ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 63 158 Number 5, 2015
http://dx.doi.org/10.11118/actaun201563051459

DETECTION OF PHTHALATES IN PACKAGES AND MONITORING THEIR MIGRATION INTO PRODUCTS

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Abstract

BOGDANOVIČOVÁ SOŇA, JAROŠOVÁ ALŽBETA. 2015. Detection of Phthalates in Packages and Monitoring Their Migration Into Products. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 63(5): 1459–1464.

The report is focused on determining the level of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in materials used for packaging meat products and monitoring the extent of PAE migration into meat products after thermal treatment. Was analyzed 50 samples of packaging. The DBP concentration ranged from 0.19 to 23.95 μg.dm^{-2} and that of DEHP was between 0.01 and 103.33 μg.dm^{-2}. Analysed packages containing higher concentrations of phthalates (5 samples) were subsequently used for monitoring the phthalate migration into the product due to thermal treatment. The determination of phthalates was conducted using high-performance liquid chromatography (HPLC) with UV detection at a wavelength of 224 nm. The DBP concentration ranged from 0.19 to 23.95 μg.dm^{-2} of the sample surface and that of DEHP was between 0.01 and 103.33 μg.dm^{-2} of sample surface. With regard to the specific migration limit (1.5 mg.kg^{-1} for DEHP and 0.3 mg.kg^{-1} for DBP), four of the analysed samples packages (a total of 5 samples) ceased to meet legal limits after thermal treatment, i.e. 70 °C in the product core for 10 minutes. Our analysis has confirmed that the migration of phthalates is influenced by temperature.

Keywords: package, Gothaer salami, phthalic acid esters, thermal treatment, migration limit, di-2-ethylhexyl phthalate, di-n-butyl phthalate

INTRODUCTION

Phthalic acid esters (phthalates/PAE) belong to the group of plasticisers which cause gelling of polymer materials, improving the flexibility, elasticity, extensibility and processability of plastic materials. Phthalates can release (by leaching) into the material with which the substance is in contact. Toxic and most abundant phthalates include those identified in this study, i.e. di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP). Substances of lipophilic nature, they accumulate in fatty tissues. The body metabolism turns phthalates into toxic metabolites that interact with biologically active substances and can negatively affect vital body functions (Wittassek *et al*., 2011).

Phthalates are used in many industries and are produced in extremely large quantities worldwide (Wang *et al*., 2008). Since phthalate-based plasticisers are not firmly bonded through covalent bonding in the material, they slowly release into the surrounding environment by volatilisation, leaching or migration (Wormuth *et al*., 2006). Foods can be contaminated either primarily during primary production, as a result of contaminated water, soil and air, or secondarily during subsequent processing and all kinds of handling (Corea-Tellez *et al*., 2008). The materials with which food comes into contact form the main source of food contamination (Wagner *et al*., 2009). Foods and raw materials can be contaminated by means of plastic equipment used for the processing or by migration from the packaging material and from the printing ink (Corea-Tellez *et al*., 2008). These have to meet the requirements of the Commission Regulation (EU) No. 10/2011 on materials and articles of plastics intended for food contact, one that defines a migration limit and a specific limit.

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 (Commission Regulation 10/2011). Exceeding the permitted limit should be avoided in materials that are used for packaging foods.

MATERIAL AND METHODS

Meat product packages were analysed at the Department of Food Technology, Mendel University in Brno. A sample of about 1 dm^2 was taken from each package (n = 50) and then subjected to double analysis: samples were analyzed in duplicate (i.e. a total of 100 analyses were done). For the material structure of the packages analysed, refer to Tab. I.

