Investigating the rate of migration of plasticizers from PET bottles into frying oil during storage

S. Asadollahi[1], E. Hosseini[2], A. Sorouri[3]*

[1] *Department of Food Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran*
[2] *Department of Food Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[3] *Department of Food Science, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran*

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ABSTRACT: Oils extracted from plants have been used since ancient times and in many cultures .frying oils are used in many countries all around the world including Iran. Therefore it is of great importance to ensure the safety of these food products which are usually delivered in polyethylene terephthalate (PET) bottles. This study has investigated the effect of storage time and temperature on the migration rate of phthalate compounds from PET bottle into frying oil. In more detail, migration rate of Bis (2-ethylhexyl) phthalate was measured in the 1st, 30th, and 60th days of storage within two different temperatures of 20 and 40 °C. At the both storage temperatures, increasing the storage time led to a statistically significant rise of the migration rate of phthalate compounds ($p<0.1$). Furthermore, it was determined that Bis (2-ethylhexyl) phthalate had a higher migration rate at 40 °C comparing to 20 °C.

Keywords: *Bis (2-ethylhexyl) phthalate; Frying oil; Migration rate; Polyethylene terephthalate; Storage temperature*

INTRODUCTION

Oils extracted from plants have been used since ancient times and in many cultures. Edible fats and oils are among the valuable foodstuffs which can supply energy. They play an important role in maintaining public health, could be used in a wide variety of consumer goods. They serve as a concentrated energy source having the highest energy resource between the foodstuffs. Fats contain almost 9 calories per gram while protein and carbohydrates contain only 4 calories per gram. They include fat-soluble vitamins such as A, D, E, and K which are crucial to improve human health (Malek, 2000). Furthermore, they contain essential fatty acids which cannot be synthesized by human body. High fat foods increase the appetite and are usually more delicious but eating of the right kinds of fat can be found in plants like nuts, seeds, vegetable oils, and seafood actually help human body slim down and feel satisfied (Pristouri, *et al.*, 2010).

Proper packaging is one of the most important strategies to inhibit food waste and spoilage. Additionally, product's packaging is the last opportunity to convince consumers and make them to buy through comparing to the other options stocked right beside them. Con-

(*),(**) Corresponding Author - e-mail: siminasadollahi5@gmail.com

ventional containers for the distribution or storage oil including tin-plated steel can, glass and plastic bottles each and every one has its own advantages and disadvantages (Parmar, *et al.*, 1988). Instead of using saturated fats, natural unsaturated fats such as vegetable oils "olive, canola, corn, or soybean oils" can be used as a healthier alternative (Klonoff, 2007). Among the family of polyunsaturated fatty acids (PUFAs), there are two different groups: the "omega-3-fatty acids" and "omega-6-fatty acids". Both are considered essential fatty acids and can be found in vegetable oils and being served for salad dressing (Krystock, 2014).

PET is a thermoplastic polymer resin of the polyester family which is used in the production of synthetic fibers for clothing and containers for liquids and foods. It is made up of polymerized units of the monomer ethylene terephthalate, with reoccurring $C_{10}H_8O_4$ units. It is naturally transparent or colorless and can be rigid or semi-rigid, depending on processing and thermal history. Plastic bottles made from PET are widely used for storing soft drinks. Research has shown that poor storage conditions such as high temperature lead to the migration of phthalate compounds from the PET bottle wall into the liquid (Sax, 2010). As mentioned earlier, PET is rigid or semi-rigid therefore softeners/plasticizers like phthalates are needed to make them more flexible. Researchers have investigated the effects of packaging material, headspace volume, oxygen, light transmission and storage time on quality of extra virgin olive oil".

Packaging materials used in this study were including clear glass, clear PET, clear PET + UV blocker, clear PET covered with aluminum foil and clear polypropylene bottles. Quality indicators of samples such as acidity, peroxide value, spectrophotometric indices (K232, K270) and color were monitored during one year of the study. According to the result, olive oil quality was well maintained for almost 9 months when it was stored in PET bottles covered with aluminum foil. Furthermore, the impacts of other parameters on olive oil quality were as follow: Temperature ≈ light > container headspace > packaging material oxygen permeability (Pristouri, *et al.*, 2010).

