

Estimating the migration of phthalate acid ester plasticizers in plastic containers for local and imported cooking oils

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Abstract

Phthalate acid esters (PAE) serve as additives in plastic formulations, enhancing flexibility and transparency. Their potential migration from plastic packaging to food can lead to endocrine disruption in consumers. This transfer is contingent on the specific usage conditions designated for each type of plastic. Plasticizers such as di-n-butyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), and di-isononyl phthalate (DiNP) play a significant role in the production of plastic bottles utilized for storing edible oils. Since phthalates are not chemically bonded to the plastic structure, they have the potential to migrate into the food contents during storage. This research delved into examining the transfer from phthalate derivatives of PET bottles to edible oil under various storage conditions. The analysis employed a Gas Chromatography-Mass Spectrometry (GC-MS) system. Findings revealed a notable increase in migration, particularly in DEHP levels, with concentrations reaching 115.3 µg/L in the bottled oils after 6 months at 45 °C. This figure surpassed the upper threshold of the maximum contaminant level (MCL) set by the US Environmental Protection Agency, which stands at 6.0 µg/L for DEHP, by a factor of 19.1.

Keywords: Phthalate acid esters, Plasticizers, Gas Chromatography-Mass.

الخلاصة

تُستخدم إسترات حمض الفثالات (PAE) كمضافات في تركيبات البلاستيك، مما يعزز المرونة والشفافية. يمكن أن يؤدي انتقالها المحتمل من العبوات البلاستيكية إلى الأغذية إلى اختلال الغدد الصماء لدى المستهلكين. يعتمد هذا الانتقال على ظروف الاستخدام المحددة لكل نوع من البلاستيك. تلعب الملدنات مثل فثالات ثنائي-ن-بوتيل (DBP) وفثالات ثنائي (2-إيثيل هكسيل) (DEHP) وفثالات ثنائي إيزونونيل (DiNP) دورًا مهمًا في إنتاج القناني البلاستيكية المستخدمة لتخزين الزيوت الصالحة للأكل. نظرًا لأن الفثالات لا ترتبط كيميائيًا بالهيكل البلاستيكي، فإن لديها القدرة على الهجرة إلى محتويات الطعام أثناء التخزين. تعمق هذا البحث في فحص انتقال مشتقات الفثالات من زجاجات البولي إيثيلين تيرفثالات إلى الزيوت الصالحة للأكل في ظل ظروف تخزين مختلفة. استخدم التحليل نظام كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS). وكشفت النتائج عن زيادة ملحوظة في الهجرة، وخاصة في مستويات DEHP، حيث وصلت التركيزات إلى 115.3 ميكروجرام/لتر في الزيوت المعبأة بعد 6 أشهر عند 45 درجة مئوية. وتجاوز هذا الرقم الحد الأعلى لمستوى الملوثات الأقصى (MCL) الذي حددته وكالة حماية البيئة الأمريكية، والذي يبلغ 6.0 ميكروجرام/لتر لـ DEHP، بعامل 19.1.

الكلمات المفتاحية: إسترات حامض الفثالات، الملدنات، كروماتوغرافيا الغاز-مطياف الكتلة.

