

## Effect of Menthol Absorption by Packaging Material on the Quality of Yogurt Drink during Storage Time

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### ABSTRACT

Interaction of menthol with polyethylene terephthalate bottles during storage time was tested at three different temperatures. Menthol is a mint flavor agent added to yogurt drinks in Iran and is considered as a factor affecting consumer acceptance. Absorption of menthol to packaging material could cause a loss of quality in the final product due to diminished flavor intensity. Tests were done on the effects of environmental conditions (storage for three months at temperatures of 4, 25, and 45°C) on flavor stability of yogurt drink samples. Absorbed flavor was extracted from PET bottles after the specified time periods and quantified using gas chromatography coupled with FID detector. Then, the diffusion coefficient of menthol into PET bottles ( $D_p$ ) was determined using concentrations of absorbed menthol. Results showed different absorption levels under various conditions. After 90 days, the absorption quantities at 4, 25, and 45°C were 38.21, 186.66 and 700.50 ng g<sup>-1</sup> of PET bottle, respectively. It was concluded that amounts of menthol absorption into PET bottles increased with storage time and higher temperature. Elevation of storage temperature resulted in significant increase in diffusion coefficient of menthol in PET bottle.

**Keywords:** Absorption, Diffusion coefficient, Menthol, PET bottle, Yogurt drink.

### INTRODUCTION

Polyethylene terephthalate (PET) is a plastic material with increasing application in food packaging. It is a simple long-chain polymer. Its unique physical properties, including chemical inertness, make it particularly suitable for packaging food. The most commonly used form of PET is that of stretched blown bottles for orange juice and other soft drinks. A disadvantage of the use of PET, which has caused some concerns, is the absorption of flavor compounds from food and drink. The absorption of flavor compounds by

polymeric materials refers to the loss of flavors and aroma components that are transferred from food into the plastic. This transfer of flavor compounds may influence the sensory quality and acceptability of food products by the consumer. Menthol is used in yogurt drinks to add a pleasant flavor. It is, therefore, important to study absorption levels of aromas in a specific plastic polymer such as PET because the absorption of favorite flavors during storage has an unpleasant effect on the quality of drinking yogurt making the final packaged product less appetizing than the fresh one. Regarding the interaction between

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PET and aroma compounds, Nielsen has studied the absorption of 1-2% of myrcene and limonene into refillable PET bottles [1], while research by Hotchkiss has demonstrated that considerable amounts of aroma compounds absorbed by plastic packaging materials may cause loss of aroma intensity or an unbalanced flavor profile [2]. Furthermore, research by Tawfik *et al.* (1998) studied the effect of D-limonene absorption on mechanical properties of refillable PET [3]. Farhoodi *et al.* (2011) investigated absorption levels of various flavors (D-limonene,  $\alpha$ -Pinene, and myrcene) from soft drinks into PET bottles [4]. Dekker *et al.* (2003) proposed a formula to calculate an equilibrium concentration of flavors in a packaging material [5]. To understand the mechanism of flavor absorption into packaging material and to predict the shelf life of food products, determination of diffusion coefficient is necessary. Hernandez *et al.* (1986) and Mohnney *et al.* (1988) applied the diffusion equation described by Crank [6-8]. They used a microbalance gravimetric technique to study the diffusion coefficient of limonene into high-density polyethylene. Taverdet and Vergnaud (1987) described the theoretical aspect as well as numerical methodology to solve Fick's second law equation [9], meanwhile Chang *et al.* (1988) presented computational aspects of the migration of packaging materials into food and described both Fickian and non-Fickian behaviors [10]. Sheung *et al.* (2007) presented a mathematical model to determine the diffusion coefficient of orange juice flavor compounds into packaging materials tested over storage times, but the study did not include the effect of temperature [11]. Selective loss of menthol in a complex mixture may result in a loss of flavor intensity or change in flavor tones. The present study was done to assess the absorption level of menthol from yogurt drink into PET bottles during storage at different temperatures to investigate the impact of undesirable environmental conditions on the quality of the product. Evaluation of the diffusion coefficient of menthol into PET bottles could be an effective

factor determining reasonable shelf life for food products packed in the PET.

## MATERIALS AND METHODS

### Materials

Menthol at purity of > %95 (GC) was purchased from the Fluka Company (Germany). Yogurt drink samples were produced in the Pegah dairy company and packaged in 1.5 L polyethylene terephthalate bottles (commercial grade). The thickness of PET bottle was 0.5 mm.

