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Original Research

Determination of Phthalate Esters in Beverages and Milk Using High Performance Liquid Chromatography (HPLC)

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Abstract

Phthalates are commonly used plasticizers in the production of polyvinyl chloride and polyethylene terephthalate plastics. However, due to their extensive use, phthalate esters are considered as ubiquitous environmental pollutants. Beverages and packaged milk are susceptible to contamination by phthalate ester during packaging, transportation, and storage process. This research aimed to quantify the leaching of phthalate esters from commercial beverages and milk samples stored in plastic bottles. Ultrasound and vortex assisted dispersive liquid-liquid microextraction, followed by high performance liquid chromatography were used to analyze selected samples stored under various environmental conditions. The findings showed that beverage bottles stored outdoor exhibited the highest chance of phthalate leaching, followed by those stored at room temperature and 4ºC. The concentration of dibutyl phthalate increased from 3.45 µg/ml (detection on purchase day) to 4.22 µg/ml (detection on expiry day) average on the milk samples, while there was a significant increase in the concentration of diethyl phthalate upon expiray compared to its concentration in fresh milk samples (3.33-11.91 µg/ml) average on all samples. An average of 9.41 and 8.55 µg/ml, 11.4 and 9.23 µg/ml, 20.75 and 17.72 µg/ml of diethyl phthalate and dibutyl phthalate were detected at 4ºC, room temperature and outdoor beverage samples after four months of their storage.This study suggests that the inappropriate storage conditions of milk and beverages enhances the leaching of phthalate esters from plastic matrix into the contained beverages and milk. Although the leaching is slow, continuous usage may pose health effect.

Keywords: beverages, phthalates, leaching, HPLC, DBP

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Introduction

In recent years, plastic packaging materials have become increasingly prevalent in human daily life due to industrialization and urbanization. Among these materials, over thirty different types of plastic are utilized by the Food industry, including polyethylene terephthalate (PET) and high-density polyethylene (HDPE) which are commonly used in food and drinking packaging. polyethylene terephthalate (PET) in particular has gained popularity due to its stability, low cost, transparent and portability making it a popular choice for producing plastic bottles for water and beverages. However, there is growing concern over the potential release of harmful chemicals from plastic materials into drinking water, beverages under certain conditions [1-4]. Moreover, the possibility of phthalate esters being present in bottled beverages increases due to various factors, such as storage durations, temperature change, sunlight exposure; and pH. Secondly, the migration process of phthalates can increase due to ageing and the breakdown of plastic packaging. Photolysis can cause abiotic degradation of phthalates in plastic water bottles and beverages [5-8].

Plastic products often contained various additives that make them flexible, durable and transparent. phthalate esters, also known as plasticizers are one such additives [9-11]. These common plasticizers are di-esters of 1,2-benzenedicarboxylic acid (phthalic acid), and are widely used in plastic industry to improve flexibility, durability and softness of plastic materials [12-16]. However, due to their nature, phthalates can easily migrate from plastic materials into drinking water, or beverages during production and storage [17-19]. The migration of PAEs' depends on their concentrations in the packaging polymer, the acidity of content in contact with packages, and the lipid content [20-23]. Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Benzyl butyl phthalate (BBP), Di-(2-ethylhexyl) phthalate (DEHP), Di-n-octyl phthalate (DnOP), Di-iso-nonyl phthalate (DiNP), and Di-iso-decyl phthalate (DiDP) are most commonly used in industry as plasticizers [24, 25].

Phthalate esters are considered as ubiquitous environmental pollutants due to extensive usage. They are known to have adverse effects on human health and considered as endocrine disruptor. The United State Environmental Protection Agency (USEPA) recognizes phthalates as endocrine disruptor and has classified as priority pollutants due to their potential risks to human health and environment. In response to these risk, the use of phthalates (DBP, DEHP, DMP, DEP and DnOP) has been banned in various consumers products by USA, EU, Australia and responsible agencies in Canada [26-30]. Phthalates have been known to caused functional impairment in development and reproduction in both animals and human, as revealed by epidemiological studies. In particular, dibutyl phthalate (DBP), di-2 (ethyl hexyl) phthalate (DEHP), butyl benzyl phthalate (BBP) and certain phthalate metabolites have been found Teratogenic in animals. In rodents, phthalates have been identified as anti-androgenic and as a cause of reduced testosterone level, as well as prolonged estrous cycles and lower estradiol levels [31]. Phthalates are a type of endocrine disruptor that can have various adverse effects on human health. They have been linked to breast cancer and prostrate cancer, infertility, obesity, asthma, liver damage, disorder of androgenic attention deficit and function, mutagenicity, teratogenicity. Additionaly, exposure to phthalate has been associated with development abnormalility and social impairments in children, including deficit hyperactivity disorder (ADHD) [32-34].