The samples were analysed using methods verified for the determination of DBP (di-n-butyl phthalate) and DEHP (di-2-ethylhexyl phthalate) (Jarošová *et al*., (1999). Once washed with a cleaning agent and distilled water, laboratory glass and equipment were rinsed three times with hexane. The samples were leached for 72 hours in a mixture of solvents, 1:1 *n*-hexane:dichloromethane, and then extracted three times (60, 30 and 30 minutes) using a mixture of solvents, 1:1 *n*-hexane:dichloromethane. The combined extraction fractions were filtered and evaporated using a rotary vacuum evaporator, the temperature being 40 °C; then there was a final drying with nitrogen. The extract was then transferred into vials using hexane (5 ml) and centrifuged for 10 minutes at 4 °C and under the speed of 3,000 rpm by means of Hettich-Zentrifugen D-78532 Tuttlingen-universal 32R. The upper portion of the extract (1.5 ml) was removed and fine-dried with nitrogen. The samples were re-centrifuged; the upper layer of the extract was removed (1.5 ml) and again subjected to final drying with nitrogen. Subsequently, acetonitrile was added into each of the vials to a volume of 1 ml. If an extract was coloured or turbid, it was purified using sulphuric acid.

The batter of meat products (Gothaer salami) was produced and then filled into packages under pilot conditions of the Department of Food Technology, Faculty of Agronomy, Mendel University in Brno. The "Gothaer salami" batter was produced under Czech national norms and contained 30% fat. Once it underwent thermal treatment (i.e. 70 °C in the product core for 10 minutes), the batter produced by means of grinder, bowl chopper, mixer, stuffer and, eventually, smokehouse, was rapidly cooled using scale ice and then stored at 4 °C.

The DBP and DEHP analysis for the sample of the batter (thermally untreated meat product: Gothaer salami) and the meat products (heat-treated meat product: Gothaer salami) was carried out using the method of Jarošová *et al*. (1999). Samples of "Gothaer salami" were homogenised and a representative sample was collected. Then the samples were admeasured by weighing and placed into aluminium bowls, each to contain 20–25 g of the material. Once frozen, the samples were lyophilised for 36 hours under vacuum of 0.080 mBar using the "Pragolab Alpha 1-2 LD plus" unit.

Once washed with a cleaning agent and distilled water, laboratory glass and equipment were rinsed three times with hexane. A mixture of organic solvents, acetone:hexane (1:1) was used for triple extraction (three times repeated extraction) of fat from the meat product examined. The solvent was removed from amalgamated extracts at 40 °C using a vacuum rotary evaporator (D-79219 Staufen, IKA Werke GmbH & Co KG, Germany) and the extracts were stored in a freezer (–18 °C) before further analysis. After dissolving 0.5 g of extract in 2 ml of mobile phase of 1:1 dichloromethane:cyclohexane phase, the samples were separated using gel permeation chromatography. One millilitre of the dissolved sample was applied on a column containing Bio-Beads S-X3. The phthalate elution time was detected by means of standard (DBP: Pestanal (USA) assay (GC) 99.6%; DEHP Pestanal (USA) assay (GC) 99.7%) at a constant flow of 1 $ml.min^{-1}$. The mobile phase was removed from the eluent at 40 °C using a vacuum rotary evaporator; the remainder after evaporating was quantitatively transferred by hexane into a vial and stored in the freezer (–18 °C). Purification of samples using concentrated sulphuric acid was done in three replicates (three times repeated purification). Acetonitrile was added into each of the fine-dried samples to obtain a volume of 1 ml.

Phthalate content in both packages and meat products was determined using the HPLC method with UV detection at a wavelength of 224 nm by means of the column ZorbaxEclipse XDB-C8, 150×4.6 mm, 5 µm (Agilent Technologies, USA). Acetonitrile was used as the mobile phase as part of the HPLC method, the flow being 0.5 $ml.min^{-1}$. The quantity of the sample sprayed on the column was 10 µl. The resulting concentrations were computed based on the calibration curve using the AgilentChemstation software for LC and LC/MS systems. The range of the calibration curve was 1.06 to 106.00 $\mu g.ml^{-1}$ for DBP and 1.01 to 100.50 $\mu g.ml^{-1}$ for DEHP. The retention time was 4 minutes for DBP and 8 minutes for DEHP. The correlation coefficient was 0.9999 for both DBP and DEHP. The detection limit was 0.05 $\mu g.ml^{-1}$ for DBP and 0.11 $\mu g.ml^{-1}$ for DEHP.

