PHTHALATE MIGRATION FROM PACKAGING MATERIALS INTO FOOD

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ABSTRACT

The content of dibutylphthalate (DBP) and di- (2-ethylhexyl) phthalate (DEHP) in samples of packages used for packaging meat productsand the phthalate migration from packaging materials to meat products were studied. Five samples of textile packaging intended for cooked meat production were analysed as well asthe final product which was filled into packages. Subsequently an analysis was carried out (after 1, 7, 14, 21, and 28 days of storage) of the finished meat products stored over the course of their intended shelf life at ambient temperature of 4 °C.Determination of phthalates was conducted by high performance liquid chromatography (HPLC) method with UV detection at a wavelength of 224 nm on the Zorbax Eclipse C8 column.The content of phthalates in the final product was below the limit of quantification. According to the Regulation of the Commission (EU) No. 10/2011, the specific migration limit of products intended for food contact is 1.5 $mg.kg^{-1}$of food simulant for DEHP and 0.3 $mg.kg^{-1}$ for DBP. After filling and the first day of storage of the meat product, four package samples release the said phthalates to an extent that it exceeded the limits of the Commission Regulation (EU) No. 10/2011. Already after the seventh day of storage, all samples (with the exception of sample 2 for DBP) exceeded SMLs. Monitoring of each phthalate migration in individual samples during storage (for 28 days) produced a rising tendency. In sample 1, DBP increased from 0.40 to 3.37 $mg.kg^{-1}$, while DEHP from 0.58 to 14.66 $mg.kg^{-1}$. In sample 2, DBP increased from ≤ 0.2 $mg.kg^{-1}$ to 4.34 $mg.kg^{-1}$, whileDEHP from 1.46 to 28.20 $mg.kg^{-1}$. In sample 3, DBP increased from ≤ 0.2 $mg.kg^{-1}$ to 8.27 $mg.kg^{-1}$, while DEHP from 1.67 to 14.84 $mg.kg^{-1}$. In sample 4, DBP increased from 0.27 to 6.12 $mg.kg^{-1}$, while DEHP from 2.37 to 13.22 $mg.kg^{-1}$. In sample 5, DBP increased from 0.32 to 11.11 $mg.kg^{-1}$, while DEHP from 1.91 to 15.42 $mg.kg^{-1}$.

INTRODUCTION

Phthalates are a group of dialkyl- or alkyl aryl esters of 1,2-benzenedicarboxylic acid derived from the trivial name for this acid, phthalic acid. Phthalates have become ubiquitous contaminants (**Net et al., 2015**) because of their volatilization and leaching (**Wormuth et al., 2006**). High molecular weight phthalates, such as di- (2-ethylhexyl) phthalate (DEHP), are primarily used as plasticizers (PVC) (**Rahman et al., 2004**) and low molecular weight phthalates, such as di-n-butyl phthalate (DBP), are added e.g. in colours to increase their adhesion to the surface (**Xue et al., 2010**).

Negative effects of phthalates on living organisms have been demonstrated. They pose teratogenic, carcinogenic, mutagenic, and reproductive hazards.Contamination of the body by phthalates may take different routes, e.g. through inhalation, skin absorption and food (**YIN et al., 2003; Borchers et al., 2010; Witassek et al., 2011**).

The highest human exposure to phthalates comes from food. One of the sources of food contamination isthe packaging material, from which phthalates migrate to food (**Cao, 2010**). **Bradley et al., (2013)** conducted an analysis of the phthalate content in 261 food samples, 20 UK Total Diet Study food groups. Phthalate diesters were confirmed to be present in 77 samples and DEHP was detected in 66 samples. Other studies confirmed that packaging materials contribute to the concentration of phthalate diesters in some food.

