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THE EFFECTS OF STORAGE TEMPERATURE AND TIME ON THE LEVELS OF PHTHALATES IN COMMERCIAL PET-BOTTLED WATER

(Kesan Suhu dan Masa Penyimpanan Terhadap Kandungan Ftalat dalam Air Berbotol PET Komersial)

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Abstract

This study was conducted to determine the effects of storage temperature and time on the levels of phthalates in commercial PET-bottled water. Freshly-produced water samples consisted of drinking, mineral, and sparkling in PET bottles were collected from a manufacturing site and subjected to different storage temperatures (refrigeration temperature of 4 °C, room temperature of 25 °C, and 40 °C) and times (0 month as control, 1.5 months, 3 months, and 6 months). Six different phthalates were analyzed using LC-MS/MS with deuterated bis(2-ethylhexyl)phthalate (DEHP) as internal standard. DEHP in the PET-bottled water was detected in the range from 2.32 to 27.6 ng/mL for 3- and 6-month storage samples; higher than di-n-octyl phthalate (DnOP) detected in the range from 1.57 to 12.6 ng/mL. Higher levels of DEHP and DnOP in PET-bottled mineral water were detected at room temperature of 25 °C when compared to refrigeration temperature of 4 °C, and 40 °C at 6-month storage. Higher level of DEHP and DnOP in drinking water was found at 6 months compared to 3 months in refrigeration temperature of 4 °C. The pronounced effects of storage temperatures on the levels of phthalates was observed only after 6 months of storage in which DEHP levels exceeded the maximum established limit of 6 ng/mL.

Keywords: PET-bottled water, phthalates, storage temperature, storage time, liquid chromatography-mass spectrometry/mass spectrometry.

Abstrak

Kajian ini dilakukan untuk mengetahui kesan suhu dan masa penyimpanan terhadap kandungan phthalates dalam air berbotol PET komersial. Sampel air yang baharu dihasilkan di pusat pengilangan terdiri daripada air minuman, air mineral, dan air soda yang dibotolkan dengan menggunakan botol PET telah dikumpulkan dan disimpan di dalam suhu (suhu penyejukan 4 °C, suhu bilik 25 °C, dan 40 °C) dan masa (0 bulan sebagai kawalan, 1.5 bulan, 3 bulan, dan 6 bulan) yang berbeza. Enam jenis ftalat dianalisa dengan menggunakan LC-MS/MS dan bis(2-etilheksilt)ftalat (DEHP) deuterasi sebagai piawai dalaman. DEHP dalam air botol PET dikesan dalam julat 2.32 hingga 27.6 ng/mL untuk sampel penyimpanan 3- dan 6 bulan; lebih tinggi daripada di-n-oktil ftalat (DnOP) yang dikesan dalam julat 1.57 hingga 12.6 ng/mL. Tahap DEHP dan DnOP yang lebih tinggi dalam air mineral botol PET dikesan pada suhu bilik 25 °C jika dibandingkan dengan suhu penyejukan 4 °C, dan 40 °C pada penyimpanan 6 bulan. Tahap DEHP dan DnOP yang lebih tinggi dalam air minuman didapati pada 6 bulan berbanding 3 bulan pada suhu penyejukan 4 °C. Kesan suhu penyimpanan yang ketara kepada kandungan ftalat diperhatikan hanya selepas penyimpanan selama 6 bulan di mana tahap DEHP melebihi had maksimum yang ditetapkan iaitu 6 ng/mL.

Kata kunci: air berbotol PET, ftalat, masa penyimpanan, suhu penyimpanan, kromatografi cecair-spektrometri jisim/spektrometri jisim

Introduction

Changes in human lifestyle have affected the development and innovation of food packaging [1]. Bottled water are mostly obtainable in two major forms of packaging material: plastic and glass [2, 3]. About 80% of plastic bottles made today are of polyethylene terephthalate (PET) due to the desired characteristics of its chemical and physical properties such as lightweight, clarity, strength, and easy recyclability [4]. A class of compounds named phthalates are utilised extensively as a plasticiser in the manufacturing of various plastics such as consumer products, including food contact applications [5, 6]. Phthalates cause severe health risks such as endocrine disruption, cancers and development and reproductive effects [7,8]. United States Environmental Protection Agency regulates bis(2-ethylhexyl)phthalate (DEHP) and bis(2-ethylhexyl)adipate (DEHA) as National Primary Drinking Water Regulations under the Safe Drinking Water Act. The maximum contaminant level of DEHP in drinking water was set at 6.0 ng/mL [9]. In addition, World Health Organization stated a guideline of 8.0 ng/mL of DEHP in fresh and drinking waters [10,11].