### Sample Preparation

Yogurt drink samples were produced from milk that had 2% fat. After fermentation, about 60% of the yogurt was mixed with 40% water and 1 g salt 100 g<sup>-1</sup>. About 100 ppm of menthol was added to the final drink and, after pasteurization, it was transferred to 1.5 lit PET bottles, which were then tightly closed with screw caps and sealed. The fat content of final yogurt drink was 1.2%. Filling and capping was performed in sterile conditions to minimize cross-contamination. Then, samples were stored at 4, 25, and 45°C for three months.

### Menthol Extraction

Bottles were cut into small pieces and rinsed with distilled water after removal of the yogurt drink solution to determine amounts of menthol in the plastic material. One gram of cut surface of a bottle was placed in 5 ml acetone solution (disperser solvent) and was rested for about 24 hours to complete the extraction of menthol from PET bottles. The method of dispersive liquid-liquid micro extraction (DLLME) was used for pre concentration of acetone extraction solution containing menthol, before GC analyses [12, 13]. For this purpose, 60  $\mu$ l of CCl<sub>4</sub> (as extraction

solution) was mixed in acetone extract solution and stirred well. One ml solution of this mixture was rapidly injected into 5 ml distilled water. A cloudy mixture resulted from dispersion of fine particles of carbon tetrachloride in water and the menthol entered into the  $\text{CCl}_4$  phase. Then, the mixture was centrifuged for 5 min at 5,000 rpm (Centurion Scientific Ltd., model 1020D, UK). Accordingly, the dispersed fine particles of the extraction phase settled as sediment on the bottom of the conical test tube ( $5.00 \pm 0.2 \mu\text{l}$ ). Finally,  $1.00 \mu\text{l}$  of the sediment phase was injected into GC.

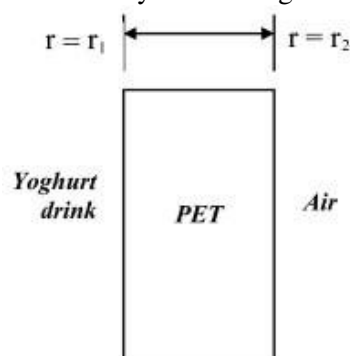
### GC Analysis

A gas chromatograph (Shimadzu GC 2010) with a split/splitless injector system and a flame ionization detector was used to separate menthol and to determine its amounts. Ultra pure helium (99.9999%, Air products, UK) was passed through a molecular sieve trap and an oxygen trap (Crs, USA) was used as the carrier gas at constant linear velocity of  $35 \text{ cm s}^{-1}$ . The injection port was held at  $250^\circ\text{C}$  and used in splitless mode for 1 minute. Separation was carried out on a BP-5,  $30 \text{ m} \times 0.22 \text{ mm}$  capillary column with a  $0.25 \mu\text{m}$  stationary film thickness, 95% methyl-5% phenyl copolymer column (SGE). The oven temperature was set as follows: initial  $100^\circ\text{C}$ , from  $100^\circ\text{C}$  (held for 1 minute) to  $160^\circ\text{C}$  at the rate of  $15^\circ\text{C min}^{-1}$ , and then held for 1 minute at  $160^\circ\text{C}$ . The total time for each GC run was 10 minutes. The FID temperature was maintained at  $300^\circ\text{C}$ ; hydrogen gas was generated with a hydrogen generator (OPGU-2200s, Shimadzu) for FID at a flow rate of  $40 \text{ ml min}^{-1}$ . The flow of zero air (99.999, Air Products) for FID was  $400 \text{ ml min}^{-1}$ .

### Diffusion Coefficient of Menthol in PET

One side of the packaging material is exposed to the yogurt drink and the other

side is exposed to air (Figure 1). PET is a polymer with very low diffusion coefficients. Therefore, the equilibrium concentrations after absorption of menthol from the liquid sample at low temperatures will be reached only after a long time.



