



Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2017 14(3): 513-524.

OPEN ACCESS

Phthalates' releasing pattern in low pH beverages of fermented milk, fruit juice, and soft drink packaged in plastic bottles

Mohamed Bedair M. Ahmed^{1*}, Gomaa Nour-Eldein Abdel-Rahman¹, Ahmed Hassan Zaghloul², Mohamed Mahmoud Naguib¹ and Magdy Moheb El-Dein Saad¹

¹Department of Food Toxins and Contaminants, National Research Centre, 33 El-Bohouth St., Dokki, Cairo, **Egypt**.

²Department of Dairy Science, National Research Centre, 33 El-Bohouth St., Dokki, Cairo, **Egypt**.

*Correspondence: m.bedair.nrc@gmail.com Accepted: 29 June, 2017 Published online: 15 Sep. 2017

The present investigation was conducted to figure out the releasing pattern and rate of five phthalates from the plastic bottles into the bottled beverages of fermented milk, fruit juice, and soft drink. The studied phthalates were di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DBP), bis(2-ethyl hexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP). Phthalates were determined in fifty-four samples (eighteen samples from each beverage type) at the beginning, middle, and end of the shelf-life period in two different bottles sizes for from each beverage type. The resulted limits of detection and quantification for phthalates were in the range of 6.5 ± 2.5 and 20 ± 5 (ng), respectively. Recovery percentages ranged from 75.77 ± 3.06 to 82.95 ± 3.28 (%) in fermented milk, 77.68 ± 4.54 to 80.51 ± 3.06 (%) in fruit juice, and 80.09 ± 5.57 to 88.70 ± 6.72 (%) in soft drink. DnOP was the major detected compound in all the tested beverages, which had the highest concentrations (0.52 – 0.82 ppm) and releasing rates ($85.5 - 2116.7 \mu\text{g week}^{-1}$) followed by DEHP in fermented milk, DMP in juice, and DBP in soft drink samples. The released phthalates in the big bottles were significantly higher than in the small bottles. The highest values of total phthalates for the three beverages were lower than the tolerable daily intake (TDI) for an adult person by 3 -135 times, which means that the detected amounts of phthalates in the three beverages were within the safe limits.

Keywords: plastic bottled beverages; phthalates; safety assessment

INTRODUCTION

Phthalates (esters of phthalic acid) compounds are used in the plastics industry in order to enhance and improve its quality properties such as improving the flexibility, softness, and transparency (Saad 2014; Senlik 2014). Human long-term exposure to the phthalates, through the daily consumption of foods and beverages packaged in plastic bottles, are a great concern. This exposure to phthalates may cause many health problems such as disturbance in the endocrine system. This

disturbance could be observed as serious problems in the reproductive system of adult males (Duty et al. 2005; Hauser et al. 2007) and females (Colón et al. 2000; Lovekamp-Swan and Davis 2003), abdominal obesity, insulin resistance, and diabetes (Stahlhut et al. 2007; Hatch et al. 2010). As well, adverse effects of phthalates on the liver, kidneys, and testes were reported (David et al. 1999; David et al. 2000a; David et al. 2000b).

Phthalates can contaminate food during processing, handling, transportation, packaging

and storage (Wittassek et al. 2011; Senlik 2014). For example, PVC (polyvinyl chloride) tubing is mostly used in milking process or in the bulk transfer of milk between storage tanks. Feng et al. (2005) found that DEHP levels in samples of the milk transferring line were 10–20 times higher than the levels of teat samples, while the levels of DEP and DBP were very similar between the two groups of samples. Briefly, the previous studies proved the presence of phthalates in milk and dairy products (Sorensen 2006; Guo et al. 2012; Serrano et al. 2014), fruit juices (Page and Lacroix 1995; Guo et al. 2010; Al-Saleh and Elkhatab 2014), and soft drinks (Guo et al. 2012; Ustun et al. 2015; Bosnir et al. 2007).

The European Union assigned 0.3 and 1.5 (mg kg^{-1}) as maximum permissible limits for DBP and DEHP, respectively in foods (Commission directive 2007/19/EC). In addition, the tolerable daily intake (TDI) of phthalate compounds have been assigned for DBP, BBP (Benzylbutyl phthalate), DINP (Di-isononyl phthalate), and DEHP as follows: 0.01, 0.5, 0.15, and 0.05 ($\text{mg kg}^{-1} \text{ bw/day}$), respectively (SCHER 2008), while no TDI is available for DMP and DNOP. WHO (2003) had set the TDI for DEP as 0.5 $\text{mg kg}^{-1} \text{ bw/day}$.

Phthalates are not chemically bound to the plastic materials and can easily be released and transmitted to the food from packaging materials that are in contact (Castle et al. 1988; Staples et al. 1997; Saad 2014; Senlik 2014; Ustun et al. 2015; Saad 2016). Chemical releasing or migration of a compound depends on the chemical properties (i.e. polarity) and functional properties of the packaging material. The fat content of food affects the migration rates, as many packaging chemicals are lipophilic (have a greater ability to be dissolved in fats) and can therefore rapidly migrate into fatty foods with higher rates and levels (Robertson 2013; Muncke 2014). As well, the food pH affects the chemical migration (Bosnir et al. 2007). Product conditions of filling, storage, and shelf-life period can also affect the degree and rate of migration into food (Robertson 2013; Muncke 2014). Mechanical damage to the food packaging containers could potentially lead to greater chemical migration through the different changes in moisture, ambient oxygen, light, and temperature (Robertson 2013; Cirillo et al. 2013).

