THE MIGRATION OF PHTHALATES FROM PACKAGING INTO FOOD DEPENDING ON THE HEAT PROCESSING AND FAT CONTENT OF MEAT PRODUCTS

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Abstract: Phthalates (PAE) are organic lipophilic compounds mostly used as plasticizers to increase the flexibility of plastic polymers. Other applications include printing ink and varnishes. Humans are mostly exposed to phthalates via food; such exposure can have adverse effects on health. The goal of this study was to investigate the migration of phthalate compounds: di-n-butyl pthalate (DBP) and di-2-ethylhexil pthalate (DEHP) in model meat products of the Bologna type sausage category depending on the packaging used and the percentage fat content due to heat processing.

Key Words: phthalates, heat processing, fat, meat product, packaging

INTRODUCTION

Phthalates are synthetic substances used mainly as plasticizers of polyvinyl chloride (PVC). As additives, they provide plastics with softness and flexibility. Their wide spectrum of use results in the contamination of the environment since pthalates are not firmly bound by a covalent bond in the plastic, and can leach out, migrate or evaporate into the surrounding air, atmosphere, food or other materials. Phthalates enter the human body via ingestion, inhalation or dermal transfer throughout life, and even during intrauterine development. Due to the potential risks posed to human health and the environment, some phthalates have been added to the list of priority pollutants of the European Union. Although phthalates are not persistent substances, due to the predominance of ingestion when compared to metabolic conversion, the parent compounds and metabolites cumulate in the bodies of both animals and humans. These substances do not remain in the body for long. However, throughout their stay, they are responsible for serious health issues (Heudorf et al. 2007).

Current legislation limits the use of phthalates in food packaging, but the legislative limits for the content of these compounds in food have not been set. The suitability of packaging for food is defined by the migration limit (ML) which determines the maximum acceptable amount of constituents of the packaging which can be released from the packaging per unit of area. According to Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food, products intended for contact with food must not release into the food their own constituents in an amount larger than 10 mg · dm^{-2} or 60 mg · kg^{-1} of food or food simulant. The regulation also defines the specific migration limit (SML), which is the highest permissible amount of substance migrating from the packaging to the food. SML equals 1.5 mg · kg^{-1} for DEHP and 0.3 mg · kg^{-1} for DBP (Commission Regulation (EU) No. 10/2011).

The contamination of food occurs through polluted environment, contaminated input raw materials or by the migration of phthalates during the production process, storage or preparation and serving. The highest concentration of phthalates can be found in food with higher fat content, such as milk, milk products, fish, meat or vegetable oils. The migration amounts of different phthalate plasticizers vary. An important factor influencing the migration behaviour of phthalates is temperature.

The study of the migration behaviour of these substances is very important, as it provides the information on what phthalate plasticizers are more suitable for use in food packaging and other plastic materials (Wang et al. 2013).

The prevention required to eliminate the risk of contamination of food by phthalates must also be maintained during processing, packaging, storage and the final preparation. Risk also arises in handling food in rubber gloves or using incorrect plastic dishes containing phthalates.

Although the use of phthalates in food packaging is significantly limited by regulations, monitoring the concentration of phthalates in the environment, raw materials, feed, food and drink, food and drink packaging and printing colours must be performed constantly and regularly.

MATERIAL AND METHODS

The packaging of meat products was obtained in cooperation with a German company producing food packaging. The packaging was then analysed on the Department of Food Technology of the Mendel University in Brno. From each package (n = 60) a sample the size of 1 dm^2 was taken and subsequently analysed in duplicate (120 analyses). The samples were leached in a mixture of *n*-hexane:dichlormethane (1:1) solvents for 72 hours and subsequently extracted three times (60, 30, 30 minutes). The combined extraction portions were filtered, evaporated on a rotary vacuum evaporator and dried with nitrogen. The extract was then transferred into vials using hexane (5 ml) and was centrifuged. The upper layer of the extract (1.5 ml) was removed and dried with nitrogen. The samples were centrifuged again, the upper layer of the extract (1.5 ml) was removed and also dried with nitrogen. The vials were then refilled up to 1 ml by acetonitrile. If the extracts were coloured or turbid, they were purified with sulphuric acid.

