EFFECT OF ENVIRONMENTAL CONDITIONS ON THE MIGRATION OF DI(2-ETHYLHEXYL)PHTHALATE FROM PET BOTTLES INTO YOGURT DRINKS: INFLUENCE OF TIME, TEMPERATURE, AND FOOD SIMULANT

Mehdi Farhoodi, Zahra Emam-Djomeh*, and Mohammad Reza Ehsani****

Department of Food Science, Technology and Engineering, University of Tehran, Iran

and Abdolrasul Oromiehie**

Iran Polymer Institute, Pazhuhesh Blvd., Peykanshahr, Tehran

الخلاصة:

سوف نستقصي - في هذا البحث - انتشار مادة (DEHP) من قوارير (PET) المستخدمة لحفظ شراب اللبن الإيراني. وقد استخدمنا حامض الأستيك بتركيز (3%) كعامل إثارة كما تقتضيه معايير الهيئة الأوروبية. وحُفظت القوارير التي تحوي الحامض عند درجات حرارة مئوية قدرها (4.25.45) لمدة أربعة أشهر ، وقمنا بإجراء التحليل الكيميائي بشكل دوري. كذلك استخدمنا تقنية DSC لدراسة مدى تأثر مادة (PET) بالأطعمة المستخدمة ، ووجدنا أنه مع ازدياد درجة الحرارة يزداد تركيز مادة (DEHP) ، وكذلك يزداد التركيز كلما ازداد زمن حفظ الأطعمة. وخلال فترة الدراسة لم يزدد التركيز عن القيم المسموح بها من قبل الهيئة الأوروبية (EEC). أضف إلى ذلك أن مادة (PET) لم تتأثر بالحامض المستخدم كما دلَّ على ذلك قياس درجة الانتقال الزجاجي (Tg) ، ونسبة التبلور في (PET).

*Address for correspondence:

Transfer Phenomena Laboratory (TPL), Department of Food Science, Technology and Engineering, University of Tehran, Agricultural Campus of the University of Tehran, Faculty of Biosystem Engineering, P. O. Box: 4111, 31587-11167 Karaj, Tehran, Iran

Tel: +98 261-224 8804

E-mail: emamj@ut.ac.ir

**E-mails: farhoodi@ut.ac.ir; mehsani@ut.ac.ir; A.Oromiehie@ippi.ac.ir (Tel.: +98 21-445 80060)

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ABSTRACT

Polyethylene terephthalate (PET) is one of the materials that are widely used for packaging of beverages and edible oils. In this study, the migration of di(2-ethylhexyl)phthalate (DEHP) from PET bottles into the Iranian yogurt drink was investigated. According to European Commission regulations, acetic acid (3% w/v) was chosen as simulant. The acetic acid samples were stored at 4°C, 25°C, and 45°C for four months and analyzed periodically by gas chromatography. Differential Scanning Calorimetry (DSC) was used to investigate if contact with the food simulant could affect the PET material. It was concluded that the storage temperature had a large effect on the migration of DEHP. Also, increasing storage time resulted in higher concentrations of migrating DEHP. The concentrations of migrating substance did not exceed its specific migration limit (Economic European Community (EEC) regulations). Determination of glass transition (*Tg*) and crystallinity percent of PET bottles using DSC method showed that the variations in the amount of migration at different storage condition did not induce any change in the PET material in contact with 3% acetic acid.