Fermented milk, fruit juices, and soft drinks are the most important beverages packaged in plastic containers, which are consumed on a daily basis. All of these beverages have low pH value,

and the fermented milk is a fat containing food as well. In addition, these beverages may be consumed within the shelf-life period, but after a long time from the production date, which indicates the length of contacting period between the food and plastic material. Previous studies checked the presence of phthalates only at random dates of shelf-life and did not expose to the tracing of phthalates at regular intervals of the shelf-life. Therefore, the present study was conducted on fermented milk, fruit juice, and soft drink packaged in plastic bottles (i) to assess the different concentrations of five phthalates through the beginning, middle, and end of the shelf-life to understand the releasing pattern of phthalates during the shelf-life period; (ii) to estimate the releasing rate for the five phthalates within the shelf-life period; (iii) to figure out whether the bottle's size has a significant impact on the released amounts of phthalates into the same food or not?; and (iv) to assess the safety of the studied beverages, containing phthalates, for human consumption.

MATERIALS AND METHODS

Low pH beverages

Fifty-four samples of commercial brands of fermented milk beverage with a fruit flavor (9 bottles with 440 mL of size + 9 bottles with 220 mL of size, pH: 4.39 ± 0.05 , 1.5% fat), fruit juice (9 bottles of 500 mL + 9 bottles of 250 mL, pH: 3.83 ± 0.02 , 0% fat), and (9 bottles of 1250 mL + 9 bottles of 400 mL, pH: 3.22 ± 0.07 , 0% fat) were obtained from local markets at Cairo city. From each beverage type, two different sizes of bottles were selected to study the impact of the bottle size on the released amounts of phthalates. The disposable bottles of the packaged beverages were made from polyethylene tere phthalate (PET), with some differences in thickness, transparency, and color based on the packaged food type. The collected samples were stored in the refrigerator from the production dates until the expiry dates (shelf-life period); 14 days for the fermented milk and 6 months for each of fruit juice and soft drink samples. During the storage time, the samples were analyzed at three intervals (beginning, middle, and end of the shelf-life) for each product.

Solvents

Acetonitrile, methanol (HPLC grade), and ethyl acetate were supplied by PA-ACS. Panreac Co,

EU. Sodium chloride was obtained from Merck Co (Darmstadt, Germany).

Standard materials

Five individual phthalates; di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DBP), bis(2-ethyl hexyl) phthalate (DEHP), and di-n-octyl phthalate (DnOP) were obtained from Restek Co, UK.

Apparatus

(a) HPLC

An Agilent 1100 series High Performance Liquid Chromatography (Agilent Technologies, Waldbronn, Germany), consists of a vacuum solvent degassing unit, a quaternary gradient pump, an automatic sample injector and diode array detector (DAD). Injection volume was 20 μ L, and the UV monitor was set to 225 nm. The system pressure was approximately 235 bar.

(b) Column

The separation was performed using Kromasil 10u 100A C18 (250 x 4.6 mm, 5 μ m particles, Phenomenex, Torrance, CA, USA.) column. The column temperature was set to 30 $^{\circ}$ C.

(c) Solvent system

The solvent system consisted of (A): acetonitrile/water 85:15 (v/v) and (B): acetonitrile. The gradient program was set as follows: 0.0 – 3.0 min (100% A), 3.0 – 6.5 min (changing from 100% A to 100% B), 6.5 – 19.5 min (100% B). The flow rate was 0.6 mL min⁻¹. The analysis time was 25 min and the run time was 45 min.

Standard preparation

(a) Individual and mixture solutions

Individual solutions of the five phthalates as well as a mixture solution for them were prepared in methanol as 1 mg mL⁻¹ for each and were stored in a deep freezer at -18 $^{\circ}$ C. Working solutions were freshly prepared daily by diluting the stock solutions using methanol HPLC grade and were stored in a refrigerator at 4-5 $^{\circ}$ C.

(b) Standard linear curves

Standards solutions containing 1, 10, 20, 50, and 100 μ g mL⁻¹ of the five phthalates in methanol were prepared and used for the preparation of calibration curves.

Extraction of phthalates

(a) Fermented milk

The five phthalates were extracted from the fermented milk according to the method of Li et al. (2011). Briefly, 10 mL acetonitrile was added to 2

g of milk in a 50 mL centrifuge tube, and then the mixture was homogenized in a vortex agitator. Sodium chloride (0.8 g) was then added to the mixture and vortexed once more. Following shaking for 15 min and sonication for 10 min, the mixture was then centrifuged at 4500 rpm for 10 min. The supernatant was transferred into a 30 mL round flask and evaporated to dryness using a vacuum rotary evaporating system. The dry residue was re-dissolved using 1.0 mL of methanol (HPLC grade) and frozen to precipitate the sodium chloride. The extract was then filtered through a 0.25 μ m syring filter before the injection into the HPLC.

