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*Communication*

# Survey on Phthalates in Beer Packaged in Aluminum Cans, PET and Glass Bottles

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**Abstract:** Phthalates are known as endocrine disruptors and are common in plastic polymers, varnishes, and printing inks. However, they most often enter the human body through food. Plastic materials that hold food contain different chemicals, and phthalates are one of them. Phthalates can also be found in microplastics since microplastic particles serve as a vector for different chemicals that can be slowly released into food and beverages. The aim of this preliminary study was to determine the concentration and types of phthalates (dimethyl phthalate, diethyl phthalate, diisobutyl phthalate, dibutyl phthalate, bis (2-ethylhexyl) phthalate, di-n-octyl-phthalate) in beer packaged in aluminum cans, PET, and glass bottles. Ten aluminum-canned beers, sixteen PET-packaged, and eighteen glass-bottled beers were bought at a local food store and subjected to GC–MS analysis to quantify and qualify phthalates. The results indicate that PET-packaged beers can contain significant amounts of phthalates; in sample P10, the total sum of phthalates reached 219.82 µg/L. Especially high concentrations of dibutyl phthalate were found in all samples, but the highest concentration was detected in sample P13 at 92.17 µg/L. However, canned beers showed even higher levels of certain phthalates, such as bis (2-ethylhexyl) phthalate, which amounted to 326.81 µg/L in sample C1. In short, phthalates pose a serious health-concerning problem and should be regarded as such.

**Keywords:** microplastics-related chemicals; GC–MS; phthalates; PET; beer



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## 1. Introduction

Phthalates (PAEs) are esters of phthalic acid which have many industrial applications; however, being the main plasticizers used in the polymer industry is their best-known characteristic [1]. They are mainly added to various plastic materials, such as polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyvinyl acetate (PVA), and polyethylene (PE), to improve the extensibility, elasticity, and workability of the polymers [1,2]. Phthalates are usually added to many products of general use such as toys, personal care and household products, car cosmetics, solvents, adhesives, glues, pesticides, food packaging, medical devices, electronics, tubing, and building materials [3,4]. In order to keep the color and scent of various products, phthalates of lower molecular weight (diethyl phthalate and dibutyl phthalate) are added as solvents. Phthalates of higher molecular weight, such as bis (1-ethylhexyl) phthalate, are added to soften PVC products [5,6].

Since phthalates are subject to bio-, photo-, and anaerobic degradation, they are not resilient to environmental conditions [7,8]. For example, depending on the length of their alkyl groups, phthalates display different water solubility, meaning that phthalates with long alkyl or aromatic parts in their side chains are shown to be less water soluble. Longer chains cause pronounced lipophilicity, and many fatty foods, such as fish, meat, dairy products, and vegetable oils, can be rich in these compounds [9].

The most efficient way for phthalates to enter into the human organism is via food or beverages. Phthalates are prone to migrate into food from plasticized PVC materials (lid gaskets for glass jars, food packaging films and papers, and board packaging), but from aluminum foil–paper laminates as well [5]. Food and beverages become contaminated during unit operations and transport, but also via the use of PVC gloves while handling food. As phthalates are contained in printing inks and adhesives on food wrappers, they are prone to migrate to food [5]. According to recent studies, phthalates can be found in alcoholic beverages such as wine, beer, spirits, brandy, and schnaps [9–12].

PET packaging is gaining popularity in Europe, even in countries that were against this idea, such as Czechia and Slovakia. The reasons behind their popularity are that they are transport-friendly, weigh less than glass bottles, have good barrier properties against gas permeation, and are cheaper [13]. According to Pira International Ltd.'s report [14], in 2015, around five billion PET bottles were used in the Central and Eastern European beer market, in contrast to Western Europe, where this number reached under one billion bottles per year. Beer quality deteriorates much more quickly in PET bottles (3-4 months) than in aluminum cans or glass bottles, but the biggest holdback for using PET bottles is the presence of microplastic material (microplastic-related chemicals or micro- and nanosized particles). Since microplastic is designated as a health-detrimental material, a growing number of studies focus on determining the influence it could have on human and animal health and the possibilities of the reduction and minimalization of such particles and chemicals in foodstuff and the environment [15–19]. Due to can coatings containing plastics, beer in aluminum cans can contain phthalates as well [20].