Researchers have reported the effects of storage time and container type on the quality of extra-virgin olive oil (Méndez & Falqué, 2007). The researchers similarly studied the quality of four commercial extra-virgin olive oil which kept in clear PET bottle, PET bottle covered with Al foil, glass bottle, tin and Tetra Brik for three and six months of storage. Olive quality was assessed through determining the parameters including acidity, peroxide index, absorption coefficients K270 and K230, humidity percentage, impurity content (%), phenols content, iodine index, saponification index, color index and fatty acid content. The outcomes showed that tin and Tetra Brik are the best containers for storing extra-virgin olive oil. Migration of plastic components and additives from food packaging into the food can be risky to the human health. Plasticizers such as Phthalates, Alkylphenols, 2, 2-Bis (4-hydroxyphenyl) propane (bisphenol A or BPA) and Di (2-ethylhexyl) adipate (DEHA) can cause "Endocrine disruptors". In this regard, study investigated the effectiveness of incubation and ultrasonic extraction methods on migration of plasticizers and additives from common plastic wine tops into liquids (Fasano, et al, 2007).

The first method was through incubation at 40 °C for 10 days (extreme conditions) and the other by Ultrasound-Assisted Extraction. The three liquid applied simulants were including: distilled water, acetic acid at 3%, and ethanol at 15% for wine top. In order to evaluate the potential of migration from wine tops, ten wine tops were incubated in extreme conditions at 40 °C for 10 days and the next ten samples of the wine tops, were incubated in an ultrasonic bath for 15 min. Based on the results, the researchers have suggested that the extraction which was carried out through incubation at 40 °C for 10 days can be more effective in detecting all the compounds. Considering this result, usage of products especially foodstuffs which contain such a compounds can pose a serious risk to the human health. Releasing these compounds into the environment and nature or their existence in human body could establish a serious environmental and biological problem. Storage time and temperature also can have a significant impact on the migration of these compounds into food products. According to this fact, the main objective of the present study is to investigate the effects of different storage times and temperatures on the rate of migration of such a compound into oil samples. In particular, the study was an attempt to evaluate the rate of immigration of Di-(2-ethyl hexyl) phthalate (DEHP) from bottles into oil samples during different storage times and different storage temperatures.

MATERIALS AND METHODS

In order to carry out this study, 15 pcs of PET bottles of Ladan Talaie Frying oil with an acceptable expiration date each having 1 lit volume beside two blank PET bottles were purchased from Behshahr Industrial Company. In this study, type of Frying oil (Ladan Talaie), storage temperature (20 °C and 40 °C) and Storage time (0, 10, 20, 30, 40, 50, and 60 days) were considered as independent variable and The level of DEHP was considered as dependent variable (Table 1). In this study, HPLC grade acetonitrile, Ethyl acetate and n-Hexane with grade of HPLC were purchased from Merck Chemical Company.

Preparation of the Internal Standard (IS)

Standardization was done through the addition of 0.0199 g of C17 fatty acid to reach to the volume of 1.5 cc. after complete dissolve, 50 µL of the solution was injected into each prepared sample and well cortex-mixed.

Oil Sample Preparation

All oil samples were heated in a water bath at 55 °C. Then, 1.5 g of each sample was separated and placed into test tubes. Afterwards, 5ml HPLC-grade acetonitrile was added to each tube and were vortex-mixed for 5 minutes. In order to separate extracted phthalate from the oil phase of compound, test tubes were centrifuged at 1,000 rpm for 30 minutes. After that, the upper layer was removed with a Pasteur pipette and transferred to a clean test tube so that it could be more concentrated on a cartridge (CHROMABOND C18 EC, USA).

Sample concentration

Foodstuffs contain very low levels of phthalate which are very difficult to determine. To overcome this problem, pre-columns are used for the initial concentration of phthalate in the foodstuffs. Concentration was done with HPLC-grade acetonitrile, as described by Sung, 2009. First of all, 3 µL acetonitrile was added to each column and sample loading was performed at flow rate of 1.5 µL/min. Then the column was washed with 3 µL acetonitrile to remove any phthalate. The Condensed phthalates was placed in an oven then dried at 100 Thereafter, 1 µL hexane was added to the sample and being vortex-mixed. The present study examined the rate of migration of phthalate from bottles into Frying oil samples at two temperatures of 40 and 20 . Some Samples kept at 20 ºC and were analyzed after 1st, 10th, 20th, 30th, 40th, 50th, and 60th days. Some Samples also sorted at 40 ºC and analyzed after the 1st, 30th, and 60th days. Thus, the samples were brought out from the incubator at the specific times and temperatures. In order to determine the level of DEHP, it was injected into a gas chromatograph after preparation, concentration and addition of the external standard.