(b) Juice

Phthalates were extracted from the fruit juice as follows: 5 mL of ethyl acetate was added to 5 mL juice sample in a 50 mL glass centrifuge tube. The tube was covered tightly, and the contents were mixed using a vortex mixer for 1 min and sonicated for 5 min followed by centrifugation for 15 min at 4500 rpm. The supernatant was transferred to 30 mL rotary flask and the residue was re-extracted using 5 mL ethyl acetate. The supernatant was combined and evaporated at 37 $^{\circ}$ C until full dryness. The dry residue was re-dissolved using 750 μ L (500 + 250) methanol (HPLC grade) and transferred to a 3 mL glass vial. The methanolic extract was finally filtered using a 0.25 μ m syring filter before the injection into the HPLC system.

(c) Soft drink

Phthalates were extracted from the soft drink as follows: A hundred milliliters of soft drink sample was evaporated in rotary apparatus at 38 $^{\circ}$ C up to 85 mL to discard the dissolved CO₂ and foaming agents. Ten milliliters of the evaporated sample was mixed with 20 mL ethyl acetate in a separating funnel, and the mixture was shaken hardly for 1 min and left for liquid-liquid partitioning. The lower layer (soft drink) was withdrawn and decanted into another separating funnel for the second extraction using 20 mL more ethyl acetate. The upper layer (ethyl acetate) of the two times of extractions was separated, then combined and evaporated to dryness at 38 $^{\circ}$ C using a vacuum rotary evaporating system. The dried extract was re-dissolved in 1 mL methanol HPLC grade, and the methanolic extract was withdrawn using a glass pasture pipette. The methanolic extract was then filtered through a 0.25 μ m syring filter before the injection into the HPLC.

Method validation

Samples of fermented milk, fruit juice, and soft drink were tested for the presence of phthalates, and the negative samples were used to estimate the recovery study for the extraction method. The extraction recovery experiment was carried out by spiking the samples with a concentration of 1000 ng mL⁻¹ using phthalates standard solution. The concentration of 1000 ng mL⁻¹ was selected as a mean concentration, where the majority of detected amounts of the five phthalates were located in the range of 0 and 2000 ng mL⁻¹. The different samples were extracted, and the concentrations of phthalates were estimated. The calculated recovery value for each food sample was a mean of 3 replicates. The recovery value was calculated using the following equation:

Extraction recovery (%) = (concentration of spiked sample – concentration of blank sample) × 100/concentration of added phthalate.

The method precision was evaluated by calculating the relative standard deviation percent (RSD%) for recovery values of the spiked replicates. The limits of detection (LODs) and the limits of quantification (LOQs) for phthalates were estimated based on the signal to noise ratios (S/N) which equal 3:1 and 10:1, respectively.

Phthalates releasing pattern

The slope values of phthalates concentrations during the three dates of shelf-life were estimated as indicators for the releasing pattern from plastic bottles into packaged food. The slope values were calculated using regression models for two covariates (storage time and concentration). Besides, the releasing rate of phthalates from the plastic bottles into the bottled food matrices was estimated as µg week⁻¹ using the following equation: Releasing rate = final concentration of phthalates/ shelf-life time as weeks.

Statistical analysis

Means, standard deviations, and relative standard deviations were calculated using Microsoft Office Excel (Microsoft Office 2007). Linear regressions were also generated using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Calibration curves and linearity

Three replicates were measured for each concentration level and the obtained calibration curves of phthalates showed a linearity

relationship between the concentrations and the obtained areas, with coefficient (R²) values ranged between 0.981 and 0.999, as listed in Table 1.

Method validation

The limits of detection and quantification (LODs and LOQs) for the five phthalates were estimated to assess the method sensitivity, which were in the range of 6.5 ± 2.5 and 20 ± 5 (ng), respectively as summarized in Table 1.

For the method selectivity, the five phthalates were well separated using HPLC-UV with a C18 column and a gradient elution with a mobile phase consisted of (A): acetonitrile/water 85:15 (v/v) and (B): acetonitrile, the retention times were shown in Table 1. The used method of estimation proved a good separation for the five phthalates, which were clearly distinguished from each other without neither an overlapping between the compounds nor an interference with the food matrices. That data ensured accuracy for the presented method of estimation. Concerning the extraction methods of phthalates from the three food matrices, it was clear from Table 2 that the recovery percentages for phthalates were in the range of 75.77±3.06 to 82.95±3.28 (%) in fermented milk, 77.68±4.54 to 80.51±3.06 (%) in fruit juice, and 80.09±5.57 to 88.70±6.72 (%) in soft drink.

In addition, the extraction method showed acceptable precision (RSD%) values at the spiked concentration with a minimum of 2.60% and a maximum of 7.58% (Table 2). Generally, the precision values did not exceed 10% as recommended by the EU directions (Commission Decision 2002/657/EC) for method performance, which assigned 10% as a maximum acceptable percent for RSD (%) with the biological samples.