The European Food Safety Authority (EFSA) has established specified tolerable daily intakes for humans for several phthalates including bis (2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), and bis (2-ethylhexyl) adipate (DEHA). However, since 2011, the European Union has not prescribed the maximum amount of phthalates in food and beverages, but only regulates the migration of some phthalates from food contact material to food. These are low amounts, with maximum residue limits of 1.5 mg/kg for DEHP, 0.3 mg/kg for DBP, 30 mg/kg for BBP, and 18 mg/kg for DEHA [21].

In Croatia, around 20% of beer is packaged in PET bottles, 68% in glass bottles, 7% in cans, and the remainder in kegs [22]. This shows that PET bottles take up a significant amount of the market. It is clear that such beer is popular among the consumers. For that reason, the aim of this preliminary research was to conduct a screening of microplastics-related chemicals, such as phthalates, that can be found in commercially available beers packaged in PET bottles.

## 2. Materials and Methods

### *2.1. Sample Preparation*

Ten aluminum-canned (500 mL), sixteen PET-packaged (2 L), and eighteen glass-bottled (500 mL) beers were bought at a local food store and subjected to GC–MS analysis to quantify and qualify their phthalates. The purchased samples were stored in a cool and dry place until analysis. Sampling was performed directly from the bottle upon opening and the phthalates were analyzed according to the following procedure.

Samples (about 5 mL) that were previously degassed using an ultrasonic bath for 15 min were transferred to a glass test tube with the addition of 5 mL of acetonitrile (ACN) and 5 mL of water. The mixture was shaken vigorously for 1 min; then, after the addition of 3 g of anhydrous $MgSO_4$ and 1 g of NaCl, the sample was shaken again and centrifuged for 5 min.

According to the QuEChERS (Quick Easy Cheap Effective Rugged Safe) procedure [23,24], 1 mL of the extract was purified with the help of the salts of 150 mg of anhydrous $MgSO_4$, 50 mg of PSA (Primary Secondary Amine), and 50 mg of C18 (Bond Elut C18). After the addition of salt for purification and vigorous shaking for 1 min, centrifugation was performed for 5 min (5000 rpm). The pure acetonitrile extract was transferred to a GC vial and injected for analysis.

### 2.2. GC–MS Analysis and Instrumentation

The identification of phthalates was based on a comparison of the retention times of the peaks and target ions with those obtained from a standard mixture of phthalates (the standards were supplied by the instrument manufacturer). The quantification was based on external calibration curves prepared from the standard solution of each of the examined phthalates [25].

The verification of the peaks was carried out based on retention times, and target ions were compared with those of external phthalates. The solvent blanks were analyzed and quantified, and some phthalates were found in these blanks, but the quantity was lower than the LOQ (limit of quantification).

The determination was performed in splitless mode at a constant flow. The carrier gas was helium, velocity: 35.698 cm/s; pressure: 7.0 psi.

### 2.3. Calibration

Standards of phthalates (PAE) investigated in this study, namely dimethyl phthalate (DMP; $C_{10}H_{10}O_4$), diethyl phthalate (DEP; $C_{12}H_{14}O_4$), diisobutyl phthalate (DiBP; $C_{16}H_{22}O_4$), dibutyl phthalate (DBP; $C_{16}H_{22}O_4$), bis (2-ethylhexyl) phthalate (DEHP; $C_{24}H_{38}O_4$), and di-n-octyl-phthalate (DnOP); $C_{24}H_{34}O_4$), were purchased from Dr. Ehrenstorfer™ GmbH (Augsburg, Germany). The n-hexane was HPLC-grade (Carlo Erba, Milan, Italy). The solutions of each phthalate were prepared at concentrations of 1 mg/mL. Phthalates solutions at different concentrations (0.005, 0.01, 0.1, 0.05, 0.5 μg/mL) were prepared by dilution in n-hexane. The solutions were stored in vials at −20 °C. In order to avoid cross-contamination due to reagents, materials, and laboratory equipment, a thorough cleaning procedure was performed prior following the standard preparation: the glassware was soaked and washed in acetone and dried at 140 °C for at least 4 h. All the solvents used in the analysis were tested in order to check for the potential presence of PAE contamination, using GC-MS analysis. Ultrapure water was produced by a Milli-Q system (Millipore, Bedford, MA, USA) [25]. The limit of quantification (LOQ) and the limit of detection (LOD) for analyzed phthalates are shown in Table 1. and the chromatogram can be seen in Figure 1. The calibration curves for the analyzed phthalates can be seen in the Supplementary Material Figure S1.