Malaysia is a tropical country with a fairly constant temperature of 28 – 32 °C all year round [12]. Storing plastic bottled water outdoor or exposing them to sun light by some markets are a very common practice in countries such as Iraq [13] and China [14], including Malaysia. Phthalates are not covalently bound to plastics and therefore, they can be easily migrated from packaging materials into foods in extreme conditions (e.g. high temperature) over time [15, 16]. Numerous research have reported that time and temperature [17,18], fat content [19,20], pH [17,21-23], contact surface [17], and exposure to sunlight [24-26] highly influence the migration of phthalates. Storage time besides the temperature are considered as the most important factors [17, 18, 27].

Keresztes et al. [17] detected DEHP after keeping PET-bottled mineral water at 22 °C for 44 days and further reported that the leaching of DEHP was the highest on samples that were kept over 1200 days. However, Al-Saleh et al. [28] observed higher levels of phthalates (i.e., DEHP, Benzyl butyl phthalate (BBP), Diethyl Phthalate (DEP), and Dimethyl phthalate (DMP)) in bottled water stored at 4 °C (1-month storage) than samples kept in room temperature (2-month storage) and outdoors (40-45 °C for 3 months). In addition, Amiridou and Voutsa [29] did not detect DMP, di-n-octyl phthalate (DnOP), and BBP in PET-bottled drinking and mineral water samples stored outdoors and directly exposed to sunlight up to 40 °C for up to 30 days. Literature reported indistinct effects of storage temperature and time on the migration of phthalates. Thus, it is worth to be further studied. Additionally, Erythropel et al. [30] cited regional differences as one of the factors that might cause the variability in the levels of phthalates reported in literature. Therefore, the prevalence in PET-bottled water in Malaysia needs to be determined.

Moreover, recent literature have predominantly reported migration of phthalates from PET bottles into acidic liquids such as verjuice, lemon juice, and vinegar [23], sparkling water [22], drinking water [28, 29], soft drink [21, 31] and mineral water [11, 17, 21, 22, 29, 32]. All the aforementioned samples were either randomly gathered from the market [11, 14, 17, 21, 22, 28, 29, 32] or prepared in the laboratory [23] or using food stimulants [24, 33, 34] and treated to different temperatures and time conditions. Studies that purchased PET-bottled water from the market were likely to suffer from limitations such as passed shelf life, therefore the results did not accurately represent the migration.

Most manufacturers recommend the shelf life for bottled water of no more than two years for non-carbonated water, and one year for sparkling water [35]. Accelerated stability test is one of the methods to estimate shelf life in which a product is subjected to elevated stress surroundings that induces the chemical and biochemical changes in shorter time [36]. It is known that phthalate migration is accelerated by heat [2, 3]. Therefore, accelerated stability test was conducted in this study to determine the extent of phthalate migration in bottled water when subjected to different storage temperatures. Furthermore, studies pertaining to the phthalates' migration from PET-bottled into drinking, mineral, and sparkling water, particularly those freshly-produced and collected from a manufacturing site and subsequently kept sealed in their original packaging throughout storage study, are in scarcity. Therefore, this study's aim to evaluate the effects of storage temperature and time on the levels of phthalates in commercial PET-bottled water.

Materials and Methods

Characteristics of samples

Seventy-two samples of different types of water, i.e., freshly-produced drinking, mineral, and sparkling water in PET bottles were collected from a manufacturing site. For each type of PET-bottled water, two different batches of production were collected from Company A. In addition, two samples of mineral water in glass bottles of the same batch number manufactured by Company B commercially available in the market were also collected. The two batches of mineral water in PET bottles were in 600-mL containers. The size of mineral water in glass bottles from company B was 325 mL. On the day of sampling at the manufacturing site, drinking and sparkling water in PET bottles of different batches of the same size were not available. Therefore, drinking water of 1 L for the first batch and 600 mL for the second batch were sampled. Similarly, sparkling water of 325 mL for the first batch and 1 L for the second batch were sampled. Company A is located in Taiping, Perak, Malaysia whereas Company B is located near Evian-les-Bains, France (46°23' 00.0"N 6°35' 00.0"E). Mineral water in glass bottles manufactured by Company B that commercially available in the market were collected since none of the manufacturers of bottled-water in Malaysia is producing this product. The 0-month control samples and mineral water in glass bottles were analyzed a soon as the samples reached the laboratory. PET-bottled water samples were subjected to different storage temperatures (4 °C refrigeration temperature, 25 °C room temperature, and 40 °C) and times (0 month as control, 1.5 months, 3 months, and 6 months). The collected mineral water in glass bottles however, were not subjected to storage study. A chiller was used to maintain the refrigeration temperature of 4 °C, whereas an oven was used to keep the storage temperature of 40 °C. Room temperature of 25 °C was attained by keeping the bottled water samples in an air-conditioned room in which the temperature of the air-conditioner was set at 25 °C. Samples were kept sealed in their original packaging and stored at room temperature, not exceeding one week prior to the time of analysis. Phthalates analysis was performed on every batch of PET-bottled water and on replicate samples of mineral water in glass bottles to obtain the levels of phthalates in the mean of duplicate determination.