Food contamination with phthalate esters has become a matter of public concern in recent year. Beverages can be easily contaminated by phthalate esters during packaging, transportation, and storage processes. Due to complexity of the sample matrix and the low levels of phthalates in food and beverages, determining phthalates in beverage samples is a challenging task [17, 35, 36]. Temperature and content of a PET bottles play a vital role in determine the rate and extent of leaching of chemicals from the bottle matrix into the water and beverages. The duration of storage, concentration of compound, its types, nature, and solubility in foodstuffs are the main factors that influence the leaching of chemicals in water or beverage contents. Storing plastic products under ideal conditions is essential. Different manufacturers advocate for optimal storage conditions such as indoor storage, keep in cool, dry place, and away from direct sunlight. The technical testing of all plastic containers focuses on two specific attributes: first ensuring minimum leaching of compounds from plastic matrix to drinking contents and the second confirming that any compounds that might leach from plastic to the liquid medium do not pose risk to the human health. The food and drug authority (FDA) has stated that exposing the bottle to higher temperature may lead to a higher degree of leaching of compounds from plastic to water or beverages. However, it is common for people to expose plastic bottled beverages to intense sunlight during transportation from production to selling points. These beverage bottles are some time left in sunlight at markets or in cars for longer periods before consumption. For that reason it is necessary to determine whether exposure to these storage factors can impact on the leaching of compounds from plastic matrix into the beverages [53-55]. Therefore, we conducted this study to access the phthalate esters in beverage bottles and milk purchased from markets of Lahore in year 2021 stored under different storage condition to evaluate the effect of these conditions on phthalate leaching through HPLC.

Materials and Methods

Chemicals

Dibutyl phthalate (DBP), Dimethyl phthalate (DMP), Di-n-octyl phthalate (DNOP), Diethyl phthalate (DEP), Acetonitrile, Chloroform, Carbon tetrachloride, Trichloroacetic acid, Lead acetate, Methanol and Sodium chloride used in research work were purchased from Sigma merk. Stock solution of phthalate esters (~99.00%) were prepared in mixture of acetonitrile: water (v/v). and stored at 4°C until analysis.

Sampling and Storage Conditions

Eighteen samples of beverages in plastic bottles from six commercial brands and six milk brands were randomly collected from market of Lahore, Pakistan. Beverage samples were stored at 4ºC, room temperature and outdoor. Milk samples were stored at 4ºC till expiry.

Sample Extraction

Beverage Samples Extraction

Phthalate esters were extracted from beverage bottles by ultrasound and vortex-assisted dispersive liquid-liquid micro extraction with some modification as describe below [37]. For this purpose, 2.5 ml of beverage samples and 0.5g of sodium chloride were added in 10 ml of conical centrifuge tube. 2 ml of methanol and 500 µl of chloroform were added in the next step. The tube was placed in ultrasonic bath for 30 sec then vortexed for 4 min and centrifuged for 3 min at 4000 rpm and chloroform phase was separated into the glass test tube and evaporated under nitrogen flow. The residue was dissolved with the mobile phase and then filter through 0.45 µm syringe filter before injected into HPLC-DAD under the following conditions given in Table 1.

Milk Samples Extraction

Phthalate esters were extracted from milk samples by dispersive liquid-liquid micro extraction according to Yan et al. [38] with some modifications. Before extraction of phthalates, lipid layer needs to be removed from milk. For this purpose, first milk samples were mixed with trichloroacetic acid and centrifuge at 10,000 rpm for 5 min. The fatty layer was removed and added lead acetate solution into supernatant, centrifuge at 10,000 rpm for 5 min. The supernatant was separated into 10 ml centrifuge tube and mixed with sodium chloride. In next step, 800 µl methanol and 40 µl carbon tetrachloride were injected rapidly into milk samples. The mixture was manually shaken for several seconds and further mixed by ultrasonic bath for 2.0 min. Then finally centrifuge at 4000 rpm for 5 min. the solvent evaporated under the nitrogen flow, residue dissolved into mobile phase and filter through 0.45 µm syringe filter injection into HPLC- DAD conditions given in Table 1.

Results and Discussions

In this study, we examined the retention time of four phthalate esters: DMP (1.819), DEP (5.596), DBP (8.683), and DnOP (14.250), as shown in Fig. 1. The HPLC method was validated in term of linearity, LOD, precision and accuracy. The linearity was obtained in the range of 0.25-100µg/ml for beverages with coefficient (R^2) ranging from 0.9965-0.9992, and for milk samples linearity range was 0.25-100 µg/ml with coefficient ranging from 0.9935 to 0.9989, indicating good linearity and LOQ was 0.5 µg/ml for all the four phthalate esters, summarized in Table 2. The 1st and 2nd day of precision and accuracy were evaluated in six replicates of beverages and milk samples at three different concentration, coefficient of varience (CV) presented as precision. For beverages, 1st and 2nd day precision was 0.34-5.93 and 0.53-5.89 respectively and the accuracy of the method was within 94-98% as shown in table 3. For milk samples 1st and 2nd

Table 1. Analytical conditions of phthalate esters by HPLC-DAD.

HPLC	LC-20 AT liquid chromatography system (Shimadzu 24-Japan)
Detector	SPD-M20A Diode Arrey detector
Analytical column	C18 column (250 mm × 4.6 mm id, 5.0 µm)
Temperature of column	25ºC
Mobile phase	Mixture of acetonitrile-water (60:40 v/v)
Flow rate of mobile phase	1.0 mL/min
Injection volume	20µL
Run time of phthalates	20 min
Detector wavelength	225 nm