**Figure 1.** Schematic of packaging in contact with media (yogurt drink and air).  
PET: Polyethylene terephthalate

In this study a simple procedure was used for determination of the diffusion coefficient from experimental results [14].

$$\frac{m_{p,t}}{A} \approx c_{L,0} \cdot \rho_L \cdot (D_p \cdot t)^{1/2} \quad (1)$$

Where,  $m_{p,t}$  is the mass of menthol that migrated into PET at time  $t$  (mg),  $A$  is the surface of package exposed to liquid (yogurt drink),  $c_{L,0}$  is initial concentration of menthol in liquid,  $\rho_L$  is the density of liquid and  $D_p$  is the diffusion coefficient of menthol in PET. This equation describes the mass transfer from liquid into polymer. It can be assumed that the diffusion coefficient in the liquid ( $D_L$ ) is much higher than in the polymer ( $D_L \gg D_p$ ) and the whole migration rate is determined by the diffusion coefficient in the polymer ( $D_p$ ). From this equation we can calculate the diffusion coefficient ( $D_p$ ):

$$D_p = \frac{1}{t} \cdot \left( \frac{m_{p,t}}{A} \cdot \frac{1}{c_{L,0} \cdot \rho_L} \right)^2 \quad (2)$$

Diffusion coefficient ( $D_p$ ) of a given organic molecule in a given polymer as a



function of the molecular weight of the molecule and the temperature could be also calculated with the Piringger equation [15, 16]:

$$D_p = 10^4 \exp \left[ \frac{A_p - 0.1351 M_r^{2/3} + 0.003 M_r}{R.T} \right] \quad (3)$$

Where,

$$A_p = A'_p - \frac{\tau}{T} \quad (4)$$

$M_r$  is the relative molecular mass of migrant (Dalton);  $T$  is temperature (K);  $A'_p$  is an upper limit polymer specific diffusion parameter;  $\tau$  is a polymer specific activation energy parameter (K);  $R$  is the gas constant ( $8.3145 \text{ J mol}^{-1} \text{ K}^{-1}$ ); and  $R 10454$  is a reference activation energy  $E_{Aref}$  (K) for the diffusion in polymer.  $A_p$  value is one of the important parameters in calculating the diffusion coefficient of migrant in polymer and investigating the kinetics of the migration. Usually, high  $A_p$  values account for high mobility of the polymer and high diffusion coefficients. In general, soft polymers with higher diffusion constants are characterized by higher  $A_p$  values, whereas stiff chain polymers such as polyesters with lower diffusion constants have lower

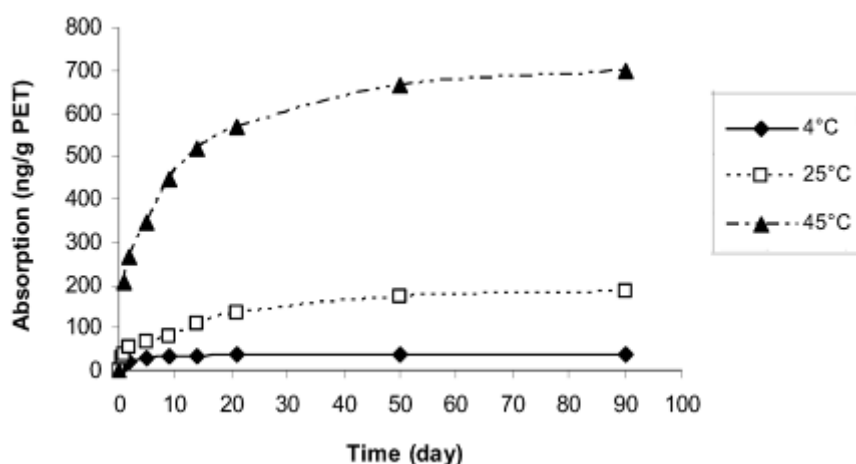
$A_p$  values.  $A_p$  is a temperature dependent parameter while  $A'_p$  is introduced as a temperature independent term. It is of high importance to note that this concept estimates upper-bound values and, therefore, leads to an over estimative migration calculation. Both the parameter  $\tau$  and the constant 10454, contribute to the diffusion activation energy  $EA \text{ (kJ mol}^{-1}\text{)} = R (10454 + \tau)$  [24]. For many important groups of plastics relevant to food packaging, e.g. polyethylene terephthalate (PET), higher activation energy is generally observed. Table 1 shows the parameters used for determination of diffusion coefficient of menthol in PET.