Phthalates analysis

Reagent and materials

EPA 606-M Phthalate Esters Mix (DEHP, DEP, DMP, DnOP, Dibutyl Phthalate (DBP), and BBP) at the 200 μg/mL concentration was obtained from Supelco (Bellefonte, PA, USA). Bis(2-ethylhexyl)phthalate-3,4,5,6-d4 analytical standard (DEHP d4) that was used as internal standard throughout the study was purchased from Sigma-Aldrich Chemicals (St. Louis, USA). High purity analytical grade of methanol was purchased from Sigma-Aldrich Chemicals (St. Louis, USA).

Phthalate Esters Mix (1 µg/mL) and DEHPd4 (1000 mg/L) stock solutions were prepared by dissolving the compound in methanol. Intermediate standard for Phthalate Esters Mix was prepared by diluting the stock solution to concentrations of 50 ng/mL and 100 ng/mL with methanol. Intermediate standard for DEHPd4 was prepared by diluting the stock solution to the concentration of 1 µg/mL with methanol. All stock solutions and intermediate standards were stored in refrigerator at 4°C for maximum of 1 year.

Analysis methods

The scheme described by Schreiber et al. [37] was followed. One mL of water sample was added into a 10-mL volumetric flask, and made into volume with methanol. Internal standard with concentration of 100 ng/mL was spiked into the volumetric flask and mixed well. The mixture was then allowed to stand for 5 minutes before being transferred into vial for LC-MS/MS analysis.

Phthalates in bottled water were detected using PerkinElmer Flexar UHPLC AS system (PerkinElmer, Waltham, Massachusetts, US) coupled with Q TRAP® 3200 Linear Ion Trap Quadrupole LC-MS/MS performed in Multiple Reaction Monitoring (MRM) mode (AB Sciex, Framingham, Massachusetts, USA). Phthalates standards were prepared in concentrations of 0.5, 1.0, 5.0, 10.0, 20.0, and 50.0 ng/mL. Phthalate internal standard ($DEHP_{D4}$) was prepared at the concentration of 10.0 ng/mL. Volume of injection was 20 µL.

Phthalates separation was achieved under gradient conditions using Phenomenex Synergi Fusion RP C_{18} (100 mm x 2.0 mm x 2 µm) column and mobile phase of fast gradient water with 0.1% formic acid and acetonitrile with 0.1% formic acid used at flow rate of 400 µL/min. The MS/MS transition monitored was m/z 279>205 for DBP, 223>177 for DEP, 195>163 for DMP, 391>261 for DnOP, 313>205 for BBP, 391>279 for DEHP, and 395>171 for $DEHP_{D4}$.

The transition m/z values of 279>205 for DBP, 223>177 for DEP, 195>163 for DMP, 391>261 for DnOP, 313>205 for BBP, 391>279 for DEHP, and 395>171 for $DEHP_{D4}$ were used for quantification. For confirmation of peak identity, m/z values of 279>149 for DBP, 223>149 for DEP, 195>133 for DMP, 391>149 for DnOP, 313>149 for BBP, 391>167 for DEHP, and 395>153 for $DEHP_{D4}$ were used. A calibration graph was constructed by plotting peak area of phthalates relative to the internal standard against corresponding ratios of amounts of analyte. Calibration curves of phthalates were linear ($R^2 > 0.999$). Phthalates levels in samples were calculated from the calibration slope and intercept value. The limit of detection (LOD) and quantitation (LOQ) were 0.5 ng/mL and 1.5 ng/mL respectively. The recoveries were in the range between 80 and 120%.

Statistical analysis

All data obtained were analysed by using SPSS Version 21.0 (SPSS Inc., Chicago, IL). Two-way ANOVA with Tukey's Test was utilized to determine the differences in the means of phthalates levels when treated at different storage time and temperature. P-value of 0.05 or less was considered significant.