RESULTS AND DISCUSSION

The objective of this study was to find out whether the content of the monitored phthalates in the packages of meat products and subsequent migration of phthalates into the meat product was in compliance with the limit provided in the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact.

The resulting concentrations of DBP, DEHP and DBP + DEHP are specified in $\mu g.dm^{-2}$ of sample area and are shown in Tab. I. The DBP concentration values per sample ranged from 0.19 to 39.52 $\mu g.dm^{-2}$ of sample area while DEHP concentrations ranged from 0.01 to 103.33 $\mu g.dm^{-2}$ of sample area. The highest DBP + DEHP concentration observed was 127.28 $\mu g.dm^{-2}$. The analysed packages complied with the limit of the aggregate migration per unit of area referred to in Commission Regulation (EU) No. 10/2011 (max. 10 $mg.dm^{-2}$ of the surface of the material/product).

To monitor the migration of phthalates from packaging to the meat product, packages were selected that based on the previous analyses contained the highest concentrations of phthalates from analyzed packages (n = 50). It involved coloured textile wraps (5 packages) designed for the packaging of boiled meat products. They are highlighted in Tab. I (package 40, 41, 42, 43 and 44). According to the previous analysis (Bogdanovičová *et al*., 2014), phthalates were most probably contained in the printing colours. Our previous studies demonstrated the variability of phthalate content in printed and non-printed portions of the package. Were analysed 17 samples packages with printing and without printing, of which 13 samples were found to have a higher content of DBP + DEHP in printed packages, while only 4 samples were determined to have a slightly increased level of phthalates in the packages without printing. The concentration in the printed portion was higher than in unprinted portion package in most cases, probably due to the addition of PAE in the printing colours (Bogdanovičová *et al*., 2014).

Phthalate migration depends on numerous factors, such as temperature and storage period, type of the package, chemical composition of the packaged food and others. Due to the lipophilic nature of the substances, the phthalate migration is also subject to the fat content in the food. Monitoring phthalate migration from packaging to meat product is additionally monitored on a meat product ("Gothaer salami" type), which has a fat content of 30%. Migration DBP and DEHP is also dependent on the total phthalate content in the analyzed samples. The analysed packages were used for the packaging of a thermally treated meat product produced at the Department of Food Technology, Mendel University in Brno, with 6 salami samples made for each package. An analysis was made of the meat product prior to product packaging and heat treatment (n = 6) and the meat product (5 packages × 6 reps = 30 samples) was analysed after the thermal treatment. Each sampling was carried out in six replicates, with a total of 30 samples being produced and packed (5 packages × 6 reps). The samples were stored at 4 °C. The fat content in the meat product was 30%. The determination of phthalates was carried out using high-performance liquid chromatography (HPLC) with spraying the sample on the column in two replicates. The results for detecting the migration of DBP and DEHP from the packages into the foods totalled 72, i.e. 60 results (5 package × 6 reps. × 2 spraying) from the analysis of meat products and 12 results (6 reps. × 2 spraying) from the analysis of the batter.

With regard to the specific migration limit for DBP (max. 0.3 $mg.kg^{-1}$ of the food simulant) and DEHP (max. 1.5 $mg.kg^{-1}$ of the food simulant), four of the analysed samples would not be meeting the above regulation. These values are provided in $mg.kg^{-1}$ and shown in Tab. II, involving samples 40, 42, 43 and 44 that after thermal treatment meat products, i.e. 70 °C in the product core for 10 minutes don't meet the legislative limits.

