University of Belgrade
Technical Faculty in Bor

28th International Conference Ecological Truth & Environmental Research

EcoTER'20

PROCEEDINGS

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Prof. Dr Snežana Šerbula

16 - 19 June 2020, Hotel Aquastar Danube, Kladovo, Serbia

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THE MIGRATION OF DEHP FROM PLASTIC PACKAGING INTO DAIRY PRODUCTS WITH DIFFERENT FAT CONTENT

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Abstract

Objective of this study was to determine the effect of fat content of dairy products on the migration of di-(2-ethylhexyl) phthalate (DEHP) from plastic packaging into different dairy products. Migration of DEHP from plastic packaging into three dairy recipients with different fat content was observed: infant formula, whey powder and water under the influence of increased temperature. Amount of leached DEHP from plastic packaging into different recipients was determined by liquid-liquid extraction followed by gas chromatography-mass spectrometry (GC/MS).The ion of base peak m/z 149 for DEHP was selected for the screening studies. The acquisition was performed at the selected ion monitoring mode (SIM). The mass-selective detector (MSD) response for GC/MS DEHP calibration standards was linear between 0.25 and 2.50 μg mL^{-1}. The study demonstrated an increase of the amount of migrated DEHP from packaging material into dairy product with higher percentage of fat. The mass of migrated DEHP was the highest in the sample of infant formula, followed by whey powder and water. Also, the results show that longer exposure to high temperature results in reaching the plateau on the graph Mass of migrated DEHP vs time, which means that further exposure to high temperature does not result in increase of DEHP leaching.

Keywords: DEHP migration, dairy products, fat content, plastic packaging, GC/MS

INTRODUCTION

Phthalates, known as dialkyl or alkyl aryl esters of 1,2-benzenedicarboxylic acid, are a type of chemical compounds used to soften plastics, making plastics more suitable for a wider range of uses. Plastics treated with such plasticizers are softer and more flexible, with a suitable odour and colour. As such, plastics are used to manufacture medical equipment, children's toys, food packaging and other different plastic packaging. However, the phthalates are not chemically bonded to the polymer but are only impregnated in the polymer, making phthalates more accessible to the environment (phthalates can easily migrate to their environment - food, drink, air, soil, *etc.*).In this way, human health can be affected by phthalates, so the European Food Safety Authority (EFSA) had set the tolerable daily intake (TDI) for the most commonly used phthalate - DEHP and amounts 0.05 mg kg^{-1} body weight of adult human day^{-1} [1]. Various studies have shown that DEHP exposure may induce reproductive toxicity, liver damage, and carcinogenesis in humans [2]. Given that DEHP affects human health, and that DEHP is easily leached into food from plastic packaging materials, it is of great importance to determine the concentration of DEHP in the investigated matrices as well as to test the impact of fat content to a DEHP migration.

Determination of DEHP can be performed using different techniques, depending on the investigated matrix. Gas chromatography coupled with mass spectrometry (GC/MS) is the most used technique for DEHP determination [3].

In this experiment, the migration of DEHP from plastic packaging materials of known percentages of DEHP was done under the influence of increased temperature, while dairy products with different fat content were used as the receiving matrix for the leached DEHP. The quantification of leached DEHP from plastic packaging into different dairy products was performed by GC/MS. In this way, the impact of fat content of dairy products on migration of DEHP from plastic packaging was determined.

MATERIALS AND METHODS

Reagents and materials

HPLC grade *n*-hexane was purchased from Sigma–Aldrich (St. Louis, MO, USA). Di(2-ethylhexyl) phthalate (DEHP) was purchased, in the highest available purity, from Sigma–Aldrich (St. Louis, MO, USA). Dibutyladipate (DBA) was purchased from Fluka (Buchs, Switzerland) and used as an internal standard. Water from a Milli-Q system (Millipore, Bedford, MA, USA) was used. Dialysis bag (Baxter, USA) was used. Whey powder and infant formula were purchased from commercial manufacturers offered at the Serbian market.