Results and Discussion

Freshly-produced drinking, mineral, and sparkling water samples in PET bottles were collected from a manufacturing site and subjected to different storage temperatures and times. Table 1 shows that all types of phthalates were not detected in the freshly produced water (0-month storage) for all types of PET bottles. The findings are in contrast with Amiridou and Voutsa [29] who detected DEHP at median level of 0.350 ng/mL, while DBP and DEP were found at lower levels of 0.044 and 0.033 ng/mL respectively. The researchers however did not detect DMP, BBP, and DnOP in PET-bottled drinking and mineral water samples in Greece that were analyzed immediately upon purchase. Similarly, Zaki and Shoeib [38] reported DEHP was the most abundant phthalate compounds, ranging from 0.062 to 0.298 ng/mL, while DBP was the second-most abundant, detected at levels ranging from 0.043 to 0.071 ng/mL. The researchers also did not detect DEP, DMP, BBP, and DnOP in

PET-bottled water samples in Egypt analyzed immediately after purchasing (~2 weeks after production). The presence of DEHP, DBP, and DEP in cited studies and absence of the contaminants in the freshly-produced samples (0-month storage) for all types of PET-bottled water in the present study may be due to different production facilities and different brands tested as observed by other researchers [28,38].

Although all types of phthalates were not detected in the 1.5-month storage samples, DEHP in PET-bottled water was detected in the range of 2.32 to 27.6 ng/mL for 3- and 6-month storage samples; higher than DnOP detected in the range of 1.57 to 12.6 ng/mL. The findings are in agreement with numerous authors who reported high levels of phthalates in PET-bottled water upon storage, especially DEHP [17,28,29]. European Authorities banned Low Molecular Weight (LMW) phthalates such as DEHP (1B Category) for use in cosmetics, children articles, toys, and medical devices as it is considered as toxic to human's reproduction system [39]. However, High Molecular Weight (HMW) phthalates such DnOP has only been prohibited on items that toddlers can suck & chew on such as children articles and toys (1999/815/EC and directive 2005/84/EC). It appears that present study detected higher levels of LMW (i.e., DEHP) phthalates when subjected to 3- and 6-month storage than HMW (i.e., DnOP) phthalates in the PET-bottled water of drinking, mineral, and sparkling samples. To protect human health, the European Food Safety Authority (EFSA) has set Tolerable Daily Intakes (TDI) for DEHP at 50 µg kg^{-1} body weight (bw) [40]. Different authors discussed that the presence of phthalates in bottled water can be attributed to several factors; among them are (i) the quality of the raw material and the technology used in bottle production [29], or the chemicals used in the production process [41] and (ii) the use of recycled PET [42].

Table 1 indicates that higher levels of DEHP and DnOP in PET-bottled mineral water (room temperature of 25 °C) were detected when compared to refrigeration temperature of 4 °C, and 40 °C at 6-month storage; the difference however, was not significant. Similarly, no significant difference in DEHP and DnOP levels was detected among different storage temperatures for drinking and sparkling water at 6-month storage. These findings are presented in Figure 1 for DEHP and Figure 2 for DnOP. The findings are consistent with the work of Leivadara et al. [25] who reported higher DEHP levels (up to 2 ng/mL) when bottled water was kept at room temperature (24 °C for 3 months in dark) as compared with DEHP was not detected when stored outdoor (30 °C for 3 months). In addition, Al-Saleh et al. [28] observed that the levels of DEHP in bottled water samples stored at 4 °C for 1 month were significantly higher than those stored at room temperature for 2 months and outdoors (> 45 °C) for 3 months. However, Schmid et al. [24] reported DEHP levels escalated in PET-bottled water kept at a higher temperature (direct sunlight, up to 34 °C for 17 hours) compared to lower temperature (room temperature in the dark). In general, high temperature accelerates the migration [2,3]. However, no clear patterns was observed with regards to storage temperature's effect on DEHP's level have been generated, with reports of contradictory observations on increasing concentrations [2, 24] and decreasing concentrations of DEHP [25, 28].

Table 1 indicates that higher levels of DEHP and DnOP in PET-bottled mineral water at room temperature of 25 °C were detected when compared to refrigeration temperature of 4 °C, and 40 °C at 6-month storage; the difference however, was not significant. Similarly, no significant difference in DEHP and DnOP levels was detected among different storage temperatures for drinking and sparkling water at 6-month storage. These findings are presented in Figure 1 for DEHP and Figure 2 for DnOP. The findings are consistent with the work of Leivadara et al. [25] who reported higher DEHP levels (up to 2 ng/mL) when bottled water was stored at room temperature (24 °C in the dark for 3 months) as compared with storage at 30 °C under outdoor conditions for 3 months (DEHP was not detected). In addition, Al-Saleh et al. [28] reported that the levels of DEHP in bottled water samples stored at 4 °C for 1 month were significantly higher than those stored at room temperature for 2 months and outdoors (> 45 °C) for 3 months. However, Schmid et al. [24] reported an increase in DEHP levels in PET-bottled water stored at a higher temperature (a maximum of 3 4°C for 17 hours under direct sunlight) compared to lower temperature (room temperature in the dark). In general, high temperature accelerates the migration [2, 3]. However, no clear or consistent trends regarding the effects of storage temperature on DEHP concentration have been produced, with reports of contradictory observations on increasing concentrations [2, 24] and decreasing concentrations of DEHP [25, 28]. Different research stated that the increase or decrease of phthalates due to different storage conditions depends mainly on the bottle's country of origin [17, 24] due to different production facilities or brands tested as mentioned above [28, 38]. Furthermore, Keresztes et al. [17] showed that different brands of PET-bottled mineral water subjected to different storage temperatures demonstrate different patterns in the increase of phthalates. The researchers performed a storage study at 22 °C, 40 °C, 50 °C, and 60 °C, and reported that the concentrations of DiBP, DBP, BBP, and DEHP for PET-bottled mineral water brand C increased by factors of 1.6, 1.4, 2.6, and 2.5, respectively, at 60 °C after 24 hours compared to the initial concentration values. This is in contrast to brand A characterized by undetectable phthalate levels at 22 °C. DEHP was the only phthalate that could be determined in significant concentration after 24 hours of storage at 60 °C.