The presence of phthalates in packaging materials and the possible migration of PAE from the package into the food have also been demonstrated by studies of other authors Zhang and Guo (2009), Xue *et al*. (2010).

Zhang and Guo (2009) found that the DEHP migration from a PVC film into meat increased with rising temperature and time, with the maximum being reached at 90 °C and 30 minutes of exposure. The aggregate migration limit (60 $mg.kg^{-1}$) was exceeded for all of the time and temperature combinations studied, except that of 10 °C and < 41 hours, where migration was not observed.

Xue *et al.* (2010) analyzed phthalates in printed and unprinted part containers. Xue *et al.* (2010) analysed 13 printed materials that are in contact with foods. For comparative purposes, four blank samples were analysed without being printed. The results show an elevated content of phthalates in printed materials compared with those without printing. For this reason, eight types of colours used for printing of packages were tested to prove that they were the main source of contamination. Printing colours were confirmed to be the main source of the examined substances that could cause a risk to food safety. Phthalates presence was confirmed in a study Bogdanovičová *et al*. (2014). As part of studying phthalic acid esters in packages of meat products, 15 packages were monitored with each sample being analysed in duplicate (30 analyses). The concentrations of the monitored phthalates ranged from 2.30 to 19.74 $\mu g.dm^{-2}$. The PAE content observed was in compliance with the limit provided in the Commission Regulation (EU) No. 10/2011 (Bogdanovičová *et al*., 2014)

I: *Average concentrations of DBP, DEHP and DBP + DEHP (μg.dm^{-2}) in the packages of meat products, including specifications*

Sample #	Specification packaging composition of packaging	DBP	DEHP	DBP + DEHP
			μg.dm^{-2}	
1	BOPET//PE HB Peel	1.32	3.81	5.13
2	BOPP/PE/PA/PE	3.17	1.8	4.97
3	BOPET//PE HB Peel	2.41	1.52	3.93
4	BOPP/PE/PA/PE	3.18	2.17	5.35
5	BOPET//PE HB Peel	0.49	5.36	5.85
6	BOPP/PE/PA/PE	1.75	2.14	3.89
7	BOPET//PE HB Peel	2.13	2.43	4.56
8	BOPET//PE HB Peel	0.72	1.98	2.7
9	BOPET//PE HB Peel	0.7	1.48	2.18
10	BOPET//PE HB Peel	0.75	1.51	2.26
11	BOPET//PE HB Peel	0.64	4.26	4.9
12	BOPET//PE HB Peel	0.83	4.11	4.94
13	BOPET//PE HB Peel	1.81	3.67	5.48
14	BOPP/PE/PA/PE	0.43	5.57	6
15	BOPET//PE HB Peel	1.43	0.28	1.71
16	BOPET//PE HB Peel	1.48	0.08	1.56
17	BOPET//PE HB Peel	2.59	0.01	2.6
18	BOPP/PE/PA/PE	1.89	1.04	2.93
19	BOPP/PE/PA/PE	1.25	1.03	2.28
20	BOPP/PE/PA/PE	0.46	4.85	5.31
21	BOPET//PE HB Peel	0.6	4.45	5.05
22	BOPET//PE HB Peel	0.28	3.47	3.75
23	BOPET//PE HB Peel	3.11	1.59	4.7
24	BOPET//PE HB Peel	2.06	2.17	4.23
25	BOPET//PE HB Peel	0.62	3.79	4.41
26	BOPP/PE/PA/PE	2.88	2.29	5.17
27	PA/EVOH/PA/PE	2.62	2.37	4.99
28	PA/EVOH/PA/PE	0.92	1.84	2.76
29	BOPET//PE HB Peel	0.36	3.13	3.49
30	PA/EVOH/PA/PE	2.55	2.97	5.52
31	BOPET//PE HB Peel	0.22	2.91	3.13
32	BOPET//PE HB Peel	0.35	0.84	1.19
33	BOPET//PE HB Peel	0.19	2.1	2.29
34	BOPP/PE/PA/PE	0.8	3.37	4.17
35	BOPET//PE HB Peel	0.57	2.18	2.75
36	BOPP/PE/PA/PE	0.83	3.8	4.63
37	BOPET//PE HB Peel	1.35	2.59	3.94
38	BOPET//PE HB Peel	0.25	2.53	2.78
39	BOPET//PE HB Peel	0.32	2.72	3.04
40	**Cotton/printing ink**	**4.35**	**19.1**	**23.45**
41	**Cotton/printing ink**	**8.26**	**16.79**	**25.05**
42	**Cotton/printing ink**	**23.95**	**103.33**	**127.28**
43	**Cotton/printing ink**	**15.09**	**26.54**	**41.63**
44	**Cotton/printing ink**	**5.26**	**0.3**	**5.56**
45	BOPP/PE/PA/PE	0.33	2.35	2.68
46	BOPP/PE/PA/PE	0.33	4.2	4.53
47	BOPET//PE HB Peel	0.91	0.34	1.25
48	BOPET//PE HB Peel	0.65	0.14	0.79
49	BOPET//PE HB Peel	0.31	0.25	0.56
50	BOPP/PE/PA/PE	0.62	1.74	2.36