Instrumental methods

In the next stage, 1 µL of the aforementioned extracted solution was injected into a gas chromatograph with a Flame Ionization Detector (FID). The applied instrument was Agilent 7890A (USA) and the capillary column for GC was HP-5 with dimensions of 0.52 mm × 30x m. Column temperature was initially set at 40 °C and the solution was held at this temperature for three minutes. After that, the temperature was raised to 150 °C (12.5 °C/min) and it was kept at this temperature for one minute. Finally, the temperature was raised up to 280 °C (3 °C/min) and the solution was retained at that temperature for two minutes. The results were reported after measuring the ratio of concentration of migrated compounds in the liquids at the designated

Table 1. Storage conditions of Ladan talaie frying oil

		Storage time (Day)						
		0	10	20	30	40	50	60
Storage	20 °C	×	×	×	×	×	×	×
temperature (°C)	40 °C	×			×			×

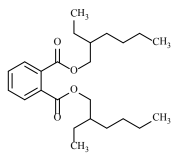
times using ppm as the unit of concentration. Measuring the ratio of residual free monomers into the polymer before migration In order to measure the rate of migration of phthalate compounds into oil samples, Firstly phthalate compounds were extracted from the PET bottles and then the migration rate was measured through the instrumental methods of analysis.

Preform and Blank PET Sample Preparation

About 1 gr of the preform and blank PET bottle was cut into small pieces and the solutions were extracted by using pure ethyl acetate solution in a Soxhlet apparatus (BUCHI 810, Switzerland) for 24 hours. Considering this fact, Polymeric containers which are usually using for packaging beverages and edible oils could have migrating compounds easily separate from them and enter into the liquids. Pure ethyl acetate was used to carry out the present study, so migratable compounds could be isolated for final identification.

The solution obtained by using Soxhlet. First extraction was condensed through distillation and then via the application of neutral Nitrogen until, it reached an ultimate volume of 2 ml. Finally, 1 µL of the solution was separated and injected into a gas chromatography machine. DEHP solution was also injected into the gas chromatography machine, exactly according to the aforementioned conditions, as the control sample. Table 2 shows characteristics of the applied phthalate compound. According to the FDA, the level of free phthalate in Polystyrene which are in contact with the food should not exceed 1 weight-percent based on the monomers. In terms of the fatty foods with types of III, IV, V, VII-A, IX, it has been recommended that the level of Styrene monomer should not exceed more than 0.5 percent by the weight of polymer.

Table 2. Characteristics of the applied phthalate compound

Chemical name	Bis(2-ethylhexyl)phthalate (DEHP)
Degree of purity	(GC)>95%
Molectular formula	C24H38O4
Molecular weight	390.56 g mol^{-1}
Melting point	385°C; 725°F; 658 K
Density (25 °C)	0.9 g/cm^3
Chemical structure	

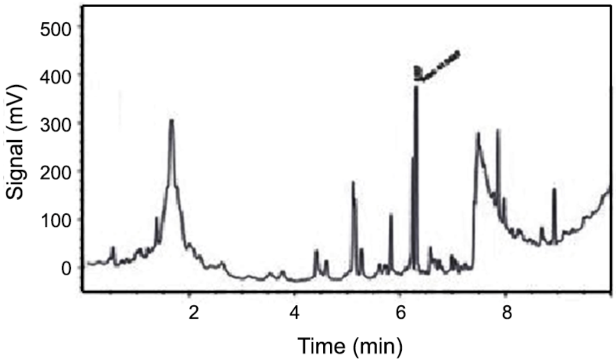


Fig. 1. Standard chromatogram.

RESULTS AND DISCUSSION

I. Statistics

To investigate the impact of storage time and temperature on the migration rate of DEHP, Duncan's multiple range tests was run by using of SPSS, Version 20.

II. Results

Measurement results of the initial level of DEHP in the PET Bottles

In the first step to determine the rate of migration of DEHP from PET bottles, phthalate compounds were extracted from the blank bottles. Then migration rate was measured through the usage of instrumental methods of analysis. The initial level of phthalate in the PET bottle, before the migration into oil, was determined for helping researchers to estimate the percentage of phthalate compounds which migrate over the storage time. Fig. 1 depicts the results of analysis of PET bottles obtained through gas chromatography.

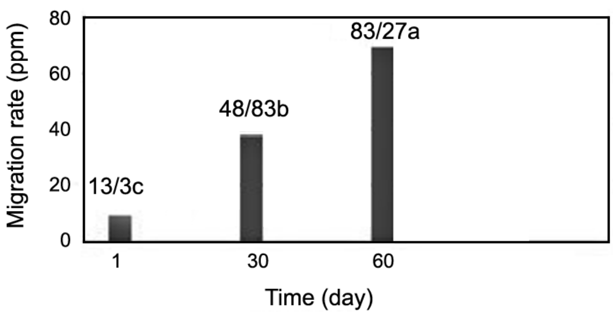


Fig 2. The effect of storage time on the migration rate of Di(2-ethylhexyl)phthalate into frying oil

Measurement the rate of Migration of DEHP into the Frying oil Samples

The results of this study indicated a statistically significant difference level of DEHP in the Frying oil samples which were kept at the different storage temperatures. The highest migration rate (83/27 ppm) was observed at 40 ºC. As Fig. 2 shows, storage time had also a statistically significant effect on the rate of migration of DEHP from PET bottles into oil samples.