Released amounts of phthalates through the different times of shelf-life

Summarized data in Fig.1. shows the released amounts of phthalates in the three studied beverages under investigation; where the fermented milk samples contained mean detectable amounts of DEP, DEHP, and DnOP at zero time of shelf-life which were 0.032, 0.335, and 0.110 (ppm), respectively with no significant differences between the samples of big and small bottles (Fig. 1. A). DMP and DBP were not detected in both sizes of fermented milk's bottles. The concentrations of DEP through the three tested dates of shelf-life were between 0.036 and 0.802 (ppm) in the big bottles, and between 0.004 and 0.701 (ppm) in the small bottles with no significant differences.

Table 1. Calibration data and detection limits of phthalates

Phthalates	Rt (Min) $\pm$ SD ¹⁾ (n=10)	Linearity range ($\mu\text{g mL}^{-1}$)	Linear equation	(R ²) ²⁾	LOD (ng)	LOQ (ng)
DMP	05.25 $\pm$ 0.09	1 - 100	y = 1048.x - 19019	0.984	4	15
DEP	06.30 $\pm$ 0.03	1 - 100	y = 1390.x - 34965	0.999	6	20
DBP	09.96 $\pm$ 0.24	1.5 - 100	y = 826.7x - 16352	0.992	9	25
DEHP	10.64 $\pm$ 0.33	1 - 100	y = 769.5x - 13482	0.981	6	20
DnOP	15.70 $\pm$ 0.11	1.5 - 100	y = 48.07x + 3286	0.999	8	25

¹⁾ Standards deviation²⁾ Correlation coefficient**Table 2. Recovery and precision percentages for phthalates extracted from fermented milk, juice, and soft drink**

Phthalates	Fermented milk		Juice		Soft drink	
	Recovery (%) $\pm$ SD (n=3)	RSD ¹⁾ (%)	Recovery (%) $\pm$ SD (n=3)	RSD (%)	Recovery (%) $\pm$ SD (n=3)	RSD (%)
DMP	77.94 $\pm$ 4.30	5.53	78.04 $\pm$ 3.26	4.18	80.51 $\pm$ 2.43	3.02
DEP	75.77 $\pm$ 3.06	4.04	77.99 $\pm$ 3.68	4.72	80.09 $\pm$ 5.57	6.96
DBP	76.51 $\pm$ 4.45	5.83	80.51 $\pm$ 3.06	3.81	88.70 $\pm$ 6.72	7.58
DEHP	80.39 $\pm$ 2.08	2.60	79.66 $\pm$ 4.78	6.00	85.09 $\pm$ 2.70	3.17
DnOP	82.95 $\pm$ 3.28	3.95	77.68 $\pm$ 4.54	5.84	86.83 $\pm$ 3.55	4.09

¹⁾ Relative standards deviation

The highest concentrations of phthalates in fermented milk were recorded for DnOP followed by DEHP. These results were in agreement with those of Feng et al. (2005) who detected DEHP at zero time of production with 0.111–0.282 ppm of concentration. In addition, Sorensen (2006) reported the same levels in milk. However, DnOP was not detected by both Feng et al. (2005) and Sorensen (2006).

For juice samples (Fig. 1. B), the released phthalates were observed in the samples of middle and end of the shelf-life. At the middle of shelf-life, DMP, DEP, and DnOP were detected with concentrations of 0.957, 0.706, and 1.396 (ppm) for the big bottles, while the small bottles contained 0.321, 0.253, and 0.951 (ppm), respectively. The five phthalates were detected in the big bottles at the end of shelf-life period with concentrations of 1.836, 1.355, 0.603, 0.617, and 2.613 (ppm), respectively for DMP, DEP, DBP,

DEHP, and DnOP. Meanwhile, DBP and DEHP were not detected in the small bottles of juice at the end of shelf-life period. These results were in accordance with Guo et al. (2010), who detected DEHP in the Chinese orange juice packaged in PVC bottle with a concentration of 0.662 ppm, at the end of shelf-life period, which was typically similar to DEHP in the present study. In another study by Al-Saleh and Elkhatib (2014), they found that averages of maximum detected amounts of DMP, DEP, DBP, DEHP, and DnOP, in apple juice of plastic bottles in Saudi Arabia, were 0.0009, 0.0102, 0.0018, 0.0018, and 0.0006 (ppm), respectively. However, the estimated levels of phthalates were lower than those of the present study.

Similarly, none of the five-phthalate compounds was observed in all samples of soft drink, from both bottle sizes, at zero time of the shelf-life (Fig. 1. C).