Note: BOPET – biaxially-oriented polyethylene terephthalate, PE – polyethylen, BOPP – bi-axially oriented polypropylene, PA – polyamide, EVOH – Ethylene vinyl alcohol, HB – high barrier films

II: *Concentration of DBP and DEHP (μg.dm^{-2}) and concentration of DBP and DEHP in the batter and in samples of meat products after thermal treatment: 70 °C in the product core for 10 minutes (μg.g^{-1})*

Sample #	Content of PAE in packages		Batter before packaging		Meat product after thermal processing	
	DBP	DEHP	DBP	DEHP	DBP	DEHP
	μg.dm^{-2}		μg.g^{-1}		μg.g^{-1}	
40	4.35	19.1	0	0	**0.4**	0.58
41	8.26	16.79	0	0	0.16	1.46
42	23.95	103.33	0	0	0.06	**1.67**
43	15.09	26.54	0	0	0.27	**2.37**
44	5.26	0.3	0	0	**0.32**	**1.91**

CONCLUSION

The project aimed at monitoring the level of phthalic acid esters in materials used for packaging meat products as well as studying the extent of PAE migration into meat products after thermal treatment. The DBP concentration ranged from 0.19 to 23.95 μg.dm^2 of the sample surface and that of DEHP was between 0.01 and 103.33 μg.dm^2 of sample surface. The analysed packages complied with the limit of the aggregate migration per unit of area stipulated in Commission Regulation (EU) No. 10/2011, i.e. max. 10 mg.dm^{-2} of the surface of the material/product. The limit, however, includes more phthalates and a number of other substances which are able to release from the material, thus migrate into the food. Commission Regulation (EU) No. 10/2011 also defines a specific migration limit that is intended for DEHP and DBP detected in this study and equals to a maximum of 1.5 mg.kg^{-1} for DEHP and a maximum of 0.3 mg.kg^{-1} for DBP. With regard to the SML, four of the analysed samples (a total of 5 samples) ceased to meet legal limits after thermal treatment, i.e. 70 °C in the product core for 10 minutes. Our analysis has confirmed that the migration of phthalates is influenced by temperature (heat treatment 70 °C in the product core for 10 minutes).

Considering the presence of phthalates in the environment and food chain as well as due to the migration of PAE from packages into the food as was proved in this study, it is desirable to accept all of the necessary legal measures to reduce migration of PAE into the environment and foodstuffs.

One of the ways of the progressive reduction of risks of phthalates is promoting the substitution of toxic phthalates by other health-safe substances, e.g. citrates, phenol alkylsulphonate or benzoates, particularly in the production of materials used in agriculture, food and health care industries.