## RESULTS AND DISCUSSION

Figure 2 shows the amount of menthol absorbed from yogurt drink samples into PET bottles at different times and temperatures. In general, the absorption level of Menthol into PET bottles ranged from 13.52 to 700.50 ng per gram of PET bottles. This study takes into account levels of menthol absorption as a function of storage temperature (Figure 2). In the case of other flavor compounds absorption into PET bottles, research by Tawfik *et al.* (1998) showed that absorption of D-limonene into refillable PET bottles

**Table 1.** The parameters used for determination of diffusion coefficient.

Parameter type	Amount	Unit
Yogurt drink		
- Density ( $\rho_L$ )	1.05	gr cm <sup>-3</sup>
- Volume	1500	lit
PET bottle		
- Denisty ( $\rho_p$ )	1.38	gr cm <sup>-3</sup>
- Thickness (d)	-0.95	cm
- $A'_p$	2.2	
- $\tau$	1577	
Menthol		
- Molecular weight ( $M_r$ )	154	gr mol <sup>-1</sup>
- Initial concentration ( $C_{L,0}$ )	100	mg kg <sup>-1</sup>



**Figure 2.** Absorption of menthol from yogurt drink into Polyethylene terephthalate (PET) bottles.

increased consistently when storage temperature of model solutions was elevated from 4 to 25 and 37°C [3]. Nielsen (1994) concluded that there were relative differences between absorption levels of PET bottles containing a soft drink type orange (60 mg limonene litre<sup>-1</sup>) stored at 25°C and those stored at 4°C evaluated at the ratio of 7:1 after seven days, decreasing to 3:1 after 85 days [1]. Farhoodi *et al.* (2011) showed that the absorption of flavors (D-limonene,  $\alpha$ -Pinene and myrcene) from soft drinks into PET bottles increased when the temperature was elevated from 4 to 25 and 40°C [4]. A slower rate of absorption at low temperature was reported by Kwapong and Hotchkiss (1987) implying that the diffusion process was temperature-dependent [17].

Results of this study showed that, after 5 days' storage, PET bottles stored at 45°C absorbed 5 and 12 times higher concentrations of menthol than PET bottles stored at 25° and 4°C, respectively. These ratios altered to 4:1 and 15:1 after 20 days and 3.7:1 and 18:1 after 90 days, respectively. Thus, the slope of the absorption curve will decrease faster at lower storage temperature and a state of equilibrium. Comparison of diffusion coefficient of menthol at three different temperatures confirms this finding (Table 2). These results show that, for all storage

temperatures, after about 20 days, the slope of the absorption curve suddenly decreases and samples reach a state of equilibrium. Since after "90 days" storage all the samples remained in their equilibrium state, 90 days has been marked as the point of equilibrium. At each storage temperature, the amount of menthol absorption was observed as increasing over time. Therefore, it can be asserted that, generally, the rate of menthol absorption into PET bottles is dependent on

**Table 2.** Diffusion coefficient of menthol at different time and temperature conditions.

Time (Day)	$D_p \times 10^{-16}$ (cm <sup>2</sup> s <sup>-1</sup> )		
	4°C	25°C	45°C
0.67	na <sup>a</sup>	64.71	na
0.75	na	61.85	na
1	10.37	77.85	2433.62
2	12.68	78.60	1983.83
5	9.64	53.38	1338.34
9	6.84	42.47	1259.65
14	5.16	49.02	1083.61
21	3.89	50.10	874.05
50	1.66	34.04	501.22
90	0.92	21.96	309.21
Mean	6.39	53.40	1222.94

<sup>a</sup> Not analyzed.



both storage time and temperature. In research by Tawfik *et al.* (1988), it was observed that the amount of *D*-limonene absorbed into PET strips ranged from 1.12-8.90 ppm and the maximum amount of absorption was achieved after 45 days with initial aroma concentration of 320 ppm in the solution [3]. Thus, it was concluded that absorption of flavors into packaging materials depended on the initial concentration. The specific type of plastic used is important in terms of how it may affect the uptake of aroma compounds, but possible interactions between flavor and food component must also be considered. Flavor compounds may undergo different changes immediately after being added to the food. They may be dissolved, adsorbed, bound, entrapped, encapsulated or retarded in their diffusion through the matrix by food components. Each of these mechanisms could have an effect on the quality of the final product before consumption. The relative importance of the mentioned phenomena varies according to the chemical properties of a flavor (functional groups, molecular size, shape and volatility) and other food components. Van Willige *et al.* (2000) reported that, after 14 days of exposure, the amount of free decanal absorbed into LLDPE, and in a model solution containing  $\beta$ -lactaglobulin and casein, significantly decreased, which means that decanal was bound by  $\beta$ -lactaglobulin and casein during storage time with respect to the existence of proteins [18]. Difference in levels of absorption of various flavors into PET bottles in different studies could be related to the different structures of flavor compounds, the structure and thickness of PET bottles used to package the food, and the complex nature of solutions containing flavor compounds. In the present study, under different storage conditions, about 0.2-0.7 percent of initial menthol contents in yogurt drink were absorbed into the PET over a 90-day period. However, results showed that this was dependent on storage temperature and the initial flavor content, while the solution itself also affects the