Table 1 also shows higher levels of DEHP and DNOP in drinking water at 6 months compared to 3 months in refrigeration temperature of 4 °C; the difference however, was not significant. The findings are in agreement with Casajuana and Lacorte [43] who reported higher concentration of DEHP after storage in PET-bottled water at 30 °C for 10 weeks while it could not be quantified in the initial samples. Furthermore, Leivadara et al. [25] observed the level of DEHP increased from the initial of less than 0.5 to 2 ng/mL when bottled water was stored at 24 °C in the dark for 3 months. The present study shows that the increase in storage time of PET-bottled drinking water stored in refrigeration temperature of 4 °C affects phthalates (i.e., DEHP and DnOP) migration. PET plastics can be subjected to various types of degradation, leading to potential leaching of phthalates [29]. Rahman and Brazel [44] also suggested that ageing and breakdown of plastic packaging might accelerate the migration process.

Table 1 shows that the levels of DEHP in the PET-bottled mineral water at 6-month storage that ranged from 9.35 to 27.6 ng/mL were the highest, followed by PET-bottled sparkling water (ranging from 6.70 to 9.75 ng/mL) and drinking (ranging from 6.63 to 7.19 ng/mL). Similarly, the levels of DnOP in the PET-bottled mineral water at 6-month storage that ranged from 4.14 to 12.6 ng/mL were the highest, followed by PET-bottled sparkling water (ranging from 2.78 to 3.76 ng/mL) and drinking (ranging from 1.94 to 2.34 ng/mL). The findings are consistent with Montuori et al. [22] who also observed slightly higher DEHP levels for PET-bottled mineral and drinking water than the sparkling water samples. Sparkling is an example of carbonated water whereas drinking and mineral are examples of non-carbonated ones. One of the differences between carbonated and non-carbonated waters is pH, which plays an important role in the acid- or base-catalysed ester hydrolysis, a slow equilibrium process at room temperature [45]. However, Lertsirisopon et al. [46] showed that there is no significant effect on the efficiency of abiotic degradation of DEHP via hydrolysis at neutral pH, acidic, or alkaline solution. It might explain why the levels of DEHP and DnOP in PET-bottled sparkling water lower than are not higher in comparison to drinking and mineral water.

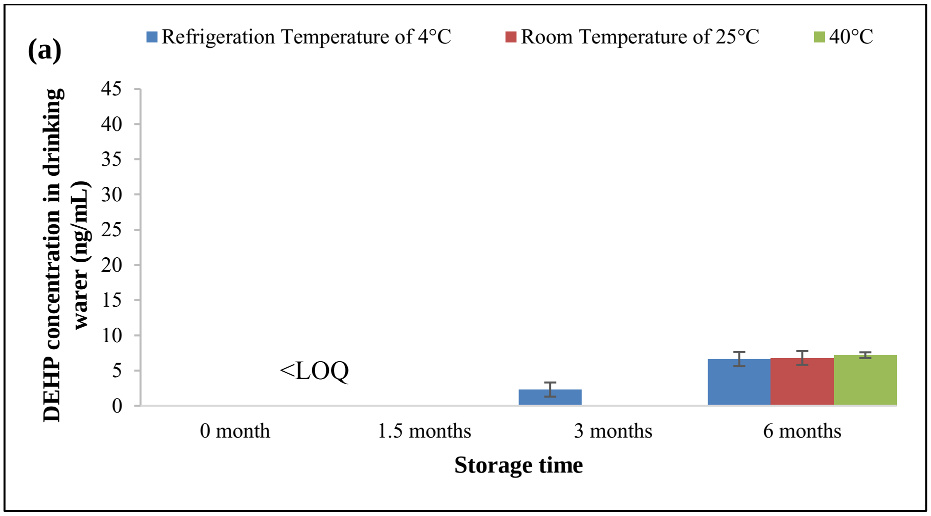
Mineral water samples in glass bottles commercially available in the market were also collected to compare the migration of phthalates from two different food contact materials; PET and glass bottles. The present study found all types of phthalates were not detected in the glass-bottled mineral water samples (data not shown). Similarly, Montuori et al. [22] did not detect DMP, DEP, or DEHP in glass-bottled water samples. Although DEHP was not detected for all types of PET-bottled water in 0-month storage and 1.5-month storage, the levels were above the maximum established limit (6 ng/mL) after 6-month storage for all temperatures. Similar results were obtained by Yousefi et al. [18] who reported the DEHP levels in