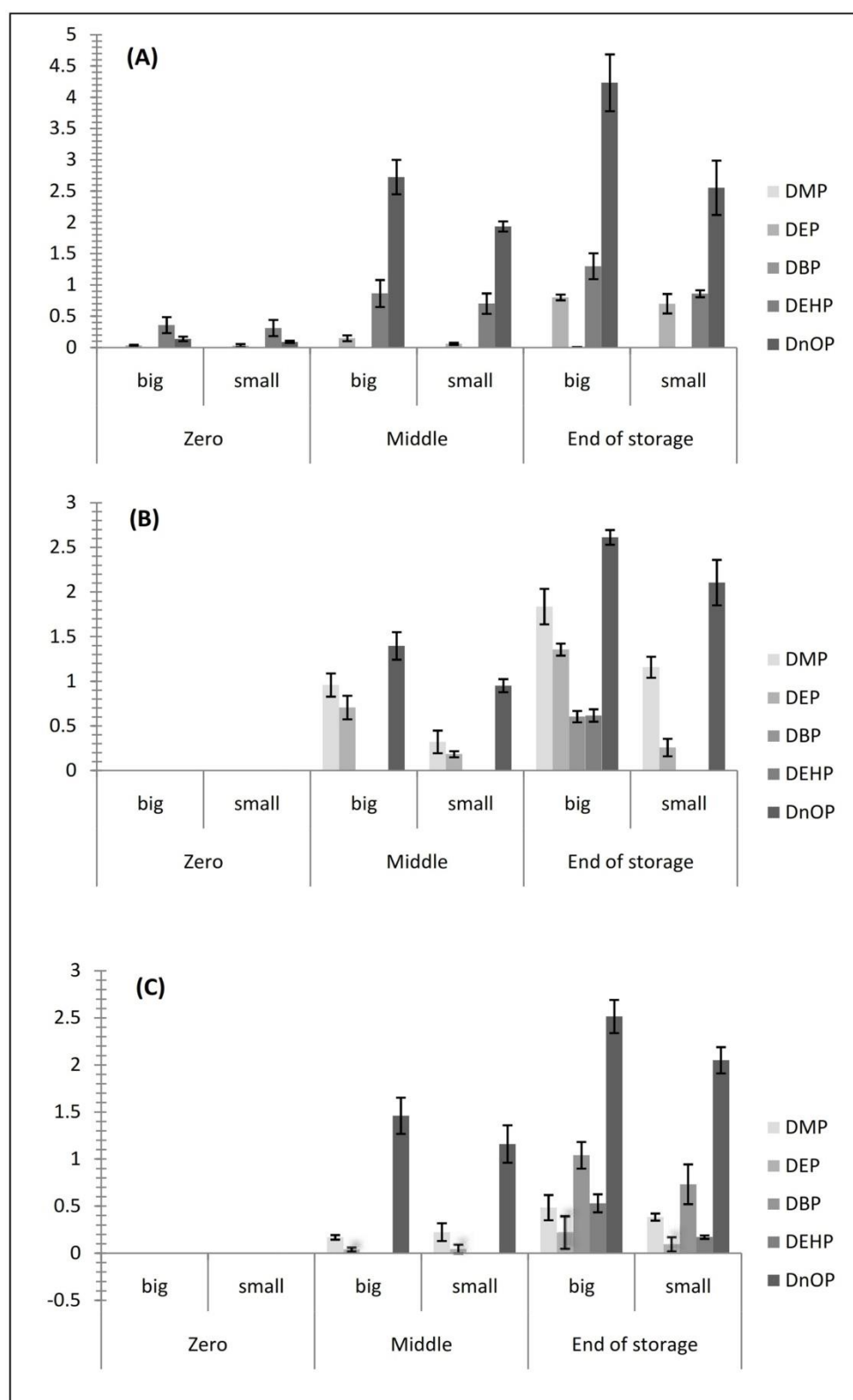


Figure. 1. Concentrations of phthalates in (A) fermented milk, (B) fruit juice, and (C) soft drink samples of big and small bottles during the shelf-life

However, DMP, DEP, and DnOP were detected in the middle of shelf-life with no significant differences between the concentrations of the detected phthalates for the big and the small bottles of soft drink. At the end of shelf-life, samples of the big and small bottles contained the five phthalates with no significant differences between the phthalates of the two different sizes of bottles except for DEHP and DnOP. DnOP, at the end of shelf-life, scored the highest concentrations in the big and small bottles, which were 2.515 and 2.051 (ppm) respectively. Ustun et al. (2015) detected DMP, DEP, DBP, and DEHP in soft drink; however, the study did not illustrate the size of plastic bottle and the time of sampling through the shelf-life period. The detected concentration of DEHP (0.633 ppm) was close to DEHP level of our study (0.531ppm) for big bottles of soft drink at the end of storage. On the other hand, Bosnir et al. (2007) detected lower concentrations of phthalates than those of our study except for DMP.

It was worthy to mention that both juice and soft drink samples had no detected amounts of the five phthalates at zero time of shelf-life. In addition, the results in the Fig. 1. revealed that, the released concentrations significantly increased in the all beverages with the storage time as confirmed by Guo et al. (2010). Moreover, the fermented milk samples contained higher concentrations of phthalates than in samples of juice and soft drink through the three dates of estimation. These elevated levels of phthalates in fermented milk samples are due to the following factors: 1) the cattle might be fed on phthalate-containing feed, and that might increase the milk content, 2) milk contamination from the contact materials (i.e. PVC tubing) during the mechanical milking, separation, pasteurization, standardization and cooling, 3) the phthalate migration can be accelerated by the applied heat during pasteurization (Wittassek et al. 2011; Heudorf et al. 2007; Fierens et al. 2013). Besides, the dairy products are rich in fat contents; and as phthalates are highly lipophilic (fat soluble) compounds, therefore the estimated concentrations of released phthalates were the highest in fermented milk (Fierens et al. 2013).