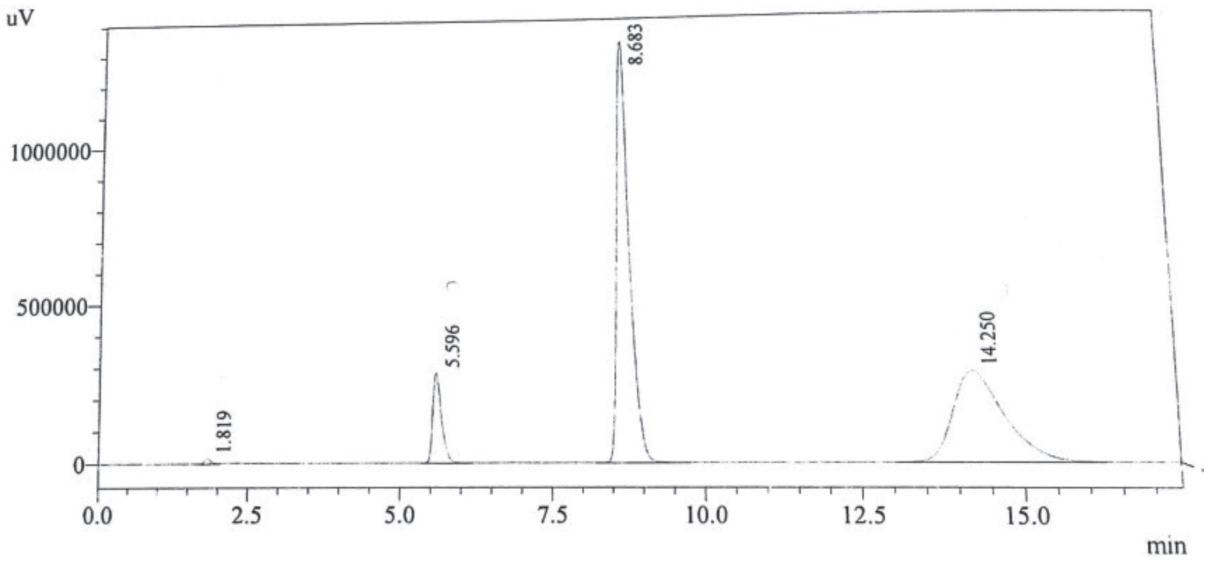

Fig. 1. HPLC chromatogram of phthalate standards 1.DMP 2. DEP 3. DBP 4. DnOP.

Table 2. Analytical performance of the developed method.

Samples	Phthalate esters	Linearity (µg/ml)	Linear equation	R^2
Beverages	DBP	0.25-100	y = 4E + 06x – 4E + 06	0.9992
	DMP	0.25-100	y = 44052x – 21236	0.9982
	DEP	0.25-100	y = 4E+06x + 5E + 06	0.9965
	DnOP	0.25-100	y = 3E+06x + 2E + 06	0.9987
Milk	DBP	2-12	y = 3E + 06x + 2E + 06	0.9987
	DMP	2-12	y = 40849x – 7333.8	0.9953
	DEP	2-12	y = 40849x – 7333.8	0.9989
	DnOP	2-12	y = 3E + 06x + 666667	0.9935

day precision was 0.74-3.64 and 0.07-3.44 respectively (Table 3) and the accuracy of the method was within 91-99%. The result indicated that analytical method is accurate and valid. Among the above-mentioned phthalate esters, only DBP and DEP could be determined quantitatively in beverages and milk samples and their detection frequency are 72% and 61% in beverage samples and 45% in milk samples. None of the fresh beverage samples contained any tested phthalates. Previous research has also failed to identify any phthalates other than diethyl hexyl phthalate (DEHP), which was not included in the list of tested phthalates in the current study. Razali et al. [8] did not find any phthalate in fresh PET bottles of sparkling (carbonated), mineral and drinking (non-carbonated) water. Montuori et al. [39] detected DMP, DEP and DBP in PET and glass water bottles and their concentration was twelve times higher in PET than in glass bottled water. However, all packaged milk samples analyzed in other countries by various researchers contained phthalate athough at low concentrations. DBP and DEHP were the most common phthalates detected in these studies, with concentrations ranging from 3.08-5.03 ng/g for DBP and 0.41-4.00 ng/g for DEHP [40-42], of which DBP is consistent with our findings. The detection of phthalates in freshly packaged milk samples is due to the contribution of severals factors along the processing chain. Mechanical milking, phthalate containing cattle feeds, commercial feeds, packaging materials and water source are the main sources of phthalate contaminations at both farm and industrial level [43-45]. Studies conducted in China, Iran and India have revealed higher concentrations of phthalates in packaged milk samples compared to raw milk samples, suggesting that the production and packaging process contribute to this contamination [45-47].

The results of phthalates concentrations in milk samples are shown in Table 4. Diethyl phthalate and dibutyl phthalate were detected in most of milk samples tested for phthalate esters on purchase day (time 1) and after expiry day (time 2). Concentrations of both the phthalates increased with storage and indicated by higher values at time 2. The concentrations differ significantly among the milk brands ranging

Table 3. 1st day and 2nd day precision and accuracy for analysis of phthalate esters in beverage and milk samples.