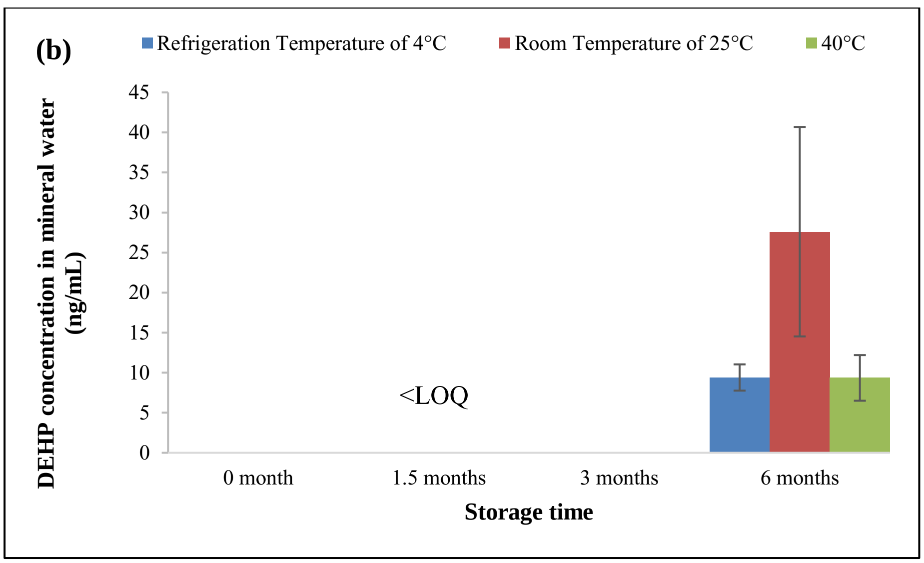
PET-bottled drinking water subjected to storage temperature of 42 °C for 15 days and 25 °C for 75 days were 10.33 and 9.62 ng/mL, respectively, higher than the established limit. It should be noted that the typical shelf life for commercial bottled water is 24 months. Thus, consumption of bottled water after expiry date may increase the dietary exposure of phthalates from bottled water.

Table 1. Concentration of phthalates in drinking, mineral, and sparkling PET-bottled water at different storage temperature and time (ng/mL)

Phthalate esters	Type of water	Drinking			Mineral			Sparkling		
	Storage Time (Month) / Temp.	Refrigeration Temp. of 4 °C	Room Temp. of 25 °C	40 °C	Refrigeration Temp. of 4 °C	Room Temp. of 25 °C	40 °C	Refrigeration Temp. of 4 °C	Room Temp. of 25 °C	40 °C
[2]BBP	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
[3]DBP	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
[4]DEHP	0	ND^{B}	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND^{B}	ND	ND	ND	ND	ND	ND	ND	ND
	3	2.32 ± 1.68^{AB}	ND	ND	ND	ND	ND	ND	ND	ND
	6	6.63 ± 1.93^{Aa}	6.78 ± 0.04^{a}	7.19 ± 0.22^{a}	9.40 ± 1.89^{a}	27.6 ±18.9^{a}	9.35 ±6.85^{a}	9.75 ± 3.36^{a}	6.70 ±0.43^{a}	8.63 ±0.18^{a}
[5]DEP	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
[6]DMP	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND	ND	ND	ND
[7]DNOP	0	ND^{A}	ND	ND	ND	ND	ND	ND	ND	ND
	1.5	ND^{A}	ND	ND	ND	ND	ND	ND	ND	ND
	3	1.57 ± 1.03^{A}	ND	ND	ND	ND	ND	ND	ND	ND
	6	2.28 ± 1.26^{Aa}	2.34 ± 0.26^{a}	1.94 ± 0.27^{a}	4.65 ± 0.27^{a}	12.6 ±9.92^{a}	4.14 ±3.46^{a}	3.76 ±1.95^{a}	2.78 ±0.16^{a}	3.38 ±0.3^{a}