In this study, an Agilent instrument 7890B/5977A MSD with a fused silica column (30 m × 0.25 μm film of HP-5M-thickness) from Agilent Technologies, Inc. (Santa Clara, CA, USA) was used. The conditions of the instrument were as follows: injection temperature 280 °C, MSD 280 °C; oven: initial temperature 90 °C (held 1 min) to 210 °C at 15 °C/min (held 2 min); then at the rate of 5 °C/min to 240 °C (held 5 min); followed by an increase of 5 °C/min to 250 °C; and then followed by an increase of 25 °C/min to 300 °C and held for 4 min.

**Table 1.** The limit of quantification (LOQ) and the limit of detection (LOD) for each analyzed phthalate.

| Compound | LOQ μg/mL | LOD μg/mL |
|---|---|---|
| Dimethyl phthalate | 0.00469 | 0.00141 |
| Diethyl phthalate | 0.00418 | 0.00030 |
| Diisobutyl phthalate | 0.00101 | 0.00030 |
| Dibutyl phthalate | 0.00101 | 0.00030 |
| Bis (2-ethylhexyl) phthalate | 0.00141 | 0.00032 |
| Di-n-octyl phthalate | 0.001101 | 0.00031 |

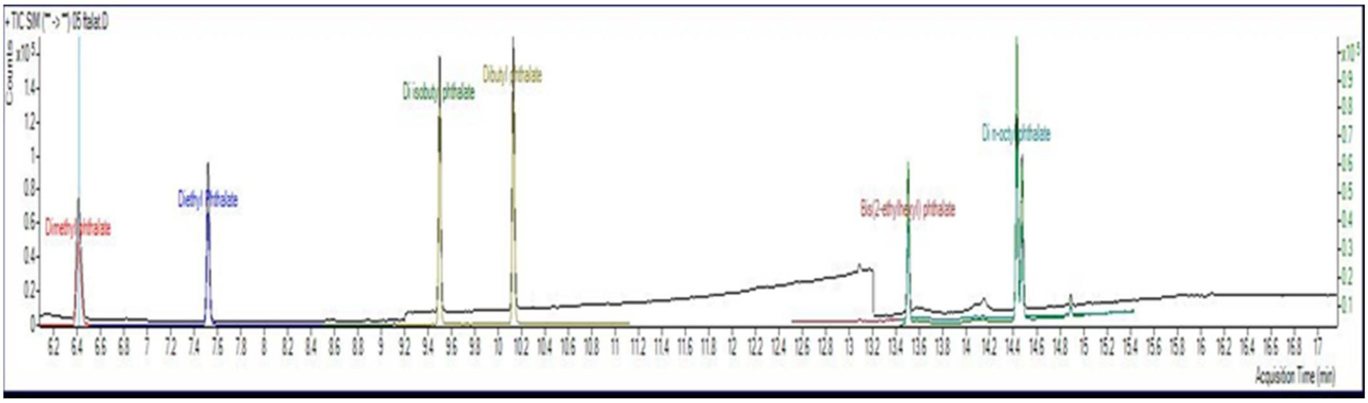


**Figure 1.** Chromatogram of analyzed phthalates.

### 2.4. Statistical Analysis

An analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) were conducted, with the least statistical significance set to $p < 0.05$. Statistica 13.1. (TIBCO Software Inc., Palo Alto, CA, USA) was the software of choice for this data set.

