Detection of di-n-butyl phthalate and di-2-ethylhexyl phthalate in packages of meat products

SONA BOGDANOVICOVA[1], ALZBETA JAROSOVA[1], JOSEF KAMENIK[2]
[1]Department of Food Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
[2]Department of Meat Hygiene and Technology
University of Veterinary and Pharmaceutical Sciences, Brno
Palackeho 1/3, 612 42 Brno
CZECH REPUBLIC

sona.bogdanovicova@mendelu.cz

Abstract: The study is focused on determining di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in materials used for packaging meat products. 80 packages were analysed with a sample of about 10 cm^2 taken from each of them and then subjected to double analysis (i.e. a total of 160 analyses). The determination of phthalates was conducted using high-performance liquid chromatography (HPLC) with the Zorbax Eclipse C8 column and UV detection at a wavelength of 224 nm. The concentrations ranged from 0.19 to 39.52 $\mu g \cdot dm^{-2}$ for DBP and from 0.01 to 103.33 $\mu g \cdot dm^{-2}$ for DEHP. The objective of this study was to determine whether the content of the monitored phthalates in packages of meat products 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 and whether or not it was posing any serious health risk for consumers.

Key-Words: – PAE, package, phthalic acid esters

Introduction

Phthalic acid esters (PAE/phthalates) are members of the group of organic environmental contaminants. Virtually ubiquitous compounds, the reason for their high production, hence their rampant spread in all the components of the environment, are suitable physico-chemical properties due to which the substances are used as plasticisers, i.e. substances improving the mechanical properties of plastics. The U.S. Environmental Protection Agency (EPA) has classified six phthalic acid esters as priority hazardous pollutants. These include dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DOP) and dibutylbenzyl phthalate (BBP) [1]. Since phthalate-based plasticisers are not firmly bonded by covalent bonding in the material, they slowly release into the surrounding environment by volatilisation, leaching or migration [2]. Large amounts of phthalates are released not only during use, but also when handling plastic waste (storing at landfills, incineration etc.) [3]. Toxic and most abundant phthalates include di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP) that are of lipophilic nature. As a result, they accumulate in fatty tissues. Events of chronic toxicity have demonstrated adverse effects of PAE – teratogenicity, spermiotoxicity, nephrotoxicity, hepatotoxicity and carcinogenicity, as well as detrimental effects on membrane function. Due to the health impact in humans, the penetration of phthalates into the environment should be controlled and the exposure reduced [4].

The materials with which food comes into contact are the main source of food contamination [5]. These have to meet the requirements of the Regulation of the Commission (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 migration limit (ML) refers to the maximum amount of packaging components that are released into a food or into a food simulant during the leaching test per unit of area of the plastic package or per unit of weight of the food or food simulant. Plastics and plastic products shall not release their ingredients into the food in a quantity greater than 60 $mg \cdot kg^{-1}$ of the food or food simulant. The limit of aggregate migration per unit of area was set at 10 $mg \cdot dm^{-2}$ of the surface of the material/product.

The specific migration limit (SML) is the maximum allowed quantity of the substance migrating from the package into the food, onto the food or into the simulant. For food simulants, the SML is 30 mg·kg^{-1} for BBP, 1.5 mg·kg^{-1} for DEHP and 0.3 mg·kg^{-1} for DBP [6].

Material and Methods

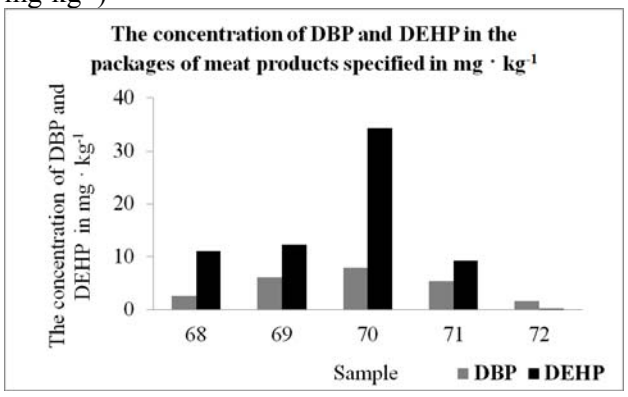
Meat product packages were provided in cooperation with the Veterinary and Pharmaceutical University in Brno and analysed at the Department of Food Technology, Mendel University in Brno. A sample of about 10 cm^2 was taken from each package (n = 80) and then subjected to double analysis (i.e. a total of 160 analyses were done).