[1]LOQ = 1.5 ng/mL
[2]Benzylbutyl phthalate
[3]Dibutyl phthalate
[4]Di-2-ethylhexyl phthalate
[5]Diethyl phthalate
[6]Dimethyl phthalate
[7]Di-n-octyl phthalate
$^{A-B}$ Values within the same column with different letters are significantly different ($p \leq 0.05$).
$^{a-b}$ Values within the same row with different letters are significantly different ($p \leq 0.05$).
Values are means of duplicate determination ± SD


(a)
Refrigeration Temperature of 4°C
Room Temperature of 25°C
40°C
DEHP concentration in drinking warer (ng/mL)
45
40
35
30
25
20
15
10
5
0
<LOQ
0 month
1.5 months
3 months
6 months
Storage time


(b)
Refrigeration Temperature of 4°C
Room Temperature of 25°C
40°C
DEHP concentration in mineral water (ng/mL)
45
40
35
30
25
20
15
10
5
0
<LOQ
0 month
1.5 months
3 months
6 months
Storage time

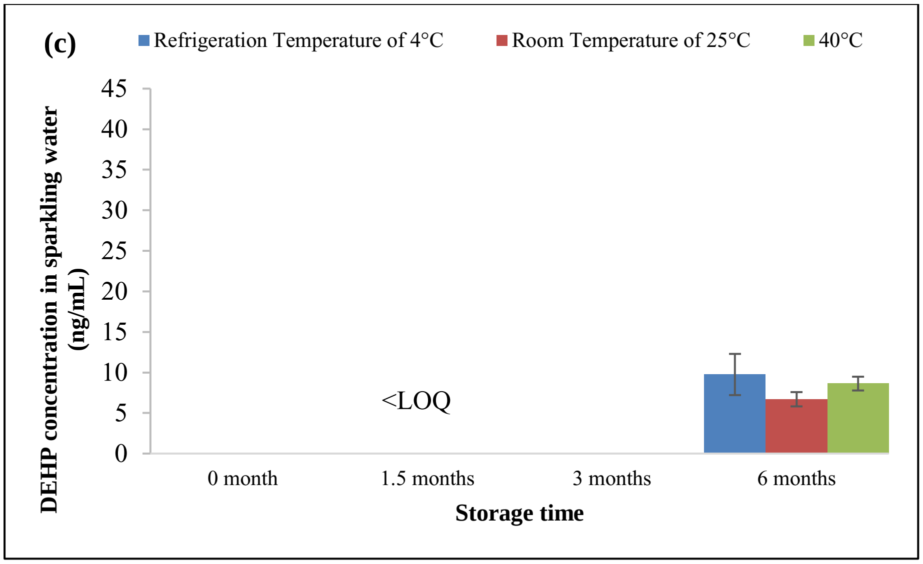
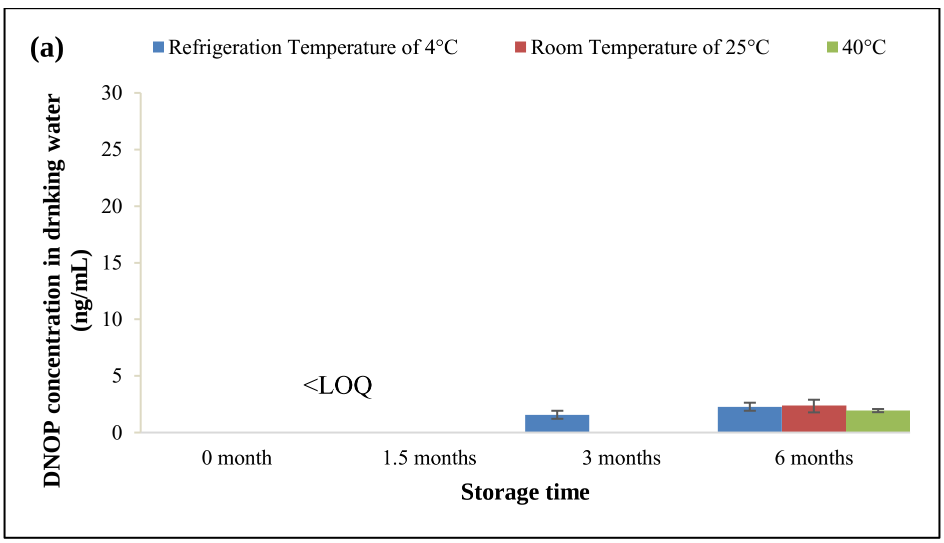
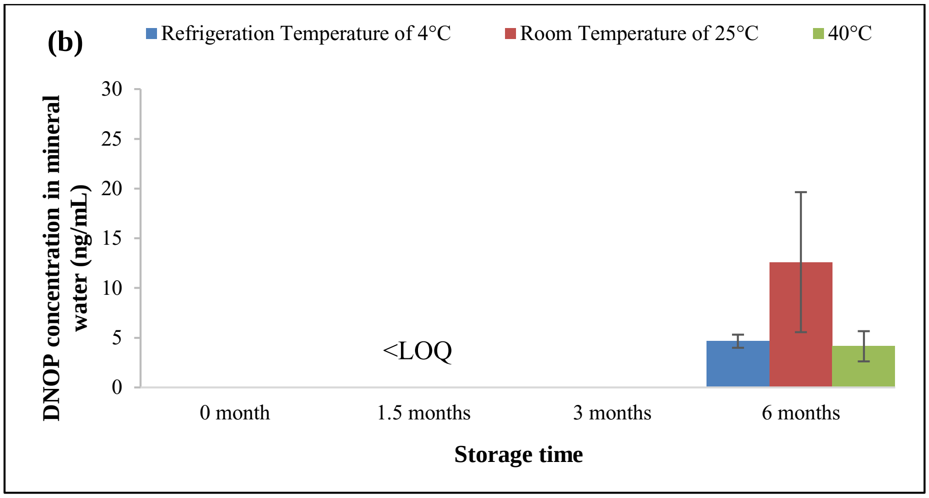