All stock, intermediate and working solutions were prepared in *n*-hexane. The stock solutions of DEHP and DBA were prepared at a concentration of 1.0 mg mL^{-1}. The stock standard was diluted stepwise with *n*-hexane to prepare working solutions of DEHP. The calibration standard series was obtained with DEHP in the concentration range of 0.25, 0.50, 1.00, 1.50 and 2.50 µg mL^{-1} with DBA as the internal standard, at a concentration of 1 µg mL^{-1}. The calibration curve was linear in the range from 0.25 to 2.5 µg mL^{-1} with a correlation coefficient higher than 0.99. The linear dynamic range was broader and covered the range from 2.50 to 50 µg mL^{-1}.

GC-MS technique

Gas chromatographic analysis was performed by gas chromatograph 6890 (Hewlett-Packard, USA) equipped with a mass selective detector (MSD) 5973 (Hewlett-Packard, USA) and Autosampler 7683 (Agilent, USA) and a DB-5 MS capillary column (30 m × 250 mm × 0.25 mm) (Agilent, USA). The gas chromatograph was operated in the split less injection mode. The oven temperature was programmed from initial temperature 60 °C (hold time 1 min) to 220 °C (hold time 1 min) at a rate of 20 °C $min^{-1}$and then to 280 °C (hold time 4 min) at a rate of 5 °C min^{-1}. Helium was the carrier gas (flow rate of 1.0 mL min^{-1}) and the inlet temperature was 250 °C. The operating temperature of the MSD was 280 °C with the electron impact ionization (EI) voltage of 70 eV. The dwell time was 100 ms. The MSD was used in the single ion-monitoring mode (SIM), the quantification ion was*m/z* 149 for DEHP and ion *m/z* 185 was chosen as representative ion of DBA. Analyte response was normalized to DBA as internal standard. The identification and quantification of target compound was based on the relative retention time, the presence of target ion and its relative abundance. Both data acquisition and processing were accomplished by Agilent MSD ChemStation® D.02.00.275 software.

Sample preparation

In the migration experiment, recipients for migrated DEHP from plastic packaging materials under the influence of temperature were infant formula, whey powder and water. Dairy products (infant formula and whey powder) were non-polar and had different fat content, unlike water which was polar and without fat, and it was taken for comparison. Fat content for infant formula and whey powder were 22.8 and 2 %, respectively.

Whey powder (65 g) and infant formula (45 g) were dissolved in 1 L of water from a Milli-Q system, respectively, according to the producer instruction. Fresh samples were always prepared immediately before analysis.

The dialysis bag was used as a plastic packaging material with high phthalate content. This reliable source of DEHP which contains about 35.8 % of DEHP compared to the total weight was used to examine DEHP migration [4]. The measured pieces of 1 cm^2 of dialysis bag were covered with 35 mL of dairy samples (infant formula and whey powder) and water.

Migration experiment and extraction procedure

The conditions of a migration experiment were set up to determine the effects of exposure time and temperature to DEHP migration into dairy products: dairy products with plastic packaging materials were heated up to 60 °C for up to 60 min, with repetition of cooling and heating processes with the total heating time up to 60 hours.

Dairy samples with 1 cm^2 of dialysis bag were vigorously stirred and heated to a temperature up to 60 °C, for 60 min. After cooling the samples at room temperature and filtering, 20 mL of *n*-hexane, as effective extraction agent[5], was added to the samples. Extracts were manually shake for 15 minutes followed by extraction inthe ultrasonic bath for 30 minutes. After clarification of blurred extracts, the *n*-hexane layers were separated and concentrated to the volume of 1 mL. DBA was added to concentrated *n*-hexane extracts such that its concentration in each sample was 1 $\mu g\ mL^{-1}$.

Pieces of dialysis bag, were subjected to repeated migration tests into dairy products, providing total heating time of 60 hours. The test was performed until the plateau was reached on the curve $m = f(t)$, where m is mass of the migrated DEHP and t time of exposure.