## 3. Results and Discussion

According to Wittassek et al. [26], phthalates are physically, not covalently, bound to polymer chains; thus, they are easily released in significant amounts in the environment. This is why they end up in food and beverages. It is clear that phthalates can end up in beer from raw materials, containers, and processing equipment, but they can also be released from plastic materials that are in a direct contact with beer [6].

The setup of this research was to determine the phthalates in PET-packaged, canned, and glass-bottled beers. The obtained data are presented in Tables 2–4. The results are somewhat expected; however, certain discrepancies from the predictions are noted. Glass-bottled beer contained fairly low concentrations of all phthalates. Namely, in relation to glass-bottled beer, PET-packaged beer (Table 2) contained significantly ($p < 0.05$) higher amounts of phthalates. Dimethyl phthalate and di-n-octyl phthalate were not detected in any of the samples.

**Table 2.** Concentrations of analyzed phthalates (µg/L) in PET-packaged beer samples.

| Sample | Dimethyl Phthalate | Diethyl Phthalate | Diisobutyl Phthalate | Dibutyl Phthalate | Bis (2-Ethylhexyl) Phthalate | Di-n-Octyl Phthalate | Sum |
|---|---|---|---|---|---|---|---|
| P1 | <LOQ | 0.46 $^{p}$ | 17.28 $^{m}$ | 29.73 $^{o}$ | 15.46 $^{n}$ | <LOQ | 62.93 $^{p}$ |
| P2 | <LOQ | 5.13 $^{g}$ | 25.93 $^{g}$ | 41.75 $^{k}$ | 27.298 i | <LOQ | 100.10 $^{l}$ |
| P3 | <LOQ | 9.16 $^{d}$ | 28.04 $^{e}$ | 51.44 $^{g}$ | 24.99 $^{k}$ | <LOQ | 113.62 $^{h}$ |
| P4 | <LOQ | 23.08 a | 25.97 $^{g}$ | 49.11 $^{h}$ | 29.11 $^{g}$ | <LOQ | 127.27 $^{g}$ |
| P5 | <LOQ | 9.97 $^{c}$ | 43.30 $^{c}$ | 73.29 $^{c}$ | 53.13 $^{d}$ | <LOQ | 179.69 $^{c}$ |
| P6 | <LOQ | 4.76 $^{j}$ | 22.73 $^{j}$ | 37.68 $^{n}$ | 9.12 $^{p}$ | <LOQ | 74.29 $^{o}$ |
| P7 | <LOQ | 4.28 $^{l}$ | 28.22 $^{e}$ | 61.10 $^{e}$ | 48.46 $^{e}$ | <LOQ | 142.06 $^{f}$ |
| P8 | <LOQ | 2.10 $^{n}$ | 25.46 $^{h}$ | 40.01 $^{l}$ | 23.07 $^{l}$ | <LOQ | 90.64 $^{m}$ |
| P9 | <LOQ | 5.61 $^{f}$ | 30.24 $^{d}$ | 62.75 $^{d}$ | 85.74 $^{b}$ | <LOQ | 184.33 $^{b}$ |
| P10 | <LOQ | 14.21 $^{b}$ | 43.72 $^{b}$ | 86.93 $^{b}$ | 74.96 $^{c}$ | <LOQ | 219.82 $^{a}$ |
| P11 | <LOQ | 4.63 $^{k}$ | 24.14 $^{i}$ | 54.65 $^{f}$ | 25.22 $^{j}$ | <LOQ | 108.64 $^{i}$ |
| P12 | <LOQ | 4.87 $^{i}$ | 20.65 $^{l}$ | 38.15 $^{m}$ | 11.15 $^{o}$ | <LOQ | 74.82 $^{n}$ |
| P13 | <LOQ | 4.95 $^{h}$ | 56.96 $^{a}$ | 92.17 $^{a}$ | 17.16 $^{m}$ | <LOQ | 171.24 $^{e}$ |
| P14 | <LOQ | 1.29 $^{o}$ | 26.88 $^{f}$ | 54.46 $^{f}$ | 95.06 $^{a}$ | <LOQ | 177.68 $^{d}$ |
| P15 | <LOQ | 6.53 $^{e}$ | 24.09 $^{i}$ | 47.61 $^{i}$ | 28.49 $^{h}$ | <LOQ | 106.72 $^{k}$ |
| P16 | <LOQ | 2.39 $^{m}$ | 22.30 $^{k}$ | 44.65 $^{j}$ | 37.95 $^{f}$ | <LOQ | 107.29 $^{j}$ |

$^{a–p}$ Means of three measurements; values with different superscripts within a row are significantly different ($p < 0.05$); LOQ—limit of quantification.