Zhang et al. (2008) determined the 2.6-diisopropylnaphthalene (DIPN) and dibutylphthalate (DBP) in 110 domestic and foreign packaging and food in marketplaces in the United States. Concentrations of DIPN and DBP in packaging ranged from 0.09 to 20.0 and from 0.14 to 55 $mg.kg^{-1}$; mostly they were less than 20 $mg.kg^{-1}$. DIPN was not detected (<0.01 mg kg^{-1}) in 41

samples of foods and DBP was only detected in two domestic and four imported food samples with a concentration from 0.01 to 0.81 mg. kg^{-1}. Phthalates were part of the packaging printing inks.
For the period of 14 days **Cirillo et al. (2013)** have analysed the diet of patients eating in the hospital for the contents of DEHP and DBP. Packaging contained the polyethylene terephthalate (PET) and the food was covered with a polypropylene (PP) foil. The study found that the highest concentration was in bread (0.307 ± 0.138) $\mu g.g^{-1}$of the original sample for DEHP, and 0.174 ± 0.091$\mu g.g^{-1}$of the original sample for DBP). Although the main route of exposure for hospital patients may be the hospital facilities, even the diet containing phthalatesmay contribute to increasing the Tolerable Daily Intake (TDI) of phthalates.
In many cases the source of phthalates may not be thematerialused for packagingfood, but the technological equipment. Such was the finding of **Bach et. al. (2012)**in case of packagedwater. Mineral water bottled in PET (polyethylene terephthalate) bottles, where phthalates as plasticisers are not used, was contaminated by equipment from bottling lines.
The aim of the study was to monitor the migration of phthalates (DEHP and DBP) from packaging to meat products during storage.

Keywords: phthalates, DBP, DEHP, packaging, migration

MATERIAL AND METHODOLOGY

Meat products packages, samples of final meat product and samples of the finished meat product were analysed. Packages of meat products (n = 5) were purchased from the company that supplies packagesfor the processing industry. These were coloured textile packages designed for the production of cooked meat products. From each type of package a sample was taken in size of 10 dm^2, when the samples were analysed in duplicate.
Final meat product intended for further heat treatment was produced in pilot conditions of the Department of Food Technology at Mendel University in Brno. Six samples of final meat product were collected, and then the final meat product was filled into packages. Thirty samples were produced for each package. After being filled into packages, the samples were stored at 4°C. Samples were taken after 1, 7, 14, 21 and 28 days of storage.
A total of 20 samples of packaging, 6 samples of final meat product before being filled into packages and 150 samples of the meat product after heat treatment and storage were analysed. All samples were analysed in duplicate, i.e. 352 analyses were performed.
Packaging samples were leached in *n*-hexane:dichloromethane (1:1) solvent mixture for 72 hours and then extracted three times (60, 30, and 30 minutes).The combined extraction shares were filtered, evaporated on a rotary vacuum evaporator and finally dried under nitrogen.The extract was then transferred into vials using hexane (5 ml) and centrifuged. The upper portion of the extract (1.5 ml) was collected into vials for HPCL (high-performance liquid chromatography) determinationand dried under nitrogen. Again, the vials were centrifuged; the upper layer of the extract (1.5 ml) was taken away and dried under nitrogen. Subsequently, the vials were supplemented with acetonitrile to make a volume of 1 ml. If the extracts were coloured or turbid, they were purified with sulphuric acid.
In order to analyse PAE in samples of final meat product and finished meat products proven methods for determining DBP and DEHP in food have been used (**Jarošová et al., 1998, 1999**).
Samples were homogenized, weighted into metal plates and frozen. Gradually, the frozen samples were lyophilized and subsequently the PAE residues were extracted with *n*-hexane. PAEs were separated from co-extractsby gel permeation chromatography on Bio Beads S-X3 gel. Eluates were purified by a cleaning process using the concentrated sulphuric acid.

Phthalates were determined by HPLC method with UV detection at a wavelength on 224 nm on the ZorbaxEclipse C8 column. The quantity of the samples sprayed on the column was 10 µl. Final concentrations were calculated based on the calibration curve in the Agilent Chemstation software for LC and LC/MS systems. The range of calibration curve was between 1.06µg.ml^{-1}and 106.00µg.ml^{-1} for DBP and between 1.01µg.ml^{-1}and 100.50µg.ml^{-1} for DEHP. The correlation coefficient was 0.9999 both for DBP and DEH. The limit of detection was 0.05µg.ml^{-1} for DBP and 0.11µg.ml^{-1} for DEHP. In the final phase, the results were statistically processed.

All the laboratory glass was flushed with hexane during sample preparation. Simultaneously dry matter and fat content were determined for each sample. Samples of the meat product contained about 30 % of fat. All samples were analysed in duplicate. Concentrations of DEHP and DBP are related to the original sample.