The packaging analysed was used for packaging heat treated model meat product: Bologna type sausage produced on the Department of Food Technology of the Mendel University in Brno. The model product was manufactured with fat content of 10%, 30% and 50%. For each packaging, 6 samples of Bologna type sausages were produced of a given fat content (six 10% samples, six 30% samples and six 50% samples). An analysis of the model product was performed (n = 18, i.e., $n_{10\%\ fat} = 6$ samples, $n_{30\%\ fat} = 6$ samples, $n_{50\%\ fat} = 6$ samples) before packaging and another analysis was performed on the meat product after heat processing. Each sampling was performed in six repetitions. A total of 90 samples was produced and packaged (30 samples with 10% fat content, 30 samples with 30% fat content and 30 samples with 50% fat content). The samples were stored at a temperature of 4°C.

Analysis of the DEBP and DEHP of the model product sample and the meat products was performed according to the method used by Jarošová et al. (1999). The samples were frozen and subsequently lyophilised. Esters of the phthalic acid were extracted from the sample three times (60, 30, 30 minutes) using an *n*-hexane:acetone (1:1) organic solvent. The combined extraction portions were filtered, evaporated on a rotary vacuum evaporator and dried with nitrogen. The samples were then separated using gel permeation chromatography. The prepared samples were then purified with concentrated sulphuric acid, centrifuged and a layer of the extract was removed and dried with nitrogen. The repurification with sulphuric acid was performed in three repetitions. The dried samples were refilled with acetonitrile up to a volume of 1 ml.

Phthalates were determined by the HPLC method with UV detection at a wavelength of 224 nm using a ZorbaxEclipse -XDB C8 column, 150x4.6 mm, 5 µm (Agilent Technologies, USA). The injection of the samples in the column used an amount of 10 µl. The resulting concentrations were calculated based on a calibration curve in AgilentChemstation for LC and LC/MS systems. The range of the calibration curve for DBP was from 1.06 $\mu g \cdot ml^{-1}$ to 106.00 $\mu g \cdot ml^{-1}$ and for DEHP from 1.01 $\mu g \cdot ml^{-1}$ to 100.50 $\mu g \cdot ml^{-1}$. The correlation coefficient was 0.9999 for DBP and also 0.9999 for DEHP. The detection limit was 0.05 $\mu g \cdot ml^{-1}$ for DBP and 0.11 $\mu g \cdot ml^{-1}$ for DEHP. In the final stage, the results were statistically processed in Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The concentration of DBP in the analysed packaging ranged from undetectable values to 89.25 $\mu g \cdot dm^{-2}$ and the concentration of DEHP ranged from undetectable values to 188 $\mu g \cdot dm^{-2}$. The highest concentration of DBP+DEHP in the analysed packaging was 205.5 $\mu g \cdot dm^{-2}$. The concentrations detected are in accordance with the limit set by Regulation No. 10/2011 (10 $mg \cdot dm^{-2}$). However, this limit also includes other phthalates and substances which can migrate into food. The migration of phthalates is influenced by a number of factors, especially by the polymer material type, temperature during storage, the presence of proteins and fats in the food, the length of the storage period and other such factors.

For the monitoring of the migration of phthalates from packaging into the meat products due to the influence of heat processing, 5 packagings were selected. Their DBP and DEHP content is provided in Table 1.