**Table 3.** Concentrations of analyzed phthalates (µg/L) in beer samples packaged in aluminum cans.

| Sample | Dimethyl Phthalate | Diethyl Phthalate | Diisobutyl Phthalate | Dibutyl Phthalate | Bis (2-Ethylhexyl) Phthalate | Di-n-Octyl Phthalate | Sum |
|---|---|---|---|---|---|---|---|
| C1 | <LOQ | 2.46 $^{bc}$ | 2.63 $^{a}$ | 2.95 $^{b}$ | 326.81 $^{a}$ | <LOQ | 334.86 $^{a}$ |
| C2 | <LOQ | 2.36 $^{c}$ | 2.39 $^{b}$ | 2.79 $^{c}$ | 313.15 $^{b}$ | <LOQ | 318.64 $^{c}$ |
| C3 | <LOQ | 2.35 $^{c}$ | 2.35 $^{bc}$ | 2.49 $^{d}$ | 326.71 $^{a}$ | <LOQ | 333.90 $^{a}$ |
| C4 | <LOQ | 2.49 $^{b}$ | 2.75 $^{a}$ | 3.07 $^{a}$ | 313.15 $^{b}$ | <LOQ | 321.47 $^{b}$ |
| C5 | <LOQ | 1.72 $^{f}$ | 2.07 $^{e}$ | 2.22 $^{e}$ | 131.20 $^{g}$ | <LOQ | 137.22 $^{h}$ |
| C6 | <LOQ | 4.15 $^{a}$ | 2.75 $^{a}$ | 2.72 $^{c}$ | 241.26 $^{d}$ | <LOQ | 250.89 $^{e}$ |
| C7 | <LOQ | 2.07 $^{d}$ | 2.22 $^{cd}$ | 4.41 $^{d}$ | 121.19 $^{h}$ | <LOQ | 127.91 $^{i}$ |
| C8 | <LOQ | 1.95 $^{e}$ | 2.07 $^{e}$ | 2.22 $^{e}$ | 95.45 $^{i}$ | <LOQ | 101.60 $^{j}$ |
| C9 | <LOQ | 1.73 $^{f}$ | 2.11 $^{de}$ | 2.39 $^{d}$ | 141.05 $^{f}$ | <LOQ | 147.28 $^{g}$ |
| C10 | <LOQ | 1.84 $^{ef}$ | 1.92 $^{f}$ | 1.99 $^{g}$ | 229.62 $^{e}$ | <LOQ | 235.38 $^{e}$ |

$^{a–j}$ Means of three measurements; values with different superscripts within a row are significantly different ($p < 0.05$); LOQ—limit of detection.