***Key words*:** DEHP; migration; PET; *Tg* point; yogurt drink

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1. INTRODUCTION

Polyethylene terephthalate (PET) is a plastic material which has found increasing applications within the packaging field. It is a simple long-chain polymer. Its chemical inertness, together with other physical properties, has made it particularly suitable for food packaging applications. It is commonly used as packaging material for drinking water, mineral water, carbonated beverages, and edible oils. Hazardous compounds are to be avoided as the bottles were manufactured by a commercial plant that produces food-contact bottles. However, PET is known to contain small amounts of low molecular weight compounds, ranging from the monomer to the pentamer. The level of these compounds depends on the type of PET. Migration from packaging materials into products generally is concerned with minor constituents that influence the quality of the contained product by sensory or toxicological hazards. There has been extensive research performed on the use of food contact PET materials. Investigations have been carried out in order to establish the type and concentration of compounds related to post-consumer PET recycling [1 – 4]. The effect of the recycling process on intentionally contaminated PET material has also been studied. Then the migration of model contaminants from the finished product, which did not include a functional barrier, to food simulants has been also determined [5 – 8]. With regard to interactions between packaging materials and contacted solutions, a higher amount of migration could relate to changes in the structure of packages. Widen *et al.* studied the effect of different simulants on the migration of model contaminants by using differential scanning calorimetry [8]. Pennarun *et al*. studied the effect of food simulants on the diffusion coefficient of migrant molecules from recycled PET bottles [9]. DEHP is used as plasticizer in some plastic materials like PVC and PET. DEHP causes liver or testicular damage in rats and mice, and the "no observed adverse effect level" (NOAEL) has been shown to be 3.7 or 14 mg/kg/day. This contaminant induces low fertility and decreases the number of litters per pair and of live pups per litter [10, 11].

Migration of chemical substances is a diffusion process subject to both kinetic and thermodynamic control and can be described by diffusion mathematics derived from Fick's Law.

The kinetic dimension of migration dictates how fast the process of migration occurs. The thermodynamic dimension dictates how extensively the transfer of substances will be when migration is finished – when the system is at equilibrium. The kinetic and thermodynamic aspects should not be confused. For example, migration may proceed at a slow rate but, if the chemical migrant has a higher affinity for the food than for the packaging material, then given enough time (*e.g.* a long shelf life) it may still migrate extensively into the food. On the other hand, if a different food or beverage is packed and the chemical is only poorly soluble in that food or beverage, then migration could be low no matter how long the shelf life is [12].

There are several determinants of chemical migration and exactly what migration occurs depends first on the identities and concentrations of the chemicals present in the packaging material. Other important parameters are the nature of the food along with the conditions of contact. Lastly, the intrinsic properties of the packaging material itself are important considerations. If a material interacts strongly with the food it could give high migration by leaching. Conversely, an inert material with low diffusivity is likely to give low migration values. It is important to understand the factors that control chemical migration because from this understanding springs the ability to prevent or limit any undesirable migration into foods [12, 13].

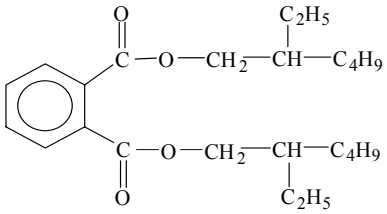
In the present study, the initial concentration of DEHP in PET bottles was determined and then the migration procedure of this compound into yogurt drink was investigated. Influence of storage time and temperature on the migration process was examined and the effect of food simulant on the PET material was investigated by differential scanning calorimetry (DSC). The traditional Iranian Yoghurt drink is known as Dough and is produced from low fat milk after mixing the yogurt with 40–60% water and about 1% salt and has a pH of about 3.5–4.0. Since this kind of beverage may be stored either under sunlight, especially in summer time, or in the refrigerator, or at room temperature, the effect of storage temperature on migration level was determined. According to European Commission regulations, we used 3% acetic acid solution as simulant (EEC 1985) [13].

2. EXPERIMENTAL

2.1. Samples and Materials

The PET bottles used in this study were made by a local bottle manufacturer using clear PET resin as major starting material. The standard compound employed was bis(2-ethylhexyl) phthalate which was purchased from Riedel–deHean Company. Properties of DEHP are listed in Table 1.

Table 1. The Characterization of DEHP

Name	Molecular weight (g/mol)	Boiling point (°C)	Structure	SML[1] (mg/kg)
DEHP	390	384		3.0

[1] SML: Specific Migration Limit

2.2. Determination of Initial Concentration of DEHP in PET Bottles

2.2.1. Sample Preparation

A total of 22 g of PET bottle wall was cut into small pieces and extracted with absolute ethanol for 48 hr using a Soxhlet apparatus [14]. Absolute ethanol was used to isolate DEHP for the identification. The Soxhlet extract was concentrated by distillation followed by nitrogen flushing, to a final volume of 2 ml (A solution).