Figs. 3, from A to F indicate that the migration rate of DEHP has increased over storage time at both temperatures. With regard to the effects of storage time and temperature, the highest migration rate (83.27 ppm) was observed after 60 days and at 40 ºC and the lowest migration rate (5.57 ppm) was observed after one day and at 20 ºC.

III. Discussion

The Influence of storage temperature on the migration rate of Di (2-ethylhexyl) phthalate from PET bottles

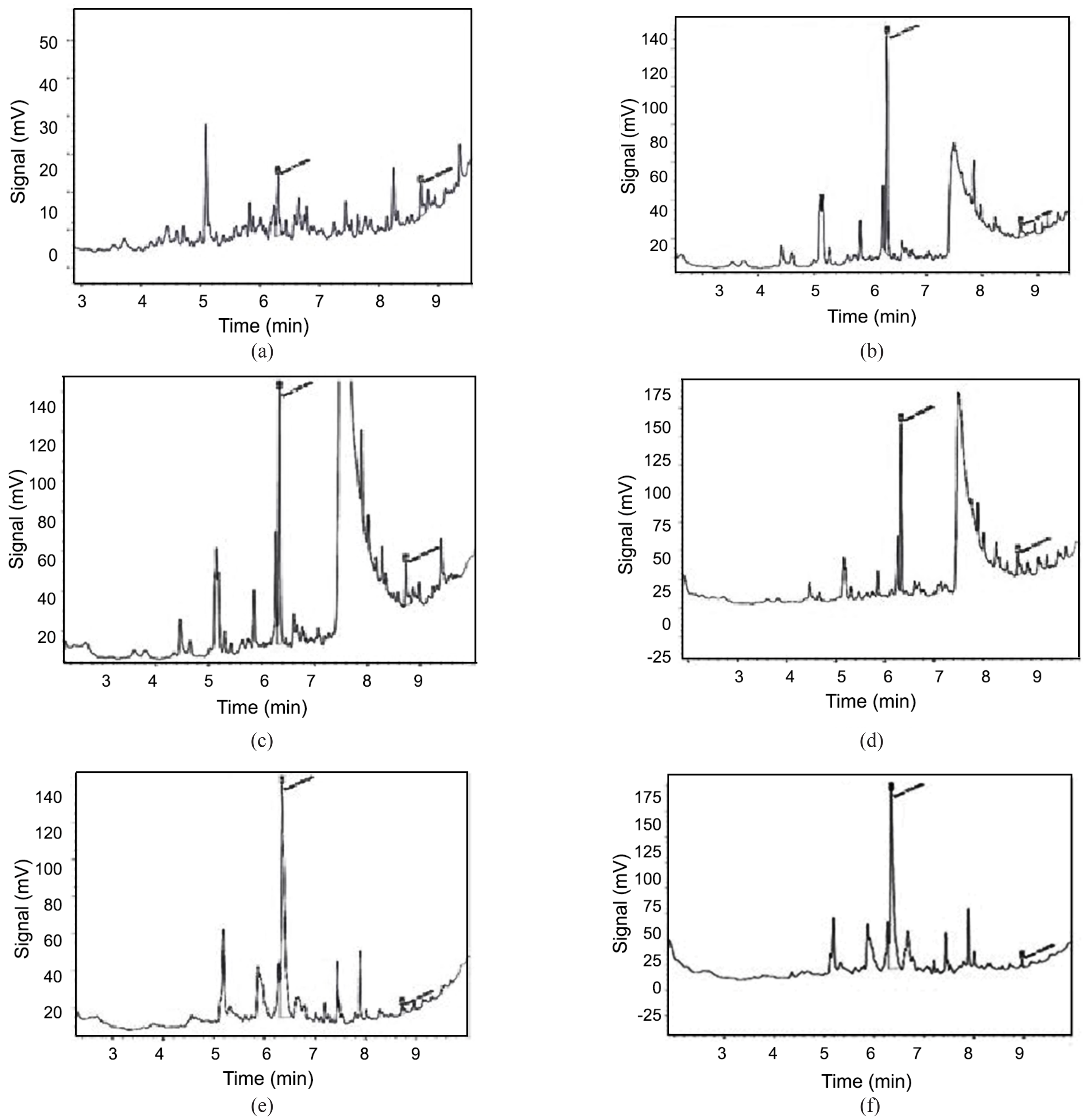


Fig 3. Chromatogram of rate of migration of DEHP into frying oil over storage time: (a) day: 60, temperature: 20 °C, (b) day: 60, temperature: 40 °C, (c) day: 30, temperature: 40 °C, (d) day: 30, temperature: 20 °C, (e) day: 1, temperature: 20 °C, (f) day: 1, temperature: 40 °C.