Compounds	Beverages						Milk					
	Conc. Added (µg/ml)	1st day		2nd day		Accuracy	Conc. Added (µg/ml)	1st day		2nd day		Accuracy
		Conc. Found*	CV	Conc. Found*	CV			Conc. Found*	CV	Conc. Found*	CV	
DMP	5	5.13 (0.042)	0.81	5.60 (0.04)	0.70	98.6	5	5.46 (0.201)	3.64	5.62 (0.03)	0.55	93.4
	40	37.79 (0.258)	0.68	40.93 (0.26)	0.64	96.5	7	0.190 (0.190)	2.63	7.79 (0.04)	0.55	94.4
	80	76.83 (0.518)	0.67	82.55 (0.52)	0.64	98.7	11	11.55 (0.208)	1.80	12.14 (0.07)	0.56	95.3
DBP	5	5.55 (0.029)	0.52	5.13 (0.03)	0.53	89.6	5	5.46 (0.201)	3.31	4.43 (0.07)	3.10	91.6
	40	39.95 (0.246)	0.62	40.30 (2.37)	5.89	97.2	7	0.190 (0.190)	2.20	6.07 (0.09)	2.10	91.7
	80	83.45 (0.521)	0.62	89.13 (3.63)	4.07	98.0	11	11.55 (0.208)	1.82	9.56 (0.15)	1.76	91.7
DEP	5	4.09 (0.074)	1.80	5.15 (0.24)	4.67	94.4	5	5.46 (0.201)	0.75	5.60 (0.17)	1.53	97.6
	40	40.11 (2.206)	5.50	41.13 (1.64)	3.99	97.8	7	0.190 (0.190)	0.74	8.07 (0.17)	1.52	92.7
	80	85.80 (1.708)	1.99	86.09 (1.29)	1.42	99.4	11	11.55 (0.208)	0.74	11.70 (0.21)	1.52	99.0
DnOP	5	5.25 (0.311)	5.93	5.40 (0.03)	0.63	96	5	5.46 (0.201)	2.81	5.29 (0.18)	3.44	96.8
	40	42.56 (0.219)	0.51	43.30 (0.27)	0.63	94.85	7	0.190 (0.190)	2.22	5.82 (0.00)	0.07	98.5
	80	90.08 (0.307)	0.34	91.59 (0.58)	0.63	95.45	11	11.55 (0.208)	2.26	9.27 (0.01	0.07	99.9

*Mean (standard deviation) n = 6

Table 4. Concentrations (μg/ml) of phthalate esters in milk samples.

Phthalates	Brand A		Brand B		Brand C		Brand D		Brand E		Brand F	
	Time 1**	Time 2***	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2
DBP	1.25	1.72	ND	ND	ND	0.77	1.01	1.27	ND	ND	2.07	2.70
DEP	1.14	4.45	1.18	1.96	ND	1.59	ND	1.68	ND	0.93	5.99	7.73
DMP	ND*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DnOP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

* Not detected, **on purchase day, ***on expiry day

Table 5. Concentrations (μg/ml) of phthalate esters in beverage brands.

Beverages Brands	DMP			DBP			DEP			DnOP		
	4ºC	RT**	Outdoor	4ºC	RT	Outdoor	4ºC	RT	Outdoor	4ºC	RT	Outdoor
A	ND	ND	ND*	ND	ND	ND	11	27	55	ND	ND	ND
B	ND	ND	ND	ND	ND	9.8	ND	ND	ND	ND	ND	ND
C	ND	ND	ND	75	77	87	ND	13	62	ND	ND	ND
D	ND	ND	ND	41	60	97	ND	76	106	ND	ND	ND
E	ND	ND	ND	ND	5.6	19	69	120	130	ND	ND	ND
F	ND	ND	ND	9.2	22	26	37	46	110	ND	ND	ND

*not detected ** room temperature/indoor

1.146-4.45 μg/ml for brand A, nd-1.963 μg/ml for brand B, nd-1.599 μg/ml for brand C, nd-1.682 μg/ml, for brand D nd-0.932 μg/ml for brand E and 2.071-7.734 μg/ml for brand F. lowest DBP concentration was detected in brand C (0.77 μg/ml) and highest in brand F (2.70 μg/ml). Maximum concentration of DEP was detected in brand F (7.73 μg/ml) and minimum in brand F (0.93 μg/ml).

Table 5 summarizes the concentrations of phthalates released in beverage brands, Diethyl phthalate (DEP) was detected in samples stored at 4ºC with concentration ranging ND-69 μg/ml. In at room temperature bottles, Diethyl phthalate (DEP) was detected with concentration ranging from ND-120 μg/ml. Outdoor storage showed DEP concentration lying in the range of ND-130 μg/ml. Similarly, dibutyl phthalate leached in beverage samples stored at 4ºC concentrations ranging from ND-75 μg/ml, but even at room temperature, range of 5.6-77 μg/ml, although outdoor storage showed its leaching in samples similar to that of DEP concentrations ND-97 μg/ml. The remaining two tested phthalates (DMP and DnOP) were not detected in any of the beverage samples stored at any of the storage conditions. The reason could be probably their limited use in industrial levels and in bottle industry. Irrespective of storage conditions, brands A, B and E give the lowest average concentrations of DBP with no detection and brand D gives the highest average concentrations of DBP equal to 97 μg/ml. DBP individual concentrations of the brands were ranged ND-9.8 μg/ml for brand B, 75-87 μg/ml for brand C, 41-97 μg/ml for brand D, ND-19 μg/ml for brand E, and 9.2-26 μg/ml for brand F. For DEP, brands B, C and D give no detection as the lowest values, while, brand E gives 130 μg/ml as the highest values. Individual ranges were 11-55 μg/ml for brand A, ND-62 μg/ml for brand C, ND-106 μg/ml for brand D, 69-130 μg/ml for brand E, 37-110 μg/ml for brand F. The detection of phthalates after 4 months of storage in beverage bottles, compared to no detection in the beverage bottles analyzed before storage, and the increase in phthalate concentration in milk samples after storage, compared to their levels detected in packaged milk samples analyzed upon purchase, are indicators of phthalate leaching from plastic matrix of the packaging materials. For both beverages and milk samples, the order of the concentration of the two detected phthalates was DEP>DBP. The increase in phthalate concentration after 45 days of storage, compared to the phthalate content measured before storage served as evidence of phthalate leaching as observed in bottle water storage under conditions by Jeddi et al. [18] and Rastkari et al. [2]. When measuring phthalate esters leaching in different types of consumable plastic products Ahmed et al. [50] also found DMP and DBP to have the highest leaching in juices and soft drinks comparable to five different phthalates. In another study, Ayofe et al. [51] observed phthalate quantity in plastic coke

Table 6. Detection frequency of DBP and DEP in beverage and milk samples.