2.2.3. Instrumental Analyses

To obtain a chromatographic profile of each sample, 2 μl of Soxhlet concentrated extract was injected into a gas chromatograph equipped with a flame ionization detector. The instrument used was a Chrompack cp9000 and the column used was a cp-sil 8 column (30m × 0.52mm i.d.). The flow rates of helium, hydrogen, and air were 4, 30, and 300 ml/min, respectively. The temperatures of the injection port and detector were kept at 250 and 280°C. The oven program is as follows, 50°C for 3min, rising at 15°C/min to 260°C, then held isothermal until the final chromatogram was obtained.

2.3 Determination of Migration from PET Bottles into 3% Acetic Acid

The simulant chosen as a surrogate for yogurt drink was acetic acid (3% w/v). Each bottle was filled with 1.5 l simulant, closed tightly with a screw stopper and placed at 4, 25, and 45°C for about four months. Samples of 75 ml were neutralized with 10 M sodium hydroxide. 20 g sodium chloride was added and the solution was extracted for 12 h in a closed vial with 3ml dichloromethane containing 50 mg/l tetradecane. The extracts were analyzed directly by on-column GC–FID with the mentioned characteristics.

2.4. DSC Measurements

The glass transition temperature, *Tg*, and the heat of fusion were determined for stored bottles. The samples were analysed with DSC (TA Instrument model TA-2010, USA). Each sample was heated from 30 to 280°C with a heating rate of 10°C/min. In order to estimate the degree of crystallinity, the measured heats of fusion were compared with published values [15].

3. RESULTS AND DISCUSSION

3.1. Determination of DEHP in PET Bottle Wall

The amount obtained by Soxhlet extraction represents the maximum level of migrating substance. In the actual contact situation such an amount may never be attained, even in long-term storage conditions. The results of a gas chromatograph analysis of the extract solution showed that DEHP was found in PET bottles with initial concentration of 670 µg per g PET. H. Kim, *et al*. [14] found that the level of DEHP in commercial amber PET bottle wall was 820µg per g PET [14]. The gas chromatographic profile of the extract solution is shown in Figure 1.

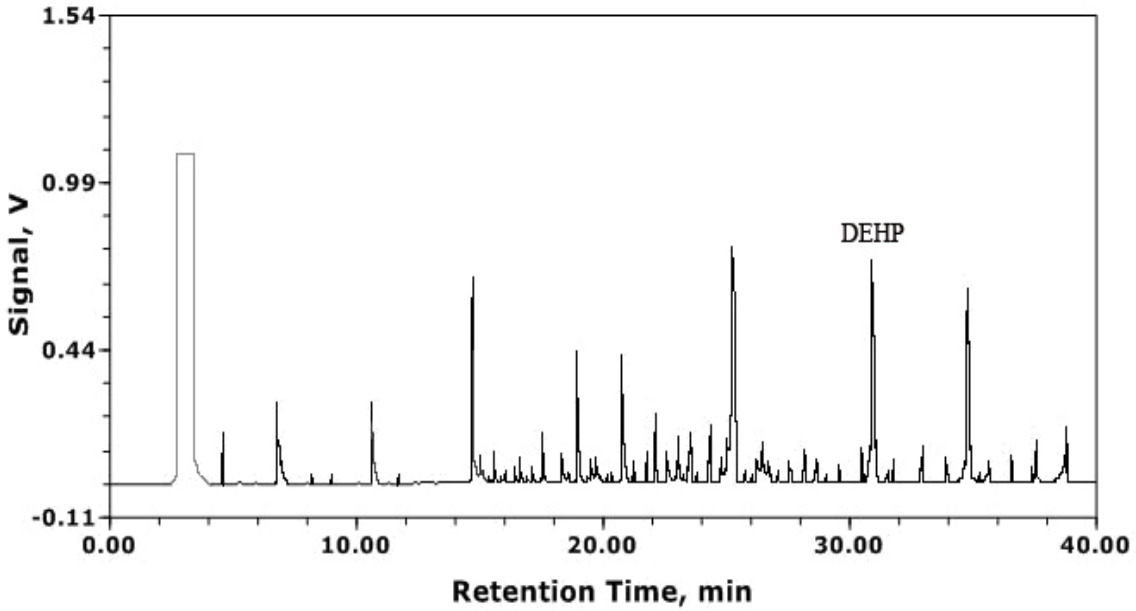


Figure 1. Gas chromatographic profile of extract solution of PET bottle wall

3.2. Storage Conditions

Typically, the shelf life of an Iranian yogurt drink is two months. So, a storage time of four months can be considered as a worst case. Yogurt drinks are often stored at room temperature, which may vary depending on location. To investigate the effect of temperature on the migration, three storage temperatures, 4, 25, and 45°C, were chosen.