Moreover, the released amounts of phthalates in the samples of big bottles were significantly higher than those of the small bottles; this may be due to the long surface of contact between the bottled beverages and the internal wall of the big bottles compared to that of the small bottles. As well, may be the phthalate content in big bottles is

higher than in small bottles to make it more flexible and resistant to the mechanical damage. DnOP was the major compound of the estimated phthalates in all samples of the three tested foods, which had the highest concentrations followed by DEHP in fermented milk, DMP in juice, and DBP in soft drink samples. The high detected levels of DnOP in all tested foods may be due to the high ratio of DnOP in the commercial phthalates' plasticizer, which is used for manufacturing of plastic bottles in Egypt.

Releasing rates of phthalates through the shelf-life

The releasing pattern of phthalates through the shelf-life was evaluated by calculating the slope values of the drawn curves for the three dates of phthalates' determination (zero, middle, and end of shelf-life). The higher value of slope refers to the higher increasing in the released amounts over time. Besides, the releasing rate was calculated to show how much quantities of phthalates (μg) were released per the unit of time (one week) as an indicator for the releasing speed.

The highest slope value of released phthalates, in the big bottles of fermented milk (pH: 4.39 ± 0.05), was recorded for DnOP followed by DEHP, DEP, and DBP with values of 2.034, 0.437, 0.383, and 0.002, respectively as shown in Table 3. While in the small bottles, the slope values were less, which were descendingly arranged as follows: 1.259, 0.348, and 0.290 for DnOP, DEP, and DEHP, respectively. The same pattern was also observed for the releasing rate values. Where DnOP scored the highest rates in the big and small bottles, which were 2116.7 and 1277.4 ($\mu\text{g week}^{-1}$), respectively. On the contrary, DEP had the lowest releasing rate in both sizes of fermented milk's bottles (Table 3).

Concerning the slope value of released phthalates in fruit juice samples (pH: 3.83 ± 0.02), DMP followed DnOP in the values of slope which were 1.307 and 0.918 in the big bottles, and were 1.053 and 0.578 in the small bottles, respectively for DnOP and DMP (Table 3). Similarly, the highest releasing rate had been scored by DnOP followed by DMP in both big and small bottles. The scored rates were 108.9 and 76.5 in the big bottles, and were 87.8 and 48.2 ($\mu\text{g week}^{-1}$) in the small bottles for DnOP and DMP, respectively. DEP and DBP had the lowest values of slope and releasing rate in the big bottles, while they were not detected in the small bottles.

Table 3. Releasing pattern of phthalates during the shelf-life

Bottle Size	Phthalates	Fermented milk		Fruit juice		Soft drink	
		Slope	Releasing rate ¹⁾	Slope	Releasing rate	Slope	Releasing rate
Big	DMP	ND ²⁾	ND	0.918	76.5	0.242	20.2
	DEP	0.383	401.1	0.677	56.5	0.108	9.1
	DBP	0.002	2.9	0.301	25.2	0.520	43.4
	DEHP	0.437	650.5	0.308	25.7	0.265	22.1
	DnOP	2.034	2116.7	1.307	108.9	1.257	104.8
Small	DMP	ND	ND	0.578	48.2	0.192	16.0
	DEP	0.348	350.9	0.128	10.7	0.047	3.9
	DBP	ND	ND	ND	ND	0.366	30.6
	DEHP	0.290	430.6	ND	ND	0.085	7.1
	DnOP	1.259	1277.4	1.053	87.8	1.025	85.5

¹⁾ Releasing rate, ($\mu\text{g week}^{-1}$)²⁾ ND, not detected or below detection limit

Obtained data in Table 3 revealed that phthalates in soft drink samples (pH: 3.22 ± 0.07) had the same pattern of those in the juice samples. Where DnOP scored the highest slope value and releasing rate, which were 1.257 and $104.8 \mu\text{g week}^{-1}$ for the big bottles and were 1.025 and $85.5 \mu\text{g week}^{-1}$ for the small bottles. On the other hand, the lowest values of releasing slope and rate in this respect were recorded for DEP in both big and small bottles. These results were in contrary with those of Bosnir et al. (2007), who stated that the highest releasing rate was recorded by DMP in soft drink.

The higher releasing rates of phthalates in fermented milk can be illustrated as mentioned above about the lipophilic characteristic of phthalates and the high-fat content of fermented milk; these characteristics can accelerate the releasing rate of phthalates from the plastic bottle into the fermented milk drink over time. Moreover, the releasing rates of phthalates in samples of both juice and soft drink were relatively similar, as they are not containing fats and their pH values were close.

Safety assessment.

Due to the different habits of food consumption between people in the different regions of Egypt and lack of data, the daily intake of phthalates from plastic bottled beverages was

calculated based on assumption. Assuming that an adult person (70 kg) could consume one small bottle from each of the three studied beverages (220 mL fermented milk, 250 mL fruit juice, and 400 mL soft drink) per day.