Sample nature	% of positive samples	% of samples > MRL of US EPA (6-8 μg/L)
Milk (n= 12)	DEP = 75	8.3
	DBP = 58.3	0
Beverages (n = 18)	DEP = 72.22	72
	DBP = 66.66	61

in decreasing mean concentration of phthalate esters in order of DEHP>DMP>DEP>DphP. Likewise in another study, Yin et al. [35] analyzed eleven phthalate esters from carbonated soft drinks, mineral water bottles, fruit juices and tea beverages through HPLC analysis. Phthalates were detected in range of 0.24-34.3 μg/L in all beverage samples.

The maximum contaminant level (MCL) for DEHP in drinking water bottles set by USEPA, EU and WHO is 6 μg/L and 8 μg/L, which have been used for comparison as standard in this study because no limits have been set as permissible or maximum contaminant levels for any of the other phthalates by any national or global authority [41, 52, 56]. In this study, the mean concentration of diethyl phthalate and dibutyl phthalate in all milk samples were below the standard value, while in beverages the mean concentration of DBP and DEP were higher than standard values. Table 6 summarizes a comparison of detection frequency of DBP and DEP in milk and beverage samples and the comparison of their concentration with MRL (6 and 8 μg/L) set by USEPA, EU and WHO. Diethyl phthalate exceed the MRL in 8.3% of the positive milk samples and in 72% of the positive beverage samples. While, dibutyl phthalate exceed MRL in none of positive milk samples although it surpass the MRL in 61% of the positive beverage samples. These values confirm with the values obtained by other researchers previously. For instance, Moradian et al. [41] detected DEP (nd-0.02 μg/l), and DBP (0.02-0.04 μg/L) in milk samples. Similarly, Ayofe et al. [51] detected DMP, DEP, and DEHP in coca cola at concentration range 1.32-10.22 μg/l, nd-2.86 μg/l, and 7.43-18.67 μg/l respectively. Likewise Yin et al. [53] found DBP in mineral water (8.98-11.5 μg/l), in carbonated soft drink (5.03-16.5 μg/l), in tea beverage (2.22-12.6 μg/l), and in fruit juice (nd-20.6 μg/l).

The average concentration of both detected phthalate was calculated separately for each of storage condition, regardless of the tested brands. These average were plotted in Fig. 2. Which illustrates that outdoor (sunlight exposure) storage is the most prone to phthalate leaching, on the other hand, refrigerated condition (4°C) is the safest in this respect, on average, as this offered the highest storage temperature. In other words, probability of both DBP and DEP leaching increases as the temperature increases. The trend of phthalate leaching in plastic bottles is affected by storage conditions, with the highest leaching occurring in bottles stored outdoor, followed by room temperature and 4ºC. This is consistent with previous studies, which have found that phthalate leaching occurs as a results of hydrolysis, with temperature acting as a catalyst. Bottles that are stored at higher temperatures are more likely to support leaching of phthalates and have higher concentrations of phthalates than bottles stored at lower temperatures. This also justifies the highest level of leaching in bottles stored outdoor. Schmid et al. [48] reported an increase in DEHP in PET-bottled water that was stored in direct sunlight upto 34ºC for 17 hours. In a study of phthalate leaching in juices during storage, Arfaeinie et al. [49] found a positive correlation between the storage temperature, time and sunlight exposure of juice containers, and the extraction of phthalate esters (DnBP and DEHP) from highly acidic juices. Jeddi et al. [18] also deduced that freezing temperature (4ºC) does not support phthalate leaching in acidic beverages, hydrolysis controls the production and leaching of phthalates whose rate increases with increase temperature imply that high temperature allows more leaching than the lower ones.

Conclusions

The present research was conducted to assess the impact of storage conditions on the potential migration and concentration of phthalate esters in beverages

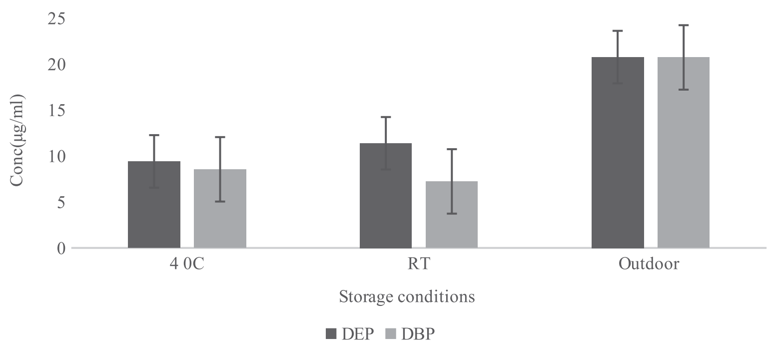

Fig. 2. Concentration of phthalate esters in beverages under each storage conditions.