3.3. Effect of Temperature

As shown in Table 2, under experimental conditions with increasing time and temperature, migration of DEHP into food simulant has increased. In fact, the storage temperature had an intense effect on the migration, with higher DEHP migration to the food simulants at 45°C than at 4 and 25°C. No detectable migration occurred for the samples stored at 4°C. The amounts of DEHP that migrated into the simulated phase at 25°C and 45°C are given in Table 2 as a function of time. Storage at 45°C could increase the diffusion of migrant compound from PET bottle wall into simulant. Widen *et al*. [8] showed that after three months storage, the migration of benzaldehyde form PET bottles into acetic acid increased by about the three times when the storage temperature increased from 20 to 40°C. Pennarun *et al*. [16] found that a rise in temperature of 20°C (from 40 to 60°C) increased the diffusion coefficients of 13 substances in PET by between six and 29 times [16].

Table 2. Migration of DEHP from PET Bottles into Simulant at 25±1°C and 45±1°C as a Function of Time

Time (day)	Amount of migration (mg/l)	
	25°C	45°C
0	0.0	0
9	0.7	1.4
16	1.0	1.8
25	1.2	2.1
52	1.4	2.3
66	1.4	2.5
86	1.4	2.4
115	1.4	2.4

3.4. Effect of Time

Increasing storage time resulted in higher concentrations of migrating DEHP. At both 25 and 45°C, the maximum level of migration was seen after 66 days storage. At 25ºC this level was 1.4 mg l^{-1} and at 45ºC the maximum level of migration was 2.4 mg l^{-1}. After 25 days, the greater part of the migration (about 80% of total migration) had already occurred. Migration into the simulant started with a rapid rate until the 25th day, while after that, the rate decreased and the migration of model contaminants into simulant reached an equilibrium state after 66 days storage (Figure 2).

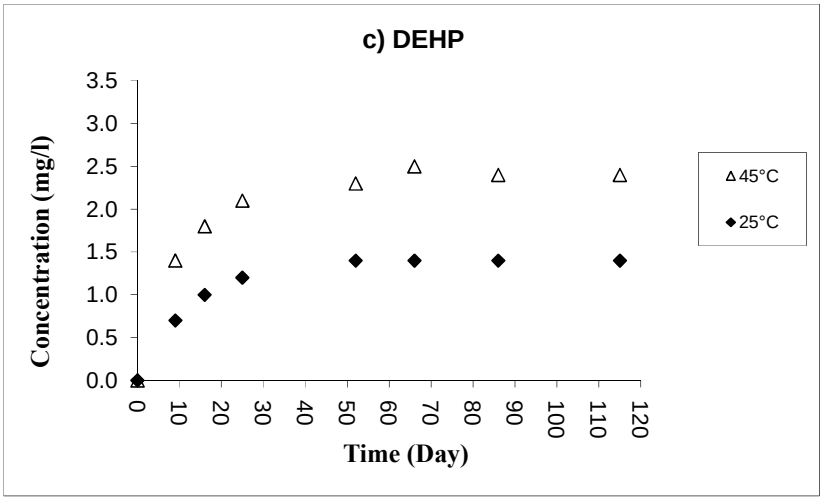


Figure 2. Migration of DEHP from PET bottles into simulant during four month storage at 25 and 45°C

It seems that the equilibrium level of migration is not just related to the primary concentration of DEHP in PET bottles, but that the solubility of this compound in the solution is a factor affecting this phenomenon. Generally, the maximum migration limit of DEHP (2.4 mg l^{-1}) was below the specific migration level established by European legislation but it approached European-approved SML (3 mg l^{-1}).