Blank samples (dairy products without plastic pieces from dialysis bag) were treated in the same way as the dairy samples with plastic pieces from dialysis bag. The laboratory contamination was thus monitored.

All experiments were performed in triplicate and quantification on GC/MS was repeated twice.

Glassware control

In order to minimize the contamination by phthalates from the laboratory, only glassware equipment was used. All glassware was washed with soap, tap water and ultrapure water, then washed with acetone and n-hexane and dried at 200 °C in the oven for 4 hours [6].

RESULTS AND DISCUSSION

The chromatogram of a standard solution of DEHP and DBA, given in Figure 1, shows good separation of DEHP and DBA occurred within a running time of 20 minand retention times for DBA and DEHP were 9.990 and 18.426 min, respectively. The calibration curve was linear in the range from 0.25 to 2.5 µg mL^{-1} with correlation coefficient of calibration curve higher than 0.990.

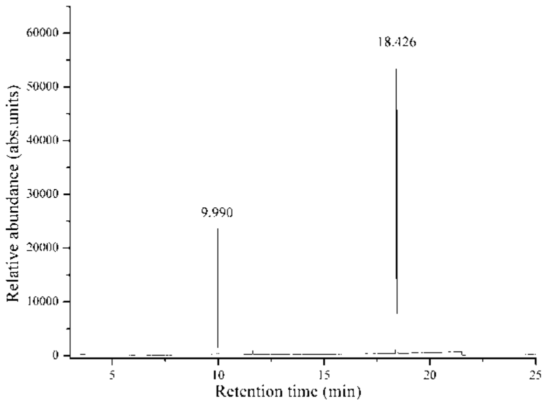
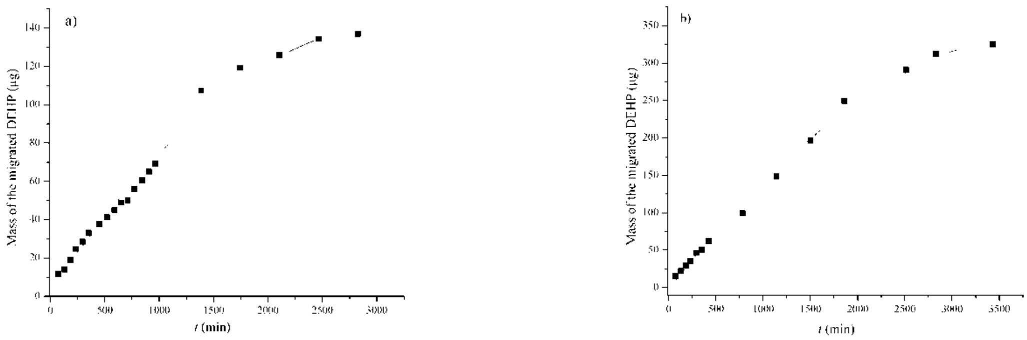


Figure 1 *GC/MS chromatogram of a standard solution of DEHP and DBA in a concentration of 2.5 µg mL^{-1} and 1.00 µg mL^{-1}, respectively*

Figure 2 shows the graphs of the mass of the migrated DEHP as the function of exposure time to increased temperature for three types of dairy products. The graphs show a linear increase of the mass of migrated DEHP over time under the influence of temperature, until the plateau is reached. Plateau indicates that after the maximum migration of DEHP has been reached DEHP no longer migrates into the food receiving matrices under the influence of increased temperature. Maximal amount of migrated DEHP, to water as recipient, is reached after 2500 min. The time required to achieve maximum DEHP migration into whey powder and infant formula were2950 and 3000 minutes, respectively.



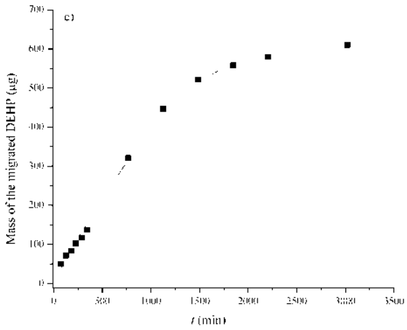


Figure 2 *The influence of temperature on the migration of DEHP in a) water, b) whey powder, c) infant formula*

The total migrated amount of DEHP follows the order: infant formula (601.29 µg), whey powder (311.83 µg) and water (135.67 µg), which is in correlation with the time of reaching plateau and with fat content of receiving matrix.