**Table 4.** Concentrations of analyzed phthalates (µg/L) in beer samples packaged in glass bottles.

| Sample | Dimethyl Phthalate | Diethyl Phthalate | Diisobutyl Phthalate | Dibutyl Phthalate | Bis (2-Ethylhexyl) Phthalate | Di-n-Octyl Phthalate | Sum |
|---|---|---|---|---|---|---|---|
| G1 | <LOQ | 0.76 $^{m}$ | 4.29 $^{a}$ | 3.99 $^{bcd}$ | 1.11 $^{b}$ | <LOQ | 10.14 $^{cd}$ |
| G2 | <LOQ | 0.74 $^{m}$ | 4.15 $^{ab}$ | 3.55 $^{g}$ | 3.65 $^{a}$ | <LOQ | 12.10 $^{a}$ |
| G3 | <LOQ | 1.52 $^{i}$ | 3.99 $^{bc}$ | 3.42 $^{gh}$ | 0.33 $^{hi}$ | <LOQ | 9.26 $^{ef}$ |
| G4 | <LOQ | 1.56 $^{i}$ | 3.90 $^{cd}$ | 3.60 $^{fg}$ | 0.39 $^{gh}$ | <LOQ | 9.45 $^{e}$ |
| G5 | <LOQ | 2.05 $^{c}$ | 2.80 $^{hi}$ | 3.05 $^{ij}$ | 0.35 $^{ghi}$ | <LOQ | 8.25 $^{g}$ |
| G6 | <LOQ | 2.55 $^{a}$ | 3.10 $^{g}$ | 2.90 $^{j}$ | 0.40 $^{g}$ | <LOQ | 8.95 $^{f}$ |
| G7 | <LOQ | 0.89 $^{l}$ | 3.55 $^{ef}$ | 4.10 $^{bc}$ | 1.01 $^{c}$ | <LOQ | 9.55 $^{e}$ |
| G8 | <LOQ | 0.99 $^{k}$ | 2.35 $^{l}$ | 3.85 $^{de}$ | 0.87 $^{d}$ | <LOQ | 8.06 $^{gh}$ |
| G9 | <LOQ | 2.45 $^{b}$ | 2.75 $^{hij}$ | 4.45 $^{a}$ | 0.41 $^{g}$ | <LOQ | 10.06 $^{d}$ |
| G10 | <LOQ | 1.61 $^{hi}$ | 3.95 $^{bcd}$ | 4.20 $^{b}$ | 0.71 $^{e}$ | <LOQ | 10.47 $^{c}$ |
| G11 | <LOQ | 1.89 $^{d}$ | 3.75 $^{de}$ | 3.95 $^{cde}$ | 0.58 $^{f}$ | <LOQ | 10.17 $^{cd}$ |
| G12 | <LOQ | 1.72 $^{fg}$ | 2.35 $^{l}$ | 3.25 $^{hi}$ | 0.52 $^{f}$ | <LOQ | 7.85 $^{h}$ |
| G13 | <LOQ | 1.83 $^{de}$ | 2.85 $^{hij}$ | 3.75 $^{ef}$ | 0.73 $^{e}$ | <LOQ | 9.06 f |
| G14 | <LOQ | 2.59 $^{a}$ | 2.94 $^{gh}$ | 4.65 $^{a}$ | 0.74 $^{e}$ | <LOQ | 10.92 $^{b}$ |
| G15 | <LOQ | 1.67 $^{gh}$ | 2.53 $^{jkl}$ | 3.11 $^{ij}$ | 0.83 $^{d}$ | <LOQ | 8.14 $^{gh}$ |
| G16 | <LOQ | 1.77 $^{ef}$ | 3.33 $^{f}$ | 3.93 $^{cde}$ | 0.97 $^{c}$ | <LOQ | 10.01 $^{d}$ |
| G17 | <LOQ | 1.27 $^{j}$ | 2.65 $^{ijk}$ | 2.88 $^{j}$ | 0.27 $^{i}$ | <LOQ | 7.07 $^{i}$ |
| G18 | <LOQ | 1.31 $^{j}$ | 2.45 $^{kl}$ | 3.07 $^{ij}$ | 0.40 $^{g}$ | <LOQ | 7.24 $^{i}$ |

$^{a–m}$ Means of three measurements; values with different superscripts within a row are significantly different ($p < 0.05$); LOQ—limit of detection.

Diethyl phthalate was found in all of the samples, with the highest concentration in PET bottles, reaching its maximum values in sample P4 with 23.08 µg/L. The lowest values were detected in beer packaged in glass bottles, with a maximum value in G3 of 1.511 µg/L, whereas canned beers generally showed values of over 2 µg/L; sample C6 had the highest amount of this chemical, 4.15 µg/L, as can be seen in Table 5.

Diisobutyl phthalate was also detected in all the samples, with a minimum value of 17.28 µg/L in P1, and the maximum value being 56.96 µg/L in sample P13. This phthalate was detected in canned beers (the maximal value was 2.75 µg/L in two samples C4 and C6) as well, with values lower than in glass-bottled beers (whose maximal value was detected in G1, 4.29 µg/L).