3. 5. Effect of Food Simulant

Pennarun, *et al*. showed that food simulant can influence the structure of PET(s) and increase the diffusion coefficient of migrants in PET bottles [9]. Widen *et al*. [8] reported that PET bottles filled with ethanol exhibited a lower *Tg* than the empty bottles and the results suggested that there was a slightly higher crystallinity in PET stored with ethanol. However, there was no considerable difference in *Tg* and crystallinity of PET bottles filled with acetic acid simulant and empty bottles [8]. From the present study it is concluded that the degree of crystallinity for the PET samples containing

3% acetic acid and stored at constant temperatures (25 or 45ºC) did not changed significantly (Table 3). *Tg* points for the samples stored at 25 and 45ºC were at the range of 70–73 and 80–84ºC, respectively.

Table 3. Δ*H* Melt and Crystallinity Percent of the Samples Stored at 25 and 45ºC

Storage time (Day)	*Tg* (ºC)		Δ*H* melt (J/g)		Crystallinity %	
	25ºC	45ºC	25ºC	45ºC	25ºC	45ºC
0	73.26	73.26	38.27	38.27	28.18	28.18
9	70.82	79.51	38.98	43.45	28.70	32.00
14	70.60	84.46	42.86	41.50	31.56	30.56
26	73.67	83.03	38.92	37.57	30.13	27.67
34	71.83	83.51	42.82	40.93	31.53	30.14
76	72.44	83.72	41.86	40.82	30.82	30.06
120	72.27	85.92	41.95	40.88	30.89	30.10

For both 25 and 45ºC storage temperatures, *Tg* and percent of crystallinity are approximately constant so the higher amount of migration into 3% acetic acid at the higher temperature does not relate to structural changes of PET bottles in contact with solution. The higher glass transition of the PET bottles stored at 45°C is attributed to the enthalpy relaxation process of amorphous regions of semicrystalline PET [17]. Figure 3 shows the DSC curves of PET bottles stored at 25 and 45ºC (after 14, 34, and 120 days storage). Solid lines describe the *Tg* points of 25ºC stored PET bottles and vacant lines the *Tg* of 45ºC stored PET bottles. Another explanation for higher *Tg* points of PET bottles at higher temperature corresponds to higher migration of DEHP at higher temperature. These small molecules act as plasticizers that increase the flexibility of the PET chain; hence decreased *Tg*. However, at high temperatures the observed loss of these small molecules reduces the polymer flexibility, leading to higher *Tg*.