and milk, using high-performance liquid chromatography to determine the risk of these phthalates on consumer health. The study found that diethyl phthalate DEP had highest frequency of leaching, followed by dibutyl phthalate (DBP). Furthermore, DEP was detected in a comparative high concentration when compared to DBP. Among the storage conditions, outdoor storage was found to be the most damaging, as it supported the highest degree of phthalate leaching from plastic bottles into beverages. On the other hand, storing beverages and milk at 4ºC was found to be safest as it did not support a high degree of hydrolysis that causes phthalate leaching. As a result, it is recommended that beverage bottles and packaged milk should be stored under 4ºC conditions if they are required to be stored for a long duration.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. MOAZZEN M., MAHVI H.A., SHARIATIFAR N., KHANIKI J.G., NAZMARA S., ALIMOHAMMADI M., AHMADKHANIKA R., RASTKARI N., AHMADLOO M., AKBARZEDEH A., DOBARADARAN S., BAGHANI, N.A. Determination of Phthalate acid esters (PAEs) in carbonated soft drinks with MSPE/GC-MS method. Journal of Toxin Reviews, **37** (4), 319, **2017**.
2. RASTKARI N., JEDDI M.Z., YUNESIAN M., AHMADKHANIHA R. The effect of storage time, Temperature and Type of packaging on the release of phthalate esters into packed acidic liquids. Food technol Biotechnol, **55** (4), 562, **2017**.
3. YANG J.F., YANG L.M., ZHENG L.Y., YING G.G., LIU C.B., LUO S.L. Phthalates in plastic bottled non-alcoholic beverages from China and estimated dietary exposure in adults. Food Additives & Contaminants: Part B, **10** (1), 44, **2017**.
4. KIANI A., AHMADLOO M., SHARIATIFAR N., MOAZZEN, M., BAGHANI A.N., KHANIKI G.J., TAGHINEZHAD A., KOUHPAYEH A., KHANEGHAH A.M., GHAJARBEYGI P. Method development for determination of migrated phthalate acid esters from polyethylene terephthalate (PET) packaging into traditional Iranian drinking beverage (Doogh) samples: a novel approach of MSPE-GC/MS technique. Environmental Science and Pollution Research, **25**, 12728, **2018**.
5. ALHADDAD F.A., ABU-DIEYEH M., DA'ANA D., HELALEH M., AL-GHOUTI M.A. Occurrence and removal characteristics of phthalate esters from bottled drinking water using silver modified roasted date pits. Journal of Environmental Health Science and Engineering, **19**, 733, **2021**.
6. FARAJZADEH M.A., PEZHHANFAR S., MOHEBBI A., AFSHAR MOGADDAM M.R. Detection and Determination of some Migrated Chemicals from Plastic Containers into Different Drinks and Liquids Using Dispersive Liquid-liquid Microextraction Prior to Gas Chromatography. Analytical and Bioanalytical Chemistry Research, **7** (3), 303, **2020**.
7. KERSZTES S., FATAR E., CZEGENGE Z., ZARAY G., MICHUCZ V.G. Study on the leaching of Phthalates from Polyethylene Terephthalate bottles into mineral water. Science of the Total Environment, **458-460**, 451, **2013**.
8. RAZALI N.A.S., ABIDIN U.F.U.Z., ABEDIN N.H.Z., OMAR S., SELAMAT J., SANNY M. The effects of storage temperature and time on the levels of phthalates in commercial pet-bottled water. Malaysian Journal of Analytical Science, **25** (3), 508, **2021**.
9. CHANG W.H., HERIANTO S., LEE C.C., HUNG H., CHEN H.L. The effects of phthalate ester exposure on human health: A review. Science of the Total Environment, **786**, 147371, **2021**.
10. BHARDWAJ L.K., SHARMA A. Evaluation of Bis (2-ethylhexyl) phthalate (DEHP) in the PET Bottled Mineral Water of Different Brands and Impact of Heat by GC-MS/MS. Chemistry Africa, **5**, 529, **2022**.
11. EZODDIN M., TAGHIZADEH T., ABDI K., JAMALI M.R. Application of modified nano γ-alumina as a solid-phase extraction sorbent combined with high-performance liquid chromatography for determination of phthalate esters in environmental water and soft drink samples. Desalination and Water Treatment, **53** (3), 671, **2015**.
12. EDJERE O., ASIBOR I.G., OTOLO S.E. Evaluation of the levels of phthalate ester plasticizers in surface water of Ethiope River System, Delta State, Nigeria. Journal of Applied Sciences Environmental Management, **20** (3), 608, **2016a**.
13. ZHANG X., TANG S., QIU T., HU X., LU Y., DU P., XIE L., YANG Y., ZHAO F., GIESY J.P. Investigation of phthalate metabolites in urine and daily phthalate intakes among three age groups in Beijing, China. Environmental Pollution, **260**, 114005, **2020**.
14. ZAATER M.F., TAHBOUB Y.R., AL SAYYED A.N. Determination of phthalates in Jordanian Bottled water using GC-MS and HPLC-UV. Journal of Chromatograhic Science, **52**, 447, **2014**.
15. KANCHANAMAYOON W., PRAPATPONG S., CHUMWANWAPEE S., CHAITHONGRAT S. Analysis of Phthalae esters contamination in drinking water samples. African Journal of Biotechnology, **11** (96), 16263, **2012**.
16. TONG Y., LIU X., ZHANG L. Green construction of Fe3O4@ GC submicrocubes for highly sensitive magnetic dispersive solid-phase extraction of five phthalate esters in beverages and plastic bottles. Food chemistry, **277**, 579, **2019**.
17. 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, **119**, 291, **2014**.
18. JEDDI Z.M., RASTKARI N., AHMADKHANIHA R., YUNESIAN M. Concentrations of Phthalates in bottled water under common storage conditions: Do they pose