The samples were leached in a mixture of solvents *n*-hexane: dichloromethane (1:1) for 72 hours and then extracted three times (60, 30 and 30 minutes). The combined extraction proportions were filtered and evaporated using a rotary vacuum evaporator; then there was a final drying with nitrogen. Subsequently, the extract was transferred into vials using hexane (5 ml) and centrifuged. The upper portion of the extract (1.5 ml) was removed and there was a final drying with nitrogen. The samples were re-centrifuged; the upper layer of the extract was removed (1.5 ml) and subjected to final drying with nitrogen as well. 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. Phthalates were determined by the HPLC method with UV detection at a wavelength of 224 nm, using the column ZorbaxEclipse XDB-C8 150 x 4.6 mm, 5 µm (Agilent Technologies, USA). The quantity of the sample sprayed on the column was 10 µl. The resulting concentrations were computed based on the calibration curve in the AgilentChemstation for LC and LC/MS systems software. The range of the calibration curve was from 1.06 to 106.00 µg·ml^{-1} for DBP and from 1.01 to 100.50 µg·ml^{-1} for DEHP. The correlation coefficient was 0.9999 for both DBP and DEHP. The limit of detection was 0.05 µg·ml^{-1} for DBP and 0.11 µg·ml^{-1} for DEHP. At the final stage, the results were statistically processed using Microsoft Office Excel 2007.

Results and discussion

The resulting concentrations of DBP, DEHP and DBP + DEHP are specified in µg·dm^{-2} of sample area and are shown in Table 1. The DBP concentration values in the samples ranged from 0.19 to 39.52 µg·dm^{-2} of sample area and DEHP concentrations ranged from 0.01 to 103.33 µg·dm^{-2} of sample area. The highest DBP + DEHP concentration observed was 127.28 µg·dm^{-2}. The analysed packages complied with the limit of the aggregate migration per unit of area referred to in the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact (max. 10 µg·dm^{-2} of the surface of the material/product). 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), five of the packages analysed would be not meeting the above regulation. These values are provided in mg·kg^{-1} and shown on Fig. 1 and involve samples 68, 69, 70, 71 and 72 – coloured textile covers that are designed for packaging boiled meat products and contained phthalates in the printing colours. In the previous studies, we demonstrated the variability of phthalate content in printed and non-printed portions of the package. The concentration in the printed portion was in most cases higher, probably due to the addition of PAE in printing colours. In the case of migration of the monitored substances into the packaged products, these packages would not be meeting the specific migration limits set by the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact.

Fig. 1 The concentration of DBP and DEHP in samples exceeding the specific migration limit (in mg·kg^{-1})



Legend: DBP = di-n-butyl phtalate, DEHP = di-2-ethylhexyl phtalate.

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 [7]. Our analysis found elevated concentrations of phthalates in five packages of meat products. Phthalic acid esters are lipophilic organic compounds, with the migration of these from the package to the packed food is influenced by a number of factors, including the type of the polymeric

Table 1 The average concentration of DBP, DEHP and DBP + DEHP in the packages of meat products specified in μg·dm^{-2}