**Table 5.** Extracted minimal, maximal and average values (μg/L) of analyzed phthalates.

| Sample | Dimethyl phthalate | Diethyl phthalate | Diisobutyl phthalate | Dibutyl phthalate | Bis (2-ethylhexyl) phthalate | Di-n-octyl phthalate | Sum |
|---|---|---|---|---|---|---|---|
| MIN_P | <LOQ | 0.46 $^{c}$ | 17.28 $^{a}$ | 27.73 $^{a}$ | 9.12 $^{b}$ | <LOQ | 62.93 $^{b}$ |
| MIN_G | <LOQ | 0.74 $^{b}$ | 2.35 $^{b}$ | 2.83 $^{b}$ | 0.27 $^{c}$ | <LOQ | 7.07 $^{c}$ |
| MIN_C | <LOQ | 1.72 $^{a}$ | 1.92 $^{c}$ | 1.99 $^{c}$ | 95.45 $^{a}$ | <LOQ | 101.60 $^{a}$ |
| MAX_P | <LOQ | 23.09 $^{a}$ | 56.96 $^{a}$ | 92.17 $^{a}$ | 95.06 $^{b}$ | <LOQ | 219.82 $^{b}$ |
| MAX_G | <LOQ | 2.59 $^{c}$ | 4.29 $^{b}$ | 4.65 $^{b}$ | 3.65 $^{c}$ | <LOQ | 12.10 $^{c}$ |
| MAX_C | <LOQ | 4.15 $^{b}$ | 2.75 $^{c}$ | 3.07 $^{c}$ | 326.90 $^{a}$ | <LOQ | 334.86 $^{a}$ |
| AVG_P | <LOQ | 6.46 $^{a}$ | 29.12 $^{a}$ | 54.09 $^{a}$ | 37.90 $^{b}$ | <LOQ | 127.57 $^{b}$ |
| AVG_G | <LOQ | 1.62 $^{c}$ | 3.20 $^{b}$ | 3.65 $^{b}$ | 0.79 $^{c}$ | <LOQ | 9.27 $^{c}$ |
| AVG_C | <LOQ | 2.31 $^{b}$ | 2.33 $^{c}$ | 2.52 $^{c}$ | 223.75 $^{a}$ | <LOQ | 230.92 $^{a}$ |

$^{a\text{–}c}$ Mean values; values with different superscripts within a row are significantly different ($p < 0.05$); LOQ—limit of detection.

Dibutyl phthalate showed especially high values in PET packaging in regard to cans (Table 3) or glass bottles (Table 4). The highest value of this chemical was detected in sample P13 with 92.17 μg/L, and the lowest value was recorded in sample P1, 29.73 μg/L. As can be seen from Tables 2 and 3, the aluminum can and glass bottle contained significantly ($p < 0.05$) lower values for dibutyl phthalate, being <3 μg/L for both beers packaged in aluminum cans and <4 μg/L for glass bottles.

Bis (2-ethylhexyl) phthalate also showed significant differences between packaging materials, as confirmed in Table 4. Namely, the maximum concentration of bis (2-ethylhexyl) phthalate was 95.06 μg/L (P14) in PET-packaged beer, whereas beer packaged in aluminum cans contained almost threefold higher values, reaching over 300 μg/L in several samples, C1-C4. In comparison, glass-bottled beer had low values of this phthalate, with the maximal value being 3.65 μg/L. Such high concentrations of this chemical in aluminum-canned beers are supported by the results published in a study [20]. Namely, they detected bis (2-ethylhexyl) phthalate as the prevalent additive in beer can bodies (71%) and lids (89%).

The sum of all phthalates was highest in aluminum-canned beer, reaching 334.86 μg/L in sample C1. The majority of the total phthalate sum is made up of bis (2-ethylhexyl) phthalate, as it was the prevalent phthalate in all samples. Samples C1 and C3 do not show statistical difference, but all the other samples exhibit statistically significant difference ($p < 0.05$) among canned beers. Beer packaged in glass bottles showed the lowest sum of phthalates, with the maximal concentration being 12.10 μg/L.