a health risk to children? Food Research International, **69**, 256-, **2015**.
19. ABTAHI M., DOBARSDARAN S., TORABBEIGI M., JORFI S. 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. Environmental Research, **173**, 469, **2019**.
20. MIRZAJANI R., KARDANI F., RAMEZANI Z. Fabrication of UMCM-1 based monolithic and hollow fiber-Metal-organic framework deep eutectic solvents/ molecularly imprinted polymers and their use in solid phase microextraction of phthalate esters in yogurt, water and edible oil by GC-FID. Food chemistry, **314**, 126179, **2020**.
21. MA T., TENG Y., CHRISTIE P., YONGMING L. Phytotoxicity in seven higher plant species exposed to di-n-butyl phthalate or bis (2-ethylhexyl) phthalate. Frontiers of Environmental Science and Engineering, **9** (2), 259, **2015**.
22. OMIDPANAH S., SAEIDNIA S., SAEEDI M., HADJIAKHONDI A., MANAYI A. Phthalate contamination of some plants and herbal products. BOLETÍN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS, **17** (1), 61, **2018**.
23. JEDDI M.Z. Phthalates mixtures in bottled water in Iran: human health risk assessment using direct and indirect exposure assessment. HYPERLINK “https://www.wur.nl/en/activity/Phthalates-mixtures-in-bottled-water-in-Iran-human-health-risk-assessment-using-direct-and-indirect-exposure-assessment-1.htm” https://www.wur.nl/en/activity/Phthalates-mixtures-in-bottled-water-in-Iran-human-health-risk-assessment-using-direct-and-indirect-exposure-assessment-1.htm. **2018**.
24. EDJERE O., ASIBOR I.G., BASSEY U. Distribution of Phthalate Easters in underground water from power transmission sites in Warri Metropolis Delta State, Nigeria. Journal of Applied Sciences and Environmental Management, **20** (3), 599, **2016b**.
25. BAŞARAN B., SOYLU G.N., CIVAN M.Y. Concentration of phthalate esters in indoor and outdoor dust in Kocaeli, Turkey: implications for human exposure and risk. Environmental Science and Pollution Research, **27** (2), 1808, **2020**.
26. FARAJZADEH M.A., KHOSHMARAM L. Development of dispersive liquid-liquid microextraction technique using ternary solvents mixture followed by heating for the rapid and sensitive analysis of phthalate esters and di (2-ethylhexyl) adipate. Journal of Chromatography A, **1379**, 24, **2015**.
27. LI H., LI C., AN T., DENG C., SU H., WANG T., JIANG Z., ZHOU J., WANG J., ZHANG C., JIN F. Phthalate esters in bottled drinking water and their human exposure in Beijing, China. Food Additives & Contaminants: Part B, **12** (1), 1, **2019**.
28. POURZAMANI H., FALAHATI M., RASTEGARI F., EBRAHIM K. Freeze-melting process significantly decreases phthalate ester plasticizer levels in drinking water stored in polyethylene terephthalate (PET) bottles. Water Science & Technology: Water Supply, **17** (3), 745, **2017**.
29. WANG Y., ZHU H., KANNAN K. A Review of Biomonitoring of Phthalate Exposures. Toxics, **7** (2), 21, **2019**.
30. ZAKHARKIV I., ZUI M., ZAITSEV V. Determination of Phthalate Esters in Water and Liquid Pharmaceutical Samples by Dispersive Liquid-Liquid Microextraction (DLLME) and Gas Chromatography with Flame Ionization Detection. Analytical Letters, **53** (10), **2020**.
31. EDJERE O., ASIBOR I.G., BASSEY U. Distribution of Phthalate Easters in underground water from power transmission sites in Warri Metropolis Delta State, Nigeria. Journal of Applied Sciences and Environmental Management, **20** (3), 599, **2016c**.
32. WU Y., ZHOU Q., YUAN Y., WANG H., TONG Y., ZHAN Y., SHENG X., SUN Y., ZHOU X. Enrichment and sensitive determination of phthalate esters in environmental water samples: A novel approach of MSPE-HPLC based on PAMAM dendrimers-functionalized magnetic-nanoparticles. Talanta, **206**, 120213, **2019**.
33. MA J.K., WEI S.L., TANG Q., HUANG X.C. A novel enrichment and sensitive method for simultaneous determination of 15 phthalate esters in milk powder samples. LWT-Food Science and Technology, **153**, 112426, **2021**.
34. REZAEI H., MOAZZEN M., SHARIATIFAR N., KHANIKI G.J., DEHGHANI M.H., ARABAMERI M., ALIKORD M. Measurement of phthalate acid esters in non-alcoholic malt beverages by MSPE-GC/MS method in Tehran city: chemometrics. Environmental Science and Pollution Research, **28** (37), 51897, **2021**.
35. YIN S., YANG Y., YANG D., LI Y., JIANG Y., WU L., SUN C. Determination of 11 phthalate esters in beverages by magnetic solid-phase extraction combined with high-performance liquid chromatography. Journal of AOAC International, **102** (5), 1624, **2019**.
36. WU Q., ZHOU X., SUN M., MA X., WANG C., WANG Z. Preparation of magnetic ordered microporous carbon for the preconcentration of some phthalate esters followed by their determination by HPLC. Microchimica Acta, **182** (3), 879, **2015**.
37. YILMAZ P.K., ERTAS A., KOLAK U. Simultaneous determination of seven phthalic acid esters in beverages using ultrasound and vortex-assisted dispersive liquid-liquid micro-extraction followed by high-performance liquid chromatography. Journal of Separation Science, **37**, 2111, **2014**.
38. YAN H., CHENG X., LIU B. Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid-liquid microextraction coupled with gas chromatography. Journal of Chromatography B, **879** (25), 2507, **2011**.
39. MONTUORI P., JOVER E., MORGANTINI M., BAYONA J.M., TRIASSI M. Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles. Food Additives & Contaminants: Part A: Chemistry, Analysis, Control, Exposure & Risk Assessment, **25**, 511, **2008**.
40. ŞENLIK D. Evaluation of phthalate esters in pasteurized milk samples and their packages by gas chromatography and mass spectroscopy (GC-MS) (Master’s thesis, Middle East Technical University). **2014**.
41. MORADIAN F., EBRAHIMPOUR K., HEIDARI Z., POURZAMANI H. Release of phthalate esters in pasteurized milk samples with plastic packaging. International Journal of Environmental Health Engineering, **9** (1), 23, **2020**.
42. FARAJZADEH M.A., DJOZAN D., REZA M., MOGADDAM A., NOROUZI J. Determination of phthalate esters in cow milk samples using dispersive