Acknowledgement

The report was produced under the assistance of the Internal Grant Agency of the Faculty of Agronomy, Mendel University in Brno, project IP 32/2015.

REFERENCES

BOGDANOVIČOVÁ, S. JAROŠOVÁ, A., KAMENÍK, J., DAŇKOVÁ, R. 2014. Phthalic acid esters in packaging meat products. In: *Proceedings of XL. Conference on the quality of food and food ingredients – Ingr days.* 4.–5. March 2014. Brno: Mendel University in Brno, 73–80.

BOGDANOVIČOVÁ, S., JAROŠOVÁ, A., KAMENÍK, J. 2014. Monitoring of phthalic acid esters in packaging of meat products [in czech]. *Meat,* 5: 56–58.

EUROPEAN UNION. 2011. *Commission Regulation No. 10/2011 of 14 January 2011 of the European Communities relating to plastic materials and articles intended to come into contact with foodstuffs*, 12: 4–12.

COREA-TELLEZ, K. S., BUSTAMANTE-MONTES, P., GARCIA-FABILA, M., HERNANDEZ-VALERO, M. A., VAZQUEZ-MORENO, F. 2008. Estimated risks of water and saliva contamination by phthalate diffusion from plasticized polyvinyl chloride. [Online]. *Environ. Health,* 71(3): 34–39. NCBI. Available at: http://ncbi.nlm.nih.gov/pubmed/18990931. [Accessed: 2015, May 17].

JAROŠOVÁ, A., GAJDŮŠKOVÁ, V., RAZSYK, 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. *Veterinary medicine,* 44: 61–70.

WAGNER, M., OEHLMANN, J. 2009. Endocrine disruptors in bottled mineral water: total extrogenic burden and migration from plastic bottles. [Online]. *Environ. Sci. Pollut. Res.,* 16(3): 278–286. Springer Link. Available at: http://link.springer.com/article/10.1007%2Fs11356-009-0107-7. [Accessed: 2015, May 17].

WANG, P., WANG, S. L., FAN, C. Q. 2008. Atmospheric distribution of particulate- and gas-phase phthalic esters (PAEs) in a Metropolitan City, Nanjing, East China. [Online]. *Chemosphere,* 72(10): 1567–1572. ScienceDirect. Available at: http://

www.sciencedirect.com/science/article/pii/S0045653508005110. [Accessed: 2015, May 15].

WITTASSEK, M., KOCH, H. M., ANGERER, J., BRÜNING, T. 2011. Assessing exposure to phthalates-the human biomonitoring approach. [Online]. *Mol. Nutr. Food Res.,* 55(1): 7–31. Wiley Online Library. Available at: http://onlinelibrary.wiley.com/doi/10.1002/mnfr.201000121/abstract;jsessionid=E9B96E30595F43038E0888C20CF5EF76.f02t02. [Accessed: 2015, May 10].

WORMUTH, M., SCHERINGER, M., VOLLENWEIDER, M., HUNGERBÜHLER, K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? [Online]. *Risk Anal.,* 26(3): 803–824. Wiley Online Library. Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1539-6924.2006.00770.x/abstract. [Accessed: 2015, May 1].

XUE, M. G., WANG, S. F., HUANG, C. X., XIA, N. N. 2010. The Analysis of Organic Contaminants in Printing Paper Food Packaging Materials. In: *Proceedings of the 17th capri world conference on packaging*. IAPRI, 360–363.

ZHANG, S. GUO, K. 2009. Migration amount of di-2-ethylhexyl phthalate from food-grade PVC film into meat at three temperatures. [Online]. *Trans. Chin. Soc. Agric. Eng.,* 25(1): 291–293. Cab-direct. Available at: http://isplb03-aux3.semantico.net/abstracts/20093127679.html;jsessionid=E9BAA558EBA0894B1D0F16313827B30F. [Accessed: 2015, May 21].

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