Nurlatifah and Nakata [20] reported high values of phthalates in beer cans from 27 countries, reaching 5300 ng/g for bis (2-ethylhexyl) phthalate in can body. This indicates that there could be significant differences of phthalate content in beers from different countries, probably due to differences in aluminum can coatings. The alcohol content in beer could help in releasing phthalates into beer [20,24,27].

Additional studies should be employed, especially since there are no legal restrictions regarding the maximum level of phthalates in food and beverages.

The results of this research are similar to previous research conducted by Carnol et al. [28]. However, their research involved glass bottles, aluminum cans and aluminum bottles, but no PET bottles. The results they reported for aluminum-canned beer were much lower than the ones found in this research. Namely, Carnol et al. reported a maximal value for total phthalates reaching 61.56 μg/L for aluminum-canned beer, whereas we found that canned beer can contain >300 μg/L of total phthalates. Bis (2-ethylhexyl) phthalate was detected as the most common phthalate in beers in general. However, the values they found for this phthalate were very different than in our research, which mainly displayed values below 1 μg/L.

Since beer is distributed to different parts of the world (it has a long distribution chain) and is stored in different conditions (summer/winter temperatures, exposure to UV light), it is probable that such changes affect the release rate of different phthalates from packaging to beer. In any case, many factors could affect the concentrations of phthalates in beers and should be taken into an account.

Table 5 shows the minimal, maximal and average values of all analyzed samples and significant statistical differences between them. It is evident that a significant difference ($p < 0.05$) exists between all types of packaging. The lowest minimal total phthalates were found in glass bottles (7.07 µg/L), whereas the highest were determined to be in cans, at 101.60 µg/L. The highest maximal value was determined to be in cans as well, reaching 334.86 µg/L. PET bottles showed a lower maximal value for total phthalates (219.82 µg/L) than canned beer, which was not expected. In comparison to PET-packaged and canned beers, glass-bottled beers showed low values for total phthalates, with the maximal value being 12.10 µg/L.

The highest minimal and maximal values for bis (2-ethylhexyl) phthalate were found in aluminum-canned beers (95.45 µg/L and 326.90 µg/L). Dibutyl phthalate also showed a relatively high maximal value for PET-bottled beers, reaching 92.17 µg/L.

The average values for total phthalates also show significant statistical differences between packaging, again with the highest value being found in aluminum-canned beer (230.92 µg/L), whereas PET-packaged beer showed almost half of this concentration with 127.57 µg/L. Glass-bottled beer, on average, has only 9.27 µg/L. It should be born in mind that 96% of the total phthalates in canned beer originated from bis (2-ethylhexyl) phthalate (223.75 µg/L), whereas for PET-packaged samples dibutyl phthalate (54.09 µg/L) made up 43% of total phthalates. From Table 4 it can be seen that even though canned beers contained higher amounts of total phthalates, PET-packaged beers contained significantly ($p < 0.05$) higher amounts of diisobutyl phthalate (29.12 µg/L). In general, this research indicates the importance of measuring phthalates in differently packaged beers, and especially in canned beers.

## 4. Conclusions

This survey confirmed that phthalates can be found in different beers. Especially high concentrations of bis (2-ethylhexyl) phthalate were found in PET-packaged beers, but even higher levels were quantified in canned beers. Overall results indicate that canned beers contain higher levels of total phthalates than PET-packaged beers. This calls for attention, since many producers turn to cans. However, canned beers had high values only of bis (2-ethylhexyl) phthalate, whereas PET-bottled beers had high values of diisobutyl phthalate, dibutyl phthalate, and bis (2-ethylhexyl) phthalate. This could be a result of weaker bonds between the PET matrix and phthalates, which results in their easier release into PET-bottled beer. Glass-bottled beer seems safest from a health perspective, since it contains the lowest amounts of phthalates.

Further and deeper research should be conducted regarding this to authenticate this finding. Differences between cans from different countries should also be further investigated, as well as the influence of different storage conditions. In any case, this is a significant preliminary finding, since not many studies are reporting data on phthalates in beer. This opens possibilities for the continuation of research.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9020125/s1, Figure S1: The calibration curves for each phthalate.

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