaging. In *Food Processing: Principles and Applications* ((Second Edition), pp. 249–273). John Wiley & Sons.

Startin, J. R., Sharman, M., Rose, M. D., Parker, I., Mercer, A. J., Castle, L., & Gilbert, J. (1987). Migration from plasticized films into foods. 1. Migration of di-(2-ethylhexyl) adipate from PVC films during home-use and microwave cooking. *Food Additives & Contaminants, 4*(4), 385–398. https://doi.org/10.1080/02652038709373647

Vidal, R. B. P., Ibañez, G. A., & Escandar, G. M. (2016). A green method for the quantification of plastics-derived endocrine disruptors in beverages by chemometrics-assisted liquid chromatography with simultaneous diode array and fluorescent detection. *Talanta, 159*, 336–343. https://doi.org/10.1016/j.talanta.2016.06.049

Wang, F., Liu, Y., Tang, Z., Hou, M., Wang, C., Wang, X., … Xiao, Q. (2017). Simultaneous determination of 15 phthalate esters in commercial beverages using dispersive liquid–liquid microextraction coupled to gas chromatography-mass spectrometry [10.1039/C7AY00123A]. *Analytical Methods, 9*(12), 1912–1919. https://doi.org/10.1039/C7AY00123A

Wang, Y., Zhu, H., & Kannan, K. (2019). A review of biomonitoring of phthalate exposures. *Toxics, 7*(2), 1–28. https://www.mdpi.com/2305-6304/7/2/21.

Yang, J., Li, Y., Wang, Y., Ruan, J., Zhang, J., & Sun, C. (2015). Recent advances in analysis of phthalate esters in foods. *Trends in Analytical Chemistry, 72*, 10–26. https://doi.org/10.1016/j.trac.2015.03.018

Ye, C.-W., Gao, J., Yang, C., Liu, X.-J., Li, X.-J., & Pan, S.-Y. (2009). Development and application of an SPME/GC method for the determination of trace phthalates in beer using a calix[6]arene fiber. *Analytica Chimica Acta, 641*(1), 64–74. https://doi.org/10.1016/j.aca.2009.02.052