Based on this assumption, the total ingested amounts of phthalates from the three beverages at the three tested dates of shelf-life period were shown in Table 4. The TDI of DEP, DBP, and DEHP were shown in the table; however, the TDI of DMP and DnOP are not available. The total amounts of released phthalates in the three beverages, at the end of shelf-life, scored the highest values compared with the values of zero time and middle of the shelf-life. Although, these amounts of DEP, DBP, and DEHP were the highest at the end of shelf-life, but they still lower than the TDI for 70 kg person by 3, 14, and 135 times, respectively. As a result, the values of zero time and middle of shelf-life are safer, taking into considerations the other sources of exposure to phthalates. Consequently, it is highly recommended to consume the plastic bottled beverages, particularly those with the low-pH and fat-containing properties, at the early dates of the shelf-life period.

Table 4. Amounts of phthalates (mg) in the small bottles of studied beverages compared to the recommended total daily intake

Phthalates	TDI1) (mg/kg bw/day)	TDI2)	Zero time of shelf-life				Middle of shelf-life				End of shelf-life			
			A	B	C	Total	A	B	C	Total	A	B	C	Total
DMP	-	-3)	ND4)	ND	ND	ND	ND	0.0804	0.0897	0.1701	ND	0.2894	0.1541	0.4435
DEP	0.01	0.7	0.001	ND	ND	0.001	0.0131	0.0633	0.0176	0.094	0.1544	0.0475	0.0376	0.2395
DBP	0.05	3.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.2933	0.2933
DEHP	0.5	35	0.0616	ND	ND	0.0616	0.1547	ND	ND	0.1547	0.1895	ND	0.0685	0.258
DnOP	-	-	0.0079	ND	ND	0.0079	0.4254	ND	0.4642	0.8896	0.5621	0.5267	0.8204	1.9092

¹⁾ Total daily intake²⁾ TDI for an adult person 70 kg³⁾ Not assigned⁴⁾ ND, Not detected

A: fermented milk (220 mL)

B: Soft drinks (400 mL)

C: Fruit juice (250 mL)

CONCLUSION

The determination method for phthalates in the present study proved good sensitivity and selectivity for the five phthalates in the samples matrices. As well, the extraction method showed elevated recovery percentages with high precision, which matching with the European criteria for method performance. It was worthy to mention that DnOP was the primary detected compound, in all the tested beverages, with the highest releasing rate followed by DEHP in fermented milk, DMP in juice, and DBP in soft drink. Furthermore, released amounts of phthalates in the big bottles were significantly higher than those in the small bottles. The highest values of total phthalates for the three beverages were lower than the tolerable daily intake (TDI) for an adult person by 3 -135 times, which ensure the safety of these beverages for human consumption.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

ACKNOWLEDGEMENT

This work was funded by the National Research Centre; research project No. 10110302 (2013 – 2016). Instrumental analyses were supported by Food Toxins and Contaminants Dept., and the Central Laboratory of the National Research Centre, Egypt

AUTHOR CONTRIBUTIONS

MBMA collected the samples, performed the method validation, analyzed of samples and data, and also wrote the manuscript. GNA and AHZ collected the samples and performed the analysis of samples and data. MMN and MMS designed the study and reviewed the manuscript. All authors read and approved the final version.

Copyrights: © 2017 @ author (s).

This is an open access article distributed under the terms of the [Creative Commons Attribution License \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author(s) and source are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

REFERENCES

- Al-Saleh I, Elkhatib R, 2014. Analysis of phthalates residues in apple juices produced in Saudi Arabia. *Food Measure.*, 8: 373–380.
- Bosnir J, Puntaric D, Galic A, Skes I, Dijanic T, Klaric M, Grgic M, Curkovic M, Smit Z, 2007. Migration of phthalates from plastic containers into soft drinks and mineral water. *Food Technol. Biotech.* 45(1): 91–95.
- Castle L, Mercer AJ, Startin JR, Gilbert J, 1988. Migration from plasticized films into foods 3. Migration of phthalate, sebacate, citrate and phosphate esters from films used for retail food packaging. *Food Addit. Contam.*, 5(1): 9-20.
- Cirillo T, Fasano E, Esposito F, Prete ED, Cocchieri RA, 2013. Study on the influence of temperature, storage time and packaging type on di-n-butylphthalate and di (2-ethylhexyl) phthalate release into packed meals. *Food Addit. Contam.: Part A.*, 30(2): 403-411.
- Colón I, Caro D, Bourdony CJ, Rosario O, 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. *Environ. Health Persp.*, 108(9): 895-900.
- Commission Decision 2002/657/EC. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official J. Europ. Union.* L221: 8-36.
- Commission directive 2007/19/EC of 30 march 2007 amending directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and council directive 85/572/EC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs. *Official J. Europ. Union.*, 91: 17-36.
- David RM, Moore MR, Cifone MA, Finney DC, Guest D, 1999. Chronic peroxisome proliferation and hepatomegaly associated with the hepatocellular tumorigenesis of di (2-ethylhexyl) phthalate and the effects of recovery. *Toxicol. Sci.*, 50(2): 195-205.
- David RM, Moore MR, Finney DC, Guest D, 2000a. Chronic toxicity of di (2-ethylhexyl) phthalate in rats. *Toxicol. Sci.*, 55(2): 433-443.
- David RM, Moore MR, Finney DC, Guest D, 2000b. Chronic toxicity of di (2-ethylhexyl) phthalate in mice. *Toxicol. Sci.*, 58(2): 377-385.