1- Introduction

Phthalates, recognized as organic compounds with plasticizing properties, are primarily utilized to enhance the flexibility, lightweight nature, durability, and softness of plastic materials (Carlos *et al.*, 2021; Farhan *et al.*, 2007). Being non-covalently bound to plastics, phthalates exhibit a lipophilic nature and tend to volatilize readily. These characteristics significantly contribute to their potential environmental and food contamination (Giuliani *et al.*, 2020; Chou and Wright., 2007). Owing to their widespread application in food-contact items, various phthalate derivatives, Diethyl phthalate (DEP), notably Dimethyl phthalate (DMP), Di-isobutyl phthalate (DIBP), Dipropyl phthalate (DPP), Butyl benzyl phthalate (BBP), Di-isodecyl phthalate (DIDP), Di-n-butyl phthalate (DBP), Dicyclohexyl phthalate (DCHP), Di-isononyl phthalate (DINP), Di - 2 - ethylhexyl phthalate (DEHP), Di - n - octyl phthalate (DOP), and Di-n-hexyl phthalate (DHP) are frequently detected as residues in food items (Wang X *et al.*, 2020; Guo *et al.*, 2012). Phthalates with elevated molecular weights like DEHP, DINP, and DIDP are employed as plasticizers to impart softness to poly(vinyl chloride) goods. On the other hand, lower molecular weight phthalates such as DBP, DEP, and BBP often function Enhancers in many personal care product (Fan Y *et al.*, 2012). Packaging materials, frequently utilized for packaging milk and dairy products, serve as significant sources of contamination, especially by phthalates like DEHP , DBP , and DINP . Phthalate derivatives contamination in food can stem not only from packaging but also from sources like soil, water, air, and various stages of food processing, transportation, storage, and even cooking within households (Mondal *et al.*, 2022; Bradley *et al.*, 2013). Due to the lipophilic nature of phthalates, Cooking oils are predisposed to accelerated phthalate migration from plastic surfaces through processing, transportation and storage (Balafas *et al.*, 1999). Consequently, the phthalate content in packaged food is influenced by factors such as the phthalate levels in packaging materials, storage duration, temperature, food fat content, and contact surfaces (Alp *et al.*, 2020; Mikula *et al.*, 2005). Hence, Cooking oils, given Its fat content and use in plastic packaging , are susceptible to phthalate migration (Halden *et al.*, 2021).

Recent animal studies have revealed the toxic impacts of certain phthalic acid esters (PAEs), their primary metabolites, and breakdown products on various organs such as the liver , reproductive system ,kidneys ,lungs , and heart .DEHP & DBP, extensively utilized phthalate esters in food production, processing, and preservation, fall under the category of 'endocrine disrupting chemicals' due to their estrogenic and antiandrogenic properties. Notably, DBP and DEHP have been linked to adverse effects on germ cell development, BBP on epididymal sperm concentration, and DINP and DiNP on liver cells . These chemicals have been associated with reduced sperm counts in males, diminished fertility, testicular alterations, Low birthweight and anomalies in females, particularly during pregnancy (Sedha *et al.*, 2021; Hauser *et al.*, 2004). The impact of phthalates on the body varies based on factors like age, duration of exposure, and level of exposure, with pregnancy, infancy, and puberty identified as particularly sensitive phases (Zota *et al.*, 2010).

Colón *et al.*, 2009 Proposed a potential correlation between PAEs exhibiting known antiandrogenic traits and estrogenic and premature breast development at young Puerto Rican girls. Furthermore, experimental studies have unveiled the carcinogenic, teratogenic, and mutagenic potential of phthalates, posing a significant threat to human health (Yıldırım *et al.*, 2020; Lovekamp *et al.*, 2003). To mitigate these risks, regulatory authorities have established legal frameworks governing the presence of phthalates in food.

DEHP , DBP , DEP, BBP and DMP phthalate esters have been classified as "Priority toxic pollutants" by the United States Environmental Protection Agency (USEPA) since 1976 , with reference values set at (20 ,200 and 100) $\mu\text{g}/\text{kg}$ body weight / day for DEHP, BBP, and DBP, respectively (Cheng *et al.*, 2016). The (EFSA)European Food Safety Authority has determined the toler able daily doses for DBP ,BBP ,DEHP ,DINP and DIDP as(0.01, 0.5, 0.05, 0.15, and 0.015) mg/kg/day, respectively (EFSA European Food Safety Authority). Additionally, the European Union directives prescribe legal thresholds for BBP,DEHP,DBP,DINP and DIDP to limit migration into food, with total phthalate levels from plastic in food capped at (60 mg/kg) (European Commision 2007). Similar regulations and thresholds have be adopted in the world through legislative measures (Official Gazette 2013).

In Iraq, cooking oil packaged in plastic containers enjoys widespread consumption across all age groups. This study aimed to assess the potential food safety and public health hazards associated with phthalate migreation over varying storage durations. Over the shelf life, cooking oil samples were scrutinized for three phthalate esters, obtained from both domestically produced and imported plastic containers.

2- Material and Methods

2-1 Storage condition

Fifteen samples of oils packed in 1000 ml plastic bottles were randomly collected from the local market of Baghdad city at the start time (production date 7-14 days).