RESULTS AND DISCUSSION

Concentrations of phthalates in the analysed packages are expressed in µg.dm^2 and are given in Table 1. Each value represents the average of two parallel determinations.

Table 1 Mean concentrations of DBP and DEHP (µg.dm^{-2}) in samples of packages used for packaging meat products

Sample	DBP	DEHP
	µg.dm^{-2}	
1	4.35	19.1
2	8.26	16.79
3	23.95	103.33
4	15.09	26.54
5	5.26	0.3

The suitability of packaging for food is defined by a migration limit (ML), which is the maximum amount of packaging components, which can be released from the packaging per unit area. According to the Commission Regulation (EU) No. 10/2011, products intended to come into contact with food and meals must not release their ingredients into the food in amounts greater than 10 mg.dm^2 or 60 mg.kg^{-1} of the food or food simulant. The said regulation also includes a specific migration limit, which is max 1.5 mg.kg^{-1} of food simulant for DEHP and max 0.3 mg.kg^{-1} of food simulant for DBP. As far as the samples of packaging given in Table 1 are concerned, four samples (1, 3, 4, and 5) would not comply with the said regulation with regard to the specificmigration limit (Table 2) after 1 day of storage of a meat product packaged in these packages.

Concentrations of monitored phthalates (DEHP and DBP) in the final meat product and in finished meat products are expressed in mg.kg-1 of the original sample and they are given in Table2. Each value represents the mean of 12 values (six parallel samples and each sample analysed in duplicate).

Table 2 Mean concentrations of DBP and DEHP (mg.kg-1) in the samples of final meat product and finished meat product (n=5) after 1, 7, 14, 21, and 28 days of storage at 4 °C.

Sample	Final meat product		Day 1		Day 7		Day 14		Day 21		Day 28	
	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP
	mg.kg^{-1}											
1	LOQ	LOQ	0.40	0.58	1.32	8.79	2.08	11.28	3.14	14.43	3.37	14.66
2	LOQ	LOQ	LOQ	1.46	0.70	11.93	1.78	19.10	2.93	21.56	4.34	28.20
3	LOQ	LOQ	LOQ	1.67	0.22	7.12	4.38	12.05	6.59	13.76	8.27	14.84
4	LOQ	LOQ	0.27	2.37	0.43	7.90	4.68	13.09	5.17	13.16	6.12	13.22
5	LOQ	LOQ	0.32	1.91	0.64	9.32	6.84	10.55	8.00	10.80	11.11	15.42

LOQ –limit of quantification of DBP and DEHP in fat matrices – 0.2 mg.kg^{-1}

The phthalate content in the final meat product and migration of phthalates after heat treatment and storage were monitored. In samples of final meat product taken immediately after mixing of the final meat product, concentrations ofboth phthalates were below the limit of quantification (≤ 0.2 mg.kg^{-1}).

After 1 day of storage DBP content moved from ≤ 0.2 to 0.40 mg.kg^{-1} and DEHP content from 0.58 to 2.37 mg.kg^{-1}. Migration limit was not exceeded in sample 2 (DBP and DEHP), sample 1 forDEHP and samples 3 and 4 forDBP. After 7 days of storage the DBP content was between 0.22 and1.32 mg.kg^{-1} and DEHP content between 7.12 and 11.93 mg.kg^{-1}. Migration limit was exceededin all analysed samples, except for sample 3, where DBP value was measured 0.22 mg.kg^{-1} below the migration limit. After 14 days of storage DBP content was between 1.78 and 6.84 mg.kg^{-1}and DEHP content between 10.55 and 19,10 mg.kg^{-1}. Migration limit was exceededin all analysed samples. After 21 days of storage DBP content was between 2.93 and 8.00 mg.kg^{-1}and DEHP content between 10.80 to 21.56 mg.kg^{-1}. Migration limit was exceededin all analysed samples. After 28 days of storage DBP content was between 3.37 and 11.11 mg.kg^{-1} and DEHP content between 13.22 to 28.20 mg.kg^{-1}. Migration limit was exceededin all analysed samples.

Monitoring of each phthalate migration in individual samples during storage produced a rising tendency.