- Duty SM, Calafat AM, Silva MJ, Ryan L, Hauser R, 2005. Phthalate exposure and reproductive hormones in adult men. *Hum. Reprod.*, 20(3): 604-610.
- Feng YL, Zhu J, Sensenstein R, 2005. Development of a headspace solid-phase microextraction method combined with gas chromatography mass spectrometry for the determination of phthalate esters in cow milk. *Anal. Chim. Acta.*, 538(1): 41-48.
- Fierens T, Van Holderbeke M, Willems H, De Henauw S, Sioen I, 2013. Transfer of eight phthalates through the milk chain—A case study. *Environ. Int.*, 51: 1-7.
- Guo Y, Zhang Z, Liu L, Li Y, Ren N, Kannan K, 2012. Occurrence and profiles of phthalates in foodstuffs from China and their implications for human exposure. *J. Agric. Food Chem.*, 60(27): 6913-6919.
- Guo Z, Wei D, Wang M, Wang S, 2010. Determination of six phthalic acid esters in orange juice packaged by PVC bottle using SPE and HPLC-UV: Application to the migration study. *J. Chromatogr. Sci.*, 48(9): 760-765.
- Hatch EE, Nelson JW, Stahlhut RW, Webster TF, 2010. Association of endocrine disruptors and obesity: perspectives from epidemiological studies. *Int. J. Androl.*, 33(2): 324-332.
- Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, Calafat AM, 2007. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Hum. Reprod.*, 22(3): 688-695.
- Heudorf U, Mersch-Sundermann V, Angerer J, 2007. Phthalates: toxicology and exposure. *Int. J. Hyg. Environ. Heal.*, 210: 623-634.
- Li Z, Xue F, Xu L, Peng C, Kuang H, Ding T, Xu C, Gong Y, Wang L, 2011. Simultaneous determination of nine types of phthalate residues in commercial milk products using HPLC-ESI-MS-MS. *J. Chromatogr. Sci.*, 49(4): 337-343.
- Lovekamp-Swan T, Davis BJ, 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. *Environ. Health Persp.*, 111(2): 139-145.
- Muncke J, 2014. Hazards in Food Contact Material: Food Packaging Contaminants. In Montarjemi Y, Moy GG, Todd ECD, eds, *The Encyclopedia of Food Safety*, Vol 2. Elsevier, Boston, pp 430-437.
- Page BD, Lacroix GM, 1995. The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985–1989: A survey. *Food Addit. Contam.*, 12(1): 129-151.
- Robertson GL, 2013. *Food packaging: Principles and Practice*. 3rd edition. CRC Press Taylor & Francis Group, Boca Raton, Florida. pp: 607-617.
- Saad MM, 2014. The most recent hazards of phthalates that threaten food safety and human health. *Int. J. of Med. Sci. Clin. Inven.*, 1(10): 527-535.
- Saad MM, 2016. The Risk of using Plastic Packaging's in Food Industry Referring to Phthalate Hazards. *Nutr. Food Tech.*, 2(2): 1-2.
- SCHER (Scientific Committee on Health and Environmental Risks), 2008. Opinion on phthalates in school supplies. European Commission. Available from: http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_106.pdf. Accessed Nov. 13, 2016.
- Senlik D, 2014. Evaluation of phthalate esters in pasteurized milk samples and their packages by gas chromatography-mass spectroscopy (GC-MS). PhD Thesis. Middle East Technical University, Ankara, Turkey. Available from: <http://etd.lib.metu.edu.tr/upload/12616725/index.pdf>. Accessed Nov. 13, 2016.
- Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S, 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. *Environ. Health.*, 13(1): 1-14.
- Sorensen LK, 2006. Determination of phthalates in milk and milk products by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Sp.*, 20(7): 1135-1143.
- Stahlhut RW, van Wijngaarden E, Dye TD, Cook S, Swan SH, 2007. Concentrations of urinary phthalate metabolites are associated with increased waist circumference and insulin resistance in adult US males. *Environ. Health Persp.*, 115(6): 876-882.
- Staples CA, Peterson DR, Parkerton TF, Adams WJ, 1997. The environmental fate of phthalate esters: a literature review. *Chemosphere.*, 35(4): 667-749.
- Ustun I, Sungur S, Okur R, Sumbul AT, Oktar S, Yilmaz N, Gokce C, 2015. Determination of phthalates migrating from plastic containers into beverages. *Food Anal. Method.*, 8: 222-228.
- Wittassek M, Koch HM, Angerer J, Brüning T, 2011. Assessing exposure to phthalates—the human biomonitoring approach. A review.

Mol. Nutr. Food Res., 55(1), 7-31.

World Health Organisation (WHO), 2003. Diethyl Phthalate. Concise International Chemical Assessment Document 52. World Health Organization, Geneva, Switzerland. Available from:

<http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf>_ Accessed Nov. 13, 2016.