Figure 1. The effect of storage temperature (refrigeration temperature of 4 °C, room temperature of 25 °C, and 40 °C) and storage time (0 month as control, 1.5 months, 3 months, and 6 months) on DEHP concentration in different types of bottled water (A: drinking, B: mineral, C: sparkling). Data are means of duplicate (*n* = 2) determination with bars indicating standard error (± SE)





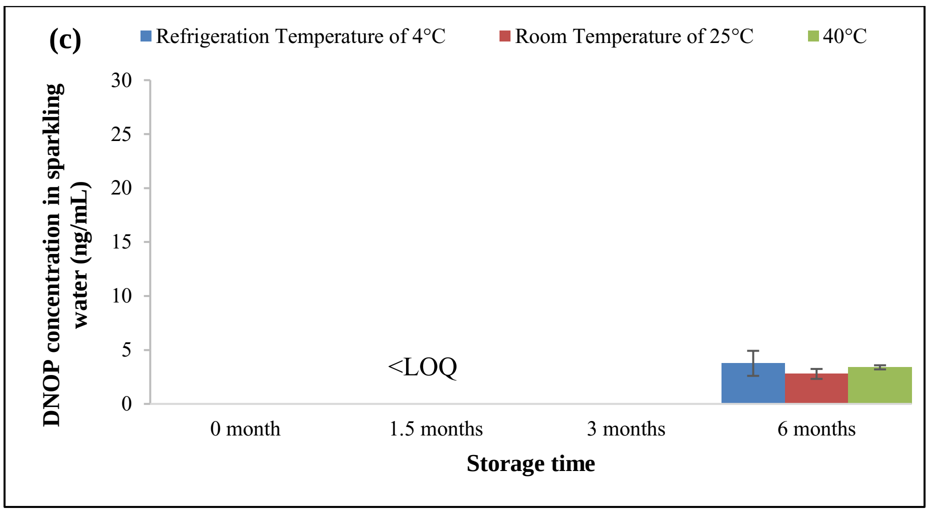


Figure 2. The effect of storage temperature (refrigeration temperature of 4 °C, room temperature of 25 °C, and 40 °C) and storage time (0 month as control, 1.5 months, 3 months, and 6 months) on DNOP concentration in different types of bottled water (A: drinking, B: mineral, C: sparkling). Data are means of duplicate (*n* = 2) determination with bars indicating standard error (± SE)

Conclusion

The present study shows that all types of phthalates were not detected in the 0 and 1.5 months of storage at different storage temperatures for all types of PET-bottled water. However, DEHP in the PET-bottled water was detected in the range between 2.32 and 27.6 ng/mL for 3- and 6-month storage samples; higher than DnOP detected in the range from 1.57 to 12.6 ng/mL. Higher levels of DEHP and DnOP in PET-bottled mineral water were detected at room temperature of 25°C when compared to refrigeration temperature of 4°C, and 40°C at 6-month storage. Similarly, higher level of DEHP in drinking water was found at 6 months compared to 3 months in refrigeration temperature of 4°C. The pronounced effects of different storage temperatures on the levels of phthalates was not observed in the first one and the half month of storage but rather after 6 months of storage in which DEHP levels were exceeded the maximum established limit of 6 ng/mL. The findings from this study may be utilized in future research that could determine the dietary exposure of phthalates from bottled water, especially in Malaysia population.

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