Sample	μg·dm^{-2}			Sample	μg·dm^{-2}		
	DBP	DEHP	DBP + DEHP		DBP	DEHP	DBP + DEHP
1	1.32	5.81	7.13	41	39.52	8.88	48.40
2	3.17	5.80	8.97	42	0.28	11.47	11.76
3	4.27	25.14	29.41	43	22.73	6.24	28.97
4	2.41	1.52	3.93	44	1.54	9.75	11.29
5	7.38	12.36	19.74	45	1.17	14.16	15.34
6	6.31	12.06	18.37	46	3.11	3.59	6.70
7	3.49	14.74	18.24	47	1.12	14.06	15.18
8	0.88	6.78	7.66	48	3.66	2.17	5.83
9	0.99	12.47	13.46	49	0.62	3.79	4.41
10	0.58	10.43	11.01	50	6.88	2.29	9.16
11	0.99	5.95	6.93	51	20.62	6.37	26.99
12	2.69	3.38	6.07	52	0.92	1.84	2.77
13	3.18	2.17	5.35	53	0.36	3.13	3.49
14	0.49	5.36	5.85	54	2.55	2.97	5.52
15	12.59	8.74	21.33	55	0.71	10.52	11.22
16	17.75	2.14	19.89	56	0.22	2.91	3.14
17	25.13	5.43	30.56	57	2.37	6.96	9.33
18	0.72	1.98	2.70	58	0.35	0.84	1.19
19	0.70	1.48	2.18	59	0.19	2.10	2.30
20	0.75	1.51	2.25	60	11.47	4.11	15.58
21	2.67	12.44	15.11	61	0.80	3.37	4.18
22	3.22	10.31	13.52	62	1.81	9.47	11.28
23	0.64	4.26	4.89	63	0.57	2.18	2.75
24	0.83	5.11	5.94	64	0.83	3.80	4.63
25	1.81	3.67	5.48	65	1.35	2.59	3.95
26	0.43	5.57	6.00	66	0.25	2.53	2.78
27	1.83	21.35	23.18	67	0.32	2.72	3.04
28	1.08	13.62	14.70	**68**	**4.35**	**19.10**	**23.45**
29	1.44	16.18	17.62	**69**	**8.26**	**16.79**	**25.05**
30	1.13	22.68	23.81	**70**	**23.95**	**103.33**	**127.28**
31	1.43	0.28	1.70	**71**	**15.09**	**26.54**	**41.63**
32	1.48	0.08	1.56	**72**	**5.26**	**0.30**	**5.56**
33	2.59	0.01	2.60	73	0.33	2.35	2.68
34	1.89	1.04	2.93	74	0.33	4.20	4.53
35	1.25	1.03	2.28	75	15.76	2.07	17.82
36	1.95	11.59	13.54	76	19.69	0.16	19.85
37	0.46	4.85	5.31	77	0.91	0.34	1.26
38	0.60	5.45	6.05	78	0.65	0.14	0.79
39	2.51	24.45	26.96	79	0.31	0.25	0.56
40	2.85	13.71	16.56	80	0.62	17.74	18.36

material, the type of food, temperature, contact duration, etc. Phthalate leaching is enhanced as the fat content in the food increases. Possible migration of DBP and DEHP from the analysed packages is further monitored for a product of the "Gothaer salami" type, depending on the packages used and storage period.

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.

Shuangling and Kangquan [8] found that the migration of DEHP from a PVC film into meat increased with increasing temperature and time, with the maximum being reached at 90˚C and 30 minutes of exposure. The aggregate migration limit (60 $mg \cdot kg^{-1}$) 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. [9] analysed 13 printed materials that are in contact with foods. For comparative purposes, four blank samples were analysed that were not 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 demonstrate that they were the main source of contamination. It was confirmed that printing colours are the main source of the examined substances that could cause a risk to food safety.

Guo et al. [10] studied the migration of DEHP from a packaging film into ham sausages that were relatively low in fat. The content of DEHP in the products was considerably decreasing with increasing distance from the surface. The concentration of DEHP in the packaging film was 8.7 $mg \cdot g^{-1}$, while that in the first outer layer of the product was reaching 206.5 $ng \cdot g^{-1}$. Layer 1 and layer 2 contained about 90% of the total amount of DEHP that had migrated from the packaging material. A significant level of DEHP in the inner layers of sausages was seen only after six months of storage.

Human exposure to phthalates can occur orally (from food/water), by inhalation (from air), by dermal absorption or parenteral application [11]. Due to their lipophilic nature, phthalates may also accumulate in animal tissues, muscle and fat by being transferred from feedstuffs and the environment, which leads to further potential threat of the chain, thus humans [12].

Conclusion

Currently, there is a lack of information about contamination of food by phthalates and their content in food packaging materials. Due to the presence of phthalates in the environment and in the food chain and their negative impact on human health, it is desirable to accept all of the necessary legislative measures to reduce migration of PAE into the environment and foodstuffs.

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

The found concentrations of PAE in the packages of meat products are in compliance with the limit of the aggregate migration per unit of area, thus do not exceed 10 $mg \cdot dm^{-2}$ of the surface of the material or product. The limit, however, includes other phthalates and many other substances which are able to release from the material and migrate into the food. The highest DBP + DEHP concentration found in this study was 127.28 $\mu g \cdot dm^{-2}$. When converting the contents of DBP and DEHP per $mg \cdot kg^{-1}$, five packages would be exceeding the specific migration limit in the event of PAE migration into the packed food, one set by the Regulation of the Commission (EU) No. 10/2011 on materials and articles of plastics intended for food contact. This analysis is the subject of another study.

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