liquid-liquid microextraction coupled with gas chromatography followed by flame ionization and mass spectrometric detection. Journal of separation science, **35** (5-6), 742, **2012**.
43. FIERENS T., VAN HOLDERBEKE M., WILLEMS H., DE HENAUW S., SIOEN I. Transfer of eight phthalates through the milk chain – A case study. Environment International, **51**, 1, **2013**.
44. FIERENS T., SERVAES K., VAN HOLDERBEKE M., GEERTS L., DE HENAUW S., SIOEN I., VANERMEN G. Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food and Chemical Toxicology, **50** (7), 2575, **2012**.
45. MONDAL R., CHAKRABORTY D., MAJUMDAR D. Phthalate Esters from Packaged Milk and Associated Human Health Risk: A Monte Carlo Probabilistic Simulation Approach. MAPAN-Journal of Metrology Society of India, **37** (2), 409, **2022**.
46. SELVARAJ K.K., MUBARAKALI H., RATHINAM M., HARIKUMAR L., SAMPATH S., SHANMUGAM G., RAMASWAMY B.R. Cumulative exposure and dietary risk assessment of phthalates in bottled water and bovine milk samples: A preliminary case study in Tamil Nadu, India. Human and Ecological Risk Assessment: An International Journal, **22** (5), 1166, **2016**.
47. JIA W., CHU X., LING Y., HUANG J., CHANG J. Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry. Journal of Chromatography A, **1362**, 110, **2014**.
48. SCHMID P., KOHLER M., MEIERHOFER R., LUZI S., WEGELIN M. Does the reuse of PET bottles during solar water disinfection pose a health risk due to the migration of plasticisers and other chemicals into the water? Water Research, **42**, 5054, **2008**.
49. ARFAEINIA L., DOBARADARAN S., NASRZADEH F., SHAMSI S., POURESHGH Y., ARFAEINIA H. Phthalate acid esters (PAEs) in highly acidic juice packaged in polyethylene terephthalate (PET) container: Occurrence, migration and estrogenic activity-associated risk assessment. Microchemical Journal, **155**, 104719, **2020**.
50. AHMED M.B.M., ABDEL-RAHMAN G.N.E., ZAGHLOUL A.H., NAGUIB M.M., SAAD M.M.E.D. Phthalates' releasing pattern in low pH beverages of fermented milk, fruit juice, and soft drink packaged in plastic bottles. Bioscience Research, **14** (3), 513, **2017**.
51. AYOFE N.A., OLADOYE P.O., JEGEDE D.O. Extraction and quantification of phthalates in plastic coca-cola soft drinks using high performance liquid chromatography (HPLC). Chemistry International, **4** (2), 85, **2018**.
52. SAJID M., BASHEER C., ALSHARAA A., NARASIMHAN K., BUHMEIDA A., AL QAHTANI M., AL-AHWAL M.S. Development of natural sorbent based micro-solid-phase extraction for determination of phthalate esters in milk samples. Analytica chimica acta, **924**, 35, **2016**.
53. AKOLAWOLE J.S., OKOYE P.A.C., OMOKPARIOLA D. Effect of storage on the levels of sodium benzoate in soft drinks sold in some Nigerian market with exposure and health risk assessment. Environmental Health and Toxicology: EHT, **37** (4), 30, **2022**.
54. The safety of beverages in plastic bottles. https://www.food-safety.com/articles/3930-the-safety-of-beverages-in-plastic-bottles.
55. UMEOCHO C.E., ONUEGBU T.U., NDUKA J.K. Effects of storage on some properties of selected soft drinks sold in Nigeria. Journal of Environmental Science, **17** (2), 184, **2021**.
56. World Health Organization Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum. https://www.who.int/publications/i/item/9789241549950 (24 April 2017).