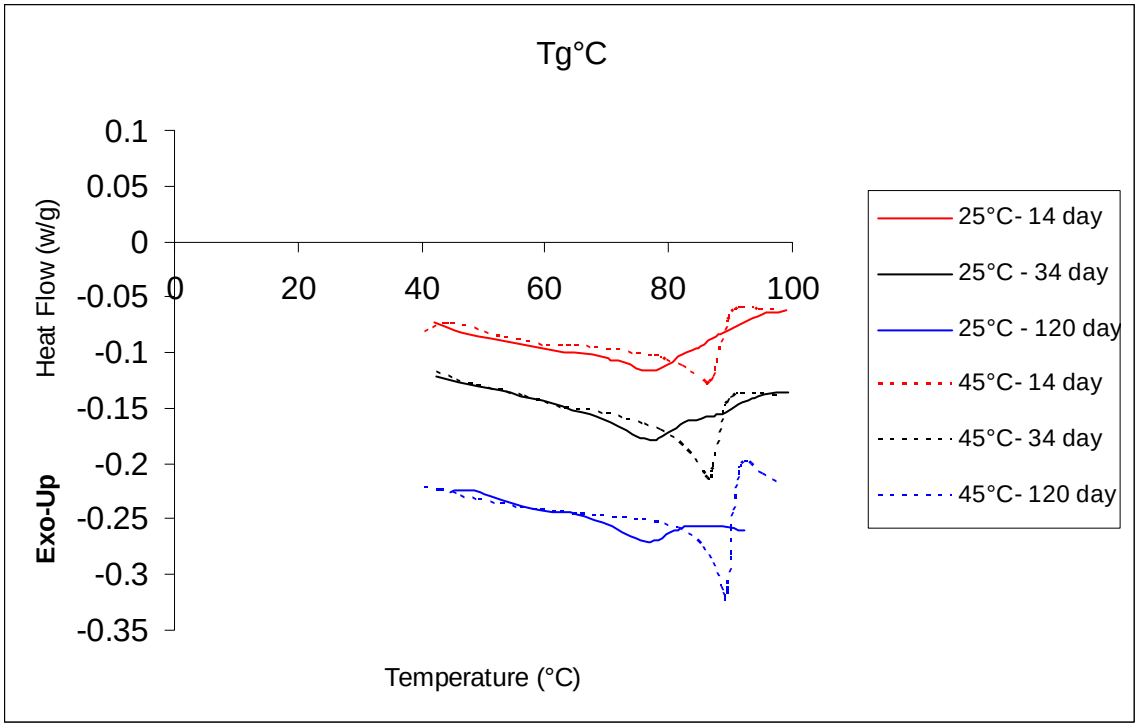


Figure 3. DSC curves of PET solvent-treated and stored at 25 and 45°C (after 14, 34, and 120 days storage)

3.6. Prediction of Diffusion Coefficients

During the diffusion process, one side of the packaging material is exposed to the simulant and the other to the air. The diffusion process of DEHP from the PET bottle into the acetic acid is given by Fick's law of diffusion:

$$J = -D\frac{\partial C}{\partial X} \tag{1}$$

Given the thickness of the PET bottles (0.5mm) and the initial concentration of the DEHP (670 µg per g PET), the diffusion coefficient of DEHP into simulant was calculated and the results are shown in Table 4.

Table 4. Diffusion Coefficient of DEHP from PET Bottle into 3% Acetic Acid

Storage Temperature	$J*10^{-10}$ (mg/cm^2s)	$D*10^{-10}$ (cm^2/s)
25	12.12	0.655
45	20.88	1.129

PET bottle density= 1.38 g/cm^3

Film thickness = 0.5mm

The results indicated that the diffusion coefficient of DEHP into the simulant stored at 45°C is about twice that for the samples stored at 25°C. It is concluded that the diffusion process of DEHP is absolutely temperature dependent and follows the Arrhenius relation (Equation (2)).

$$D = D_0.e^{-\frac{E}{RT}} \tag{2}$$

Where R is the gas constant (8.314 J/g mol K), T is absolute temperature (K), D_0 is the diffusion coefficient at reference temperature, and D is the diffusion coefficient at the determined temperature.

By considering D_0 as the diffusion coefficient at reference temperature (25°C) and drawing the curve of ln (D/D_0) with in verse temperature ($1/T$), the activation energy could be calculated from the slope of the curve ($-E/R$). The measured activation energy calculated from these data was 310.37 J/g mol.

CONCLUSIONS

The method was used to determine the migration of DEHP to 3% acetic acid as a simulant for Iranian yogurt drink. The objective was to know if the considered compounds reached upper legislation limits and if their long-time storage at different conditions is detrimental to the consumer or not. In spite of a high initial concentration of DEHP in PET bottles, it can be concluded that the amount of migration is also related to the migrant compound's molecular weight and its with the solubility in contact simulated solution. The storage temperature had a large effect on migration. An increase in the storage temperature from 25 to 45°C resulted in up to an 1.5–fold increase in migration, which could be explained by an increase in the diffusion coefficient. DSC results showed that the interaction between polymer material and 3%acetic acid simulant did not affect the structure of PET and the higher amount of migration at higher temperature is considered to be due to the higher solubility of contaminants and the higher diffusion rate. The critical effects of DEHP relate to reproduction. In this study the maximum level of migration of DEHP (2.4 mg l^{-1}) approached the European SML level (3.0 mg l^{-1}) so the requisite precautions should be considered in the storage of yogurt drinks, especially in warm weather locations, and the storage time should not exceed their determined shelf-life. Comparison of diffusion of coefficient of DEHP in simulant stored at different storage temperatures showed that the diffusion process was temperature dependent and followed the Arrhenius relation, and that the diffusion coefficients increased with increase in the storage temperature of samples.

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