According to the Commission Regulation (EU) No. 10/2011, under which the specific migration limit is 1.5 mg.kg^{-1} for DEHP and 0.3 mg.kg^{-1} for DBP, the DBP migration limit was exceeded after the first day of storage in two samples (1 and 5) and DEHP migration limit in three samples (3, 4, and 5). After the seventh day of storage all samples exceeded the migration limits set for the said phthalates,except for sample 3 for DBP.

Our experimentshave shown that the content of plasticizers leached from packages increases with temperature and time of storageand our finding is consistent with results of other authors. **Condyle et al. (1992)** examined the migration of dioctylphthalate (DOP) and dioctyladipate (DOA) from PVC into ground meat with a different fat content stored at 4 °C and -20 °C. After 8 days of storage at 4 °C from 2 to 80 mg.kg^{-1} (0.12 to 4.8 mg.dm^{-2}) plasticizers migrated to meat and from 2 to 60 mg.kg^{-2} (0.13-4 mg.dm^{-2}) migrated after 212 days pf storage at -20 °C. Their experiments demonstrated that the migration in samples with a lower fat content was proportionally lower.

Also **Moreira et al. (2015)** concluded that phthalates are released from packaging materials. They investigated the content of eight plasticizers in samples of spices and baked chicken meat stored in plastic packages. They found diisobutyl phthalate and dibutyl phthalate, which migrated from the package. Higher concentrations of plasticizers were detected in spices.
Migration of dioctyladipate (DOA) and acetyltributylcitrate (ATBC) plasticizers in ground meat due to the effect of microwave heating was studied by **Badek et al. (1998)**. The samples varied in fat, and all were packed into foil containing PVC and polyvinylidenchloride (PVDC/PVC). They were heated in a microwave oven for the period from 0.5 to 4 minutes. The values of DOA and ATBC migrationin samples with 55 % fat content and 4 minutes of heating were 846.0 $mg.kg^{-1}$ (14.7mg.dm-2) and 95.1 $mg.kg^{-1}$ (2.5 mg dm^{-2}).
Mei-Lien Chen (2008) conducted a study in Taiwan, where they monitored the level of migration of phthalates (DEHP and DBP) from PVC foil. Food was covered with PVC foil and heated in a microwave oven. Results showed that the DEHP level in food increased significantly during 3 minutes of heating.

CONCLUSION

The aim of our study was to monitor the content of phthalates (DEHP and DBP) in packaging intended for meat products and track the possible migration of phthalates from packaging into the product after heat processing and storage over the product shelf life. Given the overall migration values under the Commission Regulation (EU) No. 10/2011, themonitored packages did not exceed the migration limits. In comparison with the specific migration limits for DBP (0.3 $mg.kg^{-1}$) and DEHP (1.5 $mg.kg^{-1}$) all analysed packages exceeded the limits imposed by the said regulationalready after the seventh day of storage of the finished meat products. The DBP values ranged from $\leq$ 0.2 $mg.kg^{-1}$ to 11.11$mg.kg^{-1}$ and DEHP values from 0.58 $mg.kg^{-1}$ to 28.20 $mg.kg^{-1}$ in all the analysed samples during storage lasting 28 days.
One of the ways how to reduce the risk of food contamination with phthalates is to promote the substitution of phthalates by other non-toxic substances.