The samples were classified into three groups based on their storage conditions, which included differences in temperature and duration.

The first group included bottled oil stored at 5°C in a refrigerated environment, with extraction measurements performed initially and after three and six months.

The second group included bottled oil stored at 25°C in a controlled environment, with extraction assessments performed initially, as well as after three and six months.

The third group included bottled oil stored at 45°C in a controlled environment, with extraction assessments performed initially and after three and six months.

2-2 Preparation of oil samples

The sample volume, around (1L), is sequentially extracted with methylene chloride under alkaline conditions (pH above 11) utilizing either a separatory funnel or a continuous extraction technique as outlined in method 625(EPA , 2007) .

2-3 Preparation (DBP), (DEHP), and (DiNP)) as a standard :

Standard solutions(1.00 µg / µL) can be created using high-purity standard substances or acquired in the form of authenticated solutions.

2-4 Identification and determination of plasticizers (phthalate derivatives) using gas chromatography GC-MS

When separating phthalates using gas chromatography-mass spectrometry (GC-MS), the following detailed conditions and parameters were applied:

1. Column selection :The selection of a gas chromatography column is very important for the separation of phthalates derivatives, where a stable low-bleed and high-temperature column DB-5MS was used.
2. Temperature programming: The temperature program was used using a precise gradient to effectively separate phthalates based on their boiling points and polarity, where the initial, ramp and final temperatures were carefully adjusted to achieve optimal separation.
3. Carrier gas: A high-purity carrier gas (helium) was used to carry the sample through the column. The flow rate was for effective separation and detection in the mass spectrometer.
4. Injector temperature: The injector temperature was set at (280C) to ensure effective evaporation of the sample without causing degradation.
5. Mass Spectrometry Conditions: We determined the appropriate ionization mode for phthalates (chemical ionization) which is the optimal ionization for phthalate detection. Ionization energy, ion source temperature, and other mass spectrometer parameters were adjusted for sensitive and selective detection.
6. Sample Preparation: Samples were prepared using appropriate extraction methods using liquid-to-liquid extraction to isolate phthalates from the sample matrix.
7. Injection Volume: Optimize the injection volume to introduce a representative amount of sample without overloading the system. The injection volume should be within the linear range of the detector for accurate quantification where the injection volume was (1 uL)
8. Calibration: The GC-MS system was calibrated using standard solutions of phthalates to create calibration curves for quantification, where a range of concentrations were used to cover the expected concentrations of phthalates in the samples.
9. Data Analysis: GC-MS data were processed using a special software to identify and quantify phthalate compounds based on their mass spectra and retention times and

compared to experimental mass spectra from reference libraries for compound identification.

By carefully tuning and optimizing these detailed conditions, we were able to efficiently separate, identify and quantify phthalate compounds using gas chromatography-mass spectrometry (GC-MS).

3-Result and Discussion:

The notion of the total migration limit have historically served to oversee the collective transfer of substances from plastic to food items and to as certain the potential health implications of these substances (Arvanitoyannis&Bosnea 2004). Regarding the migration process, it can be delineated into four primary phases: the diffusion of chemical compounds across polymers, the adsorption of diffused molecules from the polymer surface, the interaction of compounds at the interface between plastic and food, and the absorption of compounds into the food product (Ferrara *et al.*, 2001). Research delved into the migration of plasticizer compounds from PET bottles to samples procured from local markets, with the outcomes detailed in Table 1. The investigation observed the transfer of PET derivatives to samples housed in PET containers under varying temperature conditions and durations. At an initial storage temperature of 5°C, contamination with plasticizer compounds from PET bottles was evident for DEHP and DPB derivatives at concentrations of 2.11 and 1.31, respectively, while no contamination was noted with DiNP. In contrast, at 25°C, all PET derivatives examined in this study (DEHP, DBP, and DiNP) manifested at concentrations of 6.13, 3.51, and 1.18 µg/L. However, at 45°C, concentrations of DEHP, BBP, and DiNP surged to 7.55, 4.87, and 1.93 µg/L, respectively, as depicted in Table 1. The migration of PET derivatives like DEHP, DBP, and DiNP exhibited a notable escalation from the outset to three months at 5°C by 7.73 , 3.61 and 0.83 µg/L . For instance, when oil was stored initially at 20°C, PET derivatives such as DEHP, DBP and DiNP were detected at concentrations of 11.76 , 6.0 and 3.22 µg/L, respectively. At 45°C, concentrations of DEHP, DBP, and DiNP recored to 13.86 , 8.57 and 5.74 µg/L, respectively, as outlined in Table 1 .