into Frying oil samples

As being reported, there has been a statistically meaningful difference in the amount of DEHP in the oil samples which were kept at different temperatures. The highest migration rate was 83/27 ppm and has occurred at 40 ºC. It is obvious that under laboratory conditions, an increase in the storage temperature has led to an increase in the migration rate of phthalate compounds; therefore, the level of migrated compounds has been higher in samples stored at 40 ºC than those stockpiled at 20 ºC. The storage temperature of 40 ºC can lead to a change in the bottle structure. The increase in the migration rate of phthalate compounds at 40 ºC occurs as a response to the increase of molecular movement belongs to the components of PET bottles and break down of the polymer chains which causes the release of phthalate compounds from PET bottles. PET bottles become wrinkled at high temperatures due to an increase in the mobility of the polymer chains (Pristouri, *et al.*, 2010). Statistical analysis results showed that the storage temperature had a significant effect on the migration rate of phthalate compounds especially at 40 ºC. The result was comparable with the other researcher's findings (Widen, *et al.*, 2004, Gajdos, *et al.*, 1999).

The effect of storage time on the migration rate of Di (2-ethylhexyl) phthalate from PET bottles into Frying oil samples

Fig. 2 shows that storage time has had a statistically significant effect on the migration rate of phthalate compounds and the highest migration rate (83/27 ppm) has occurred after 60 days. Based on statistical analysis results, storage time has had a statistically significant effect on the migration rate of DEHP ($p<0.1$).

According to the result of other study that had shown an increment in the storage time would lead to an increase in the migration rate of DEHA is in agreement with this study (Goulas, *et al.*, 2000). Hence, the result of this study and previous studies have indicated, increasing the contact time between phthalate compounds and the product would lead to an increase in the rate of migration of these compounds into the product. With regard to changes of migration rate, initially a linear relationship between migration rate and storage time was observed. However, over time, the relationship between migration rate and storage time became nonlinear until migration rate gradually reaches equilibrium. In the study that was carried out by other researchers who reported that storage time has a statistically substantial effect on the migration rate of phthalate compounds (Yen, *et al.*, 2011).

The interactive influence of storage time and temperature on the rate of Migration of DEHP from PET bottles into Frying oil samples

As it is mentioned earlier and can be seen in Figs. 3 (from A to F), at both temperatures an increase in the storage time has led to a statistically significant increase in the migration rate of DEHP. In addition, there was a relationship between storage time, temperature and the migration rate of the compound. Accordingly, the highest migration rate (83.27 ppm) had occurred after 60 days and at 40 ºC while the lowest migration rate (5.57 ppm) had happened after one day at 20 ºC. These results are in agreement with other researcher's findings who reported that the migration rate of phthalate compounds significantly affected by the storage time and temperature, and also food type (Widen, *et al.*, 2004, Gajdos, *et al.*, 1999, Yen, *et al.*, 2011).

CONCLUSIONS

The widespread use of packaged foods in the world and the cautious attitude towards consuming processed foods make the monitoring and control of the food safety more inevitable. Based on the results of this study, an increase in the storage time led to increment in the migration rate of DEHP ($p<.01$) from PET bottles into the edible oil samples at both temperatures of 20 and 40 °C. The highest migration rate observed after 60 days of storage. Additionally, comparison between samples stored at 20 °C and those were kept at 40 °C showed that "the migration rate of DEHP enhanced by an increase in storage temperature". Regarding to the interactive effect of storage time and temperature, the highest migration rate was reported after 60 days within the oil sample kept at 40 °C. Accordingly, the selection of more suitable alternatives for food packaging, lower storage temperature and recently produced foodstuffs with a limited shelf life can help to reduce the migration rate of DEHP from PET bottle into Frying oil.

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AUTHOR (S) BIOSKETCHES

Simin Asadollahi, Assistant Professor, Department of Food Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, Email: siminasadollahi5@gmail.com

Elahe Sadat Hosseini, PhD Research Student, Department of Food Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

Amirhossein Sorouri, MSc., Department of Food Science, Varamin- Pishva Branch, Islamic Azad University, Varamin, Iran