REFERENCES

Badeka A.B., Kontominas M.G. 1998. Effect of microwave heating on the migration of dioctyladipate and acetyltributylcitrate plasticizers from food-grade PVC and PVDC/PVC films into ground meat. *Developments in Food Science*, 40, P. 759–765. doi:10.1016/S0167-4501(98)80096-4. ISBN: 978-0-444-52742-4.
Bach, C., Dauchy, X., Chagnon, M. C., Etienne, S. 2012. Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephtalate (PET) bottles: A source of controversy reviewed.*Water Research*, 46, p. 571-583. ISSN: 0043-1354.
Borchers A., Teuber S.S., Keen C.L., Gershwin M.E. 2010.Clinic. Food safety.*Rev. Allerg. Immunol.*, 39, p. 98–141. ISSN: 1080-0549.
Bradley EL, Burden RA, Leon I, Mortimer DN, Speck DR, Castle L.2013. Determination of phthalate diesters in foods. *Food Additives & Contaminants*,30(4), p. 722-34. doi: 10.1080/19440049.2013.781683. Epub 2013 Apr 24. ISSN:1944-0049.
Cao, X. L., 2010. Phthalate esters in foods: sources, occurrence, and analytical methods.*Comprehensive Reviews in Food Science and Food Safety*, 9, p. 21-43. ISSN: 1541-4337.
Cirillo, T., Fasano, E., Esposito, F., Montuori, P., Cocchieri, R. A. 2013. DI(2-ethylhexyl) phthalate (DEHP) and di-n-butylphthalate (DBP) exposure through diet in hospital patients. *Food and Chemical Toxicology*, 51, p. 434-438. ISSN: 0278-6915.
Commission Regulation No. 2011/10/EC of 14 January 2011 relating to plastic materials and articles intended to come into contact with foodstuffs 2011; OJ L 12, p. 4-12.

EfthimiaKondyli, Panagiotis G. Demertzis, Michael G. Kontominas. 1992. Migration of dioctylphthalate and dioctyladipate plasticizers from food-grade PVC films into ground-meat products. *Food Chemistry*, 45, p.163-168. doi:10.1016/0308-8146(92)90108-E, ISSN: 0308-8146.
Jarošová, A., Gajdůšková, V., Raszyk, J., Ševela, K. 1998.*Determination of phthalic acid esters (PAEs) in biological materials by HPLC.Czech Journal of Food Sciences*, 16, p. 122-130. ISSN 1805-9317.
Jarošová, A., Gajdůšková, V., Raszyk, J., Ševela, K. 1999. Di-2-ethylhexyl phthalate and di-n-butyl phthalate in the tissues of pigs and broiler chicks after their oral administration.*Veterinárnímedicína*, 44, p. 61-70. ISSN: 1805-9392.
Mei-Lien Chen, Jing-Shieng Chen, Chia-Ling Tang, I-Fang Mao. 2008. The internal exposure of Taiwanese to phthalate-An evidence of intensive use of plastic materials.*Environment International*, 34, p. 79-85. ISSN: 0160-4120.
Moreira MA, André LC, CardealZde L. 2015. Analysis of plasticiser migration to meat roasted in plastic bags by PME–GC/MS. *Food Chemistry*, 178, p. 195-200. doi: 10.1016/j.foodchem.2015.01.078. Epub 2015 Jan 21. ISSN: 0308-8146.
Net, S., Delmont, A., Sempéré, R., Paluselli, A., Ouddane, B. 2015. Reliable quantification of phthalates in environmental matrices (air, water, sludge, sediment and soil): A review. *Science of The Total Environment*,515–516, p. 162-180. ISSN: 0048-9697.
Rahman M, Brazel CHS. 2004. The plasticizer market: an assessment of traditional plasticizers and research trend to meet challenges.*Progress in Polymer Science*, 29(12), p. 1223-1248. ISSN: 0079-6700.
Wittassek, M., Koch, H. M., Angerer, J., Brüningm T. 2011. Assessing exposure to phthalates – The human biomonitoring approach.*Molecular Nutrition and Food Research*, 55(1), p 7-31. ISSN: 1613-4133.
Wormuth M, Scheringer M, Vollenweider M, Hungerbühler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal*ysis, 26(3), p. 803-824. ISSN: 1539-6924
Xue MG, Wang SF, Huang CX, Xia NN. 2010. The Analysis of Organic Contaminants in Printing Paper Food Packaging Materials. *Proceedings of the 17th IAPRI World Conference on Packaging*, pp. 360-363.
Yin R., Lin X.G., Wang S.G., Zhang H.Y. 2003.Effect of DBP/DEHP in vegetable planted soil on the quality of capsicum fruit. *Chemosphere*, 50, p. 801-805. ISSN: 0045-6535.
Zhang, K., Noonan, G. O., Begley, T. H. 2008. Determination of 2,6-diisopropylnaphtalene (DIPN) and n-dibutylphthalate (DBP) in food and paper packaging materials from US marketplaces.*Food Additives and Contaminants*, 25, p. 1416-1423. ISSN 1944-0049.

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