Figure 3 shows two regions in the mass vs. time graphs. Graph (a) shows the dependence of the mass of the migrated DEHP relative to the total exposure time of the samples to increased temperature and graph (b) shows the dependence of the mass of migrated DEHP in the first 450 min of exposure. The second graph shows higher slope of curve of migrated DEHP over time and curve is almost linear. During this period of 450 min about 30 % of total amount of migrated DEHP migrated into recipients. Unlike this rapid migration in the first 450 min, the migration in the remaining exposure time is slower, and slope of curve of migrated DEHP over time was smaller then of the above noted.

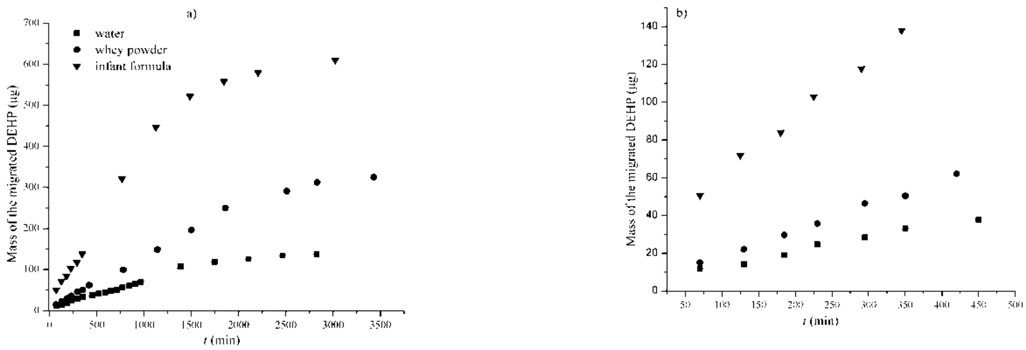


Figure 3 *Mass of the migrated DEHP under the influence of temperature a) in a time period of 3500 min and b) in a time period of 450 min*

The aim of this migration experiment was to determine the total mass of the migrated DEHP from plastic packaging materials into dairy products after repeated migration, related to temperature. The effects of temperature and fat content of dairy products on DEHP leaching are presented in Table 1.The results show that the highest mass of migrated DEHP was determined in the dairy product with the highest percentage of fat, which was expected, since DEHP is a non-polar molecule and is more soluble in non-polar media such as the infant formula with 22 % of fat. Water as a polar medium was not a suitable recipient for migrated

DEHP, while whey powder was second recipient for DEHP due to a fat content of 2 %.In all three cases, the percentage of DEHP leaching was low and ranged from to 0.02-0.12 % relative to the mass of DEHP in plastic materials.

Table 1 *Migration of DEHP influenced by temperature and fat content of food receiving matrix*

Food receiving matrix	Mass of dialysis bag (g)	Mass of DEHP in dialysis bag (g)[4]	Maximal amount of migrated DEHP (µg)	Percentage of migrated DEHP (%)
Water	1.7410	0.6233	135.67±1.23	0.02
Whey powder	1.6113	0.5768	311.83±1.40	0.05
Infant formula	1.4560	0.5212	601.29±2.73	0.12

CONCLUSION

Migration of DEHP from plastic packaging materials to dairy products depends on exposure temperature, exposure time and fat content of dairy product. Obtained data showed the following: amount of migrated DEHP decreases by following order infant formula > whey powder > water. In this order the fat content also decreases, which indicates that the highest migration is for dairy product with highest fat content. Results indicate that increasing temperature increases the migration of phthalates into diary matrices. Thus, during period of 450 min about 30 % of total amount of DEHP migrated into recipients, while the migration in the remaining exposure time is slower.

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