flavor. Nevertheless, no other reports were found in the literature about the absorption percentage of menthol in yogurt drink into PET bottles. This study showed the procedure of absorption from a dairy product into PET bottles and determined the possibility of organoleptic changes during storage times that may increase under undesirable storage conditions. Table 2 demonstrates the diffusion coefficient of menthol during the absorption process at different storage temperatures. The average  $D_p$  derived from Equation (1) gives an approximation for diffusion coefficient of menthol in PET. The results showed that an increase in the storage temperature from 4 to 25 and 45°C resulted in up to 8.3-fold and 191-fold increase in diffusion coefficient of menthol in PET, respectively. It can be concluded that the diffusion process of menthol is absolutely temperature dependent.

The diffusion coefficients were also obtained from the Equation (3). For PET, a mean value  $A'_p = 2.2$  and  $\tau = 1577$  (Table 1) can be used according to literature [14]. With these values, the diffusion coefficient of menthol ( $\text{cm}^2 \text{s}^{-1}$ ) at 4, 25 and 45°C is  $4.2 \times 10^{-16}$ ,  $8.6 \times 10^{-15}$  and  $1.12 \times 10^{-13} \text{ cm}^2 \text{ s}^{-1}$ , respectively. These values are in good agreement with the mean values obtained from the experimental data. Sheung *et al.* (2007) concluded that the diffusion coefficient of ethyl butyrate and octanal had significant variations during the diffusion process, while the diffusion coefficient of *d*-limonene and  $\alpha$ -pinene did not change significantly in terms of length of time [11]. The difference between diffusion coefficients of various flavor compounds under similar circumstances could be related to differences in molecular structure.

## CONCLUSIONS

Absorption of flavor compounds into packaging material may result in products with an imbalance of flavor and aroma. The absorption of menthol from yogurt drink

into PET bottles had not been previously investigated. From the result of this study, it can be concluded that for the absorption level in products such as yogurt drink that are packed in plastics packs, it is necessary to establish proper time and temperature conditions. The loss of aroma and flavor during storage time could be considered as a factor affecting shelf life. Comparison of diffusion coefficients of menthol into PET bottles stored at different storage temperatures showed that the diffusion process was time- and temperature-dependent.

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## تأثیر جذب منتول بوسیله ماده بسته بندی بر روی کیفیت نوشابه دوغ در طول دوره

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### چکیده

فعل و انفعال منتول با بطریهای پلی اتیلن ترفتالات در سه شرایط دمایی مورد بررسی قرار گرفت. منتول عامل طعم نعنا بوده و در دوغهای ایرانی مورد استفاده قرار گرفته و به عنوان یک فاکتور مقبولیت برای مصرف کننده تلقی می شود. جذب منتول به ماده بسته بندی سبب افت کیفیت محصول نهایی به علت کاهش شدت عطر و طعم آن می شود. به منظور بررسی تاثیر شرایط محیطی بر روی پایداری طعم، نمونه های دوغ به مدت سه ماه در سه دمای ۴، ۲۵ و ۴۵ درجه سانتیگراد نگهداری شدند. بعد از دوره های زمانی مختلف ترکیب عطر و طعمی جذب شده (منتول) از بطریهای PET استخراج شد و با استفاده از کروماتوگرافی گازی مجهز به دتکتور FID اندازه گیری شد. سپس ضریب نفوذ منتول به بطریهای PET تعیین شد. بعد از مدت زمان ۹۰ روز میزان منتول جذب شده در سه دمای ۴، ۲۵ و ۴۵ درجه سانتیگراد بترتیب ۳۸.۲۱، ۱۸۶.۶۶ و ۷۰۰.۵۰ نانوگرم بر گرم بطری PET بود. این نتیجه حاصل شد که میزان منتول جذب شده به بطری با گذشت زمان و افزایش دما افزایش می یابد. افزایش دمای نگهداری سبب افزایش قابل ملاحظه در ضریب نفوذ منتول در بطری PET شد.