It was observed that all the polyethylene terephthalate derivatives were transferred to the bottled oils stored in polyethylene terephthalate bottles after six months, as Table (1) showed an increase in concentration with increasing temperatures, where DEHP represented the maximum concentration of polyethylene terephthalate derivative (115.3) µg/L, while DBP showed a high increase after six months by (36.01) µg/L, while the concentration of DiNP was (19.0) µg/L.

The maximum allowable contaminant level (MCL) for DEHP has been set by (EPA) standards and (WHO) guidelines (6 / 8 µg/L) respectively, (FDA,2011 & EPA,2009). The concentration of DEHP in this study exceeded the (MCL) set by EPA standards and WHO guidelines by (19

, 14) times. The (US EPA) has proposed a MCL of (10 µg/L) for BBP ,the BBP values in this study exceeded the MCL as well as for DiNP.

Table 1 displays the concentrations of PET derivatives (DEHP, DBP, and DiNP) in bottled oil across varying storage durations (initial time, after 3 months, and after 6 months) under different temperature conditions (5°C, 25°C, and 45°C).

PET derivatives (µg/L)	Temperature	Concentration for Three Periods (ppb)		
		Start Time	After 3 Months	After 6 Months
DEHP	5 °C	2.11	7.73	31.31
	25 °C	6.13	11.76	88.0
	45 °C	7.55	13.8	115.3
DBP	5 °C	1.31	3.61	21.81
	25 °C	3.51	6.00	29.2
	45 °C	4.87	8.57	36.01
	5 °C	0.00	0.83	2.21

Estimating the migration of phthalate acid ester plasticizers in plastic containers for local and imported cooking oils

DiNP	25 °C	1.18	3.22	6.33
	45 °C	0	1.93	19.0

3- Conclusion

Based on the results obtained from the study on the migration of plasticizer compounds from PET bottles to food samples stored under various conditions, several conclusions can be drawn:

- ✓ Migration of PET Derivatives: The study revealed a significant migration of PET derivatives, including DEHP, DBP, and DiNP, from PET bottles to the samples stored within them. The concentrations of these compounds increased with higher temperatures and longer durations of storage, indicating a higher risk of contamination as environmental conditions became more severe.
- ✓ Temperature Influence on Migration: The temperature played a crucial role in the migration process. Higher temperatures led to greater migration of plasticizer compounds into the food samples. For instance, at 45°C, the concentrations of DEHP, BBP, and DiNP significantly increased, showing a clear correlation between temperature and migration levels.
- ✓ Long-Term Effects: Over an extended period of six months, all PET derivatives were observed to transfer to the oils stored in PET bottles. This long-term exposure resulted in a substantial increase in concentrations, with DEHP exhibiting the highest concentration among the PET derivatives studied.
- ✓ Violation of Maximum Allowable Contaminant Levels (MCL): The concentrations of DEHP exceeded the MCL set by EPA standards and WHO guidelines by a significant margin, indicating a potential health risk associated with the migration of this compound. Similarly, the concentrations of BBP and DiNP also surpassed the proposed MCL values, highlighting the need for regulatory action to address these elevated levels of contamination.

In summary, the study underscores the importance of monitoring and regulating the migration of plasticizer compounds from PET bottles to food products, especially considering the

potential health implications associated with the exceedance of MCL values for certain compounds like DEHP, BBP, and DiNP. These findings emphasize the necessity of stringent regulations and quality control measures to ensure the safety of food products stored in plastic containers.

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Estimating the migration of phthalate acid ester plasticizers in plastic containers for local and imported cooking oils

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Estimating the migration of phthalate acid ester plasticizers in plastic containers for local and imported cooking oils

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