Table 1 DBP and DEHP concentration ($\mu g \cdot dm^{-2}$) in the selected packaging for meat products

Sample number	PAE content in packaging	
	DBP	DEHP
	$\mu g \cdot dm^{-2}$	
1	21.55	95.45
2	14.12	64.75
3	18.35	88.12
4	39.13	134.97
5	27.43	108.61

Table 2 The concentration of DBP and DEHP in the model product and in the samples of meat products after heat processing ($\mu g \cdot g^{-1}$)

Sample number	Model product (before packaging)					
	10% fat		30% fat		50% fat	
	DBP	DEHP	DBP	DEHP	DBP	DEHP
	$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$	
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
Sample number	Meat product after heat processing					
	10% fat		30% fat		50% fat	
	DBP	DEHP	DBP	DEHP	DBP	DEHP
	$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$	
1	0.21	2.19	2.07	3.26	3.64	4.96
2	ND	ND	0.60	1.23	2.78	7.25
3	ND	ND	ND	0.69	ND	1.26
4	0.72	0.77	0.90	1.85	3.46	3.87
5	0.69	1.44	1.81	2.18	3.88	7.12

Note: ND: not detected

The aim of the study was to prove the migration of phthalates depending on the fat content in the meat product. The concentration of di-n-butyl and di-2-ethylhexyl phthalate in the model product and in the samples of meat product after heat processing ($\mu g \cdot g^{-1}$) is provided in Table 2.

The concentrations of DBP and DEHP listed in Table 2 are an average of 6 repetitions. The DBP concentration in the 10% fat meat product ranged from undetectable values to 0.72 $\mu g \cdot g^{-1}$ and the DEHP concentration in the 10% fat meat product ranged from undetectable values to 2.19 $\mu g \cdot g^{-1}$. The migration of phthalates increased in the case of a 30% fat meat product, where the DBP concentration ranged from undetectable values to 2.07 $\mu g \cdot g^{-1}$ and the concentration of DEHP ranged from 0.69 $\mu g \cdot g^{-1}$ to 3.26 $\mu g \cdot g^{-1}$. The highest migration of phthalates was detected in the 50% fat meat product, where the DBP concentration ranged from undetectable values to 3.88 $\mu g \cdot g^{-1}$ and the concentration of DEHP ranged from 1.26 $\mu g \cdot g^{-1}$ to 7.19 $\mu g \cdot g^{-1}$.

Based on these results, we have been able to confirm that phthalates migrate into food after heat processing and that their migration is also dependent on the fat content of the meat product. The releasing of phthalates from packaging grows with increasing content of fat in the meat product.

The migration of phthalates is also dependent on the length of the storage period as was demonstrated in a study by Jarošová, Bogdanovičová (2015), where 5 samples of textile packaging were analysed which were designed for cooked meat production. A product was packed into the packaging and analysis was subsequently performed (after the 1st, 7th, 14th, 21st and 28th day of storage) of the finished meat products stored at 4°C during the shelf-life. The packaging was first analysed for DBP and DEHP content, where the concentration was found to be in accordance with legislation (did not exceed the limit of 10 $mg \cdot dm^{-2}$). With regard to the specific migration limit, which sets the limits specifically for the phthalates analysed, all samples already exceeded the specific migration limits after the seventh day of storage (except for sample 2's DBP content). The monitoring of the migration of each phthalate in the individual samples during the storage period (28 days) revealed an increasing tendency.

Studies of other authors also demonstrated the migration of phthalates from packaging into food. Guo et al. (2010) proved a decreasing tendency in DEHP content with increasing distance from the surface. The authors monitored the migration of DEHP from the packaging film into ham sausages with relatively low fat content. The DEHP content in the sausages dropped significantly as the distance from the surface increased. The DEHP concentration was 8.7 $mg \cdot g^{-1}$ in the packaging film and 206.5 $ng \cdot g^{-1}$ in the first outer layer of the sausage. The first and second layer contained approximately 90% of the total DEHP amount which migrated from the packaging. Significant levels of DEHP in the inner layers of the sausages were detected only after six months of storage.

A study by Wang et al. (2015) investigated the presence of phthalates in greenhouse soils and vegetables. Wang et al. monitored dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DnOP) content which was analysed in 44 vegetables grown in greenhouses made of plastic film and in the corresponding soil. The total phthalate content ranged from 0.51 to 7.16 $mg \cdot kg^{-1}$ in vegetables and from 0.4 to 6.20 $mg \cdot kg^{-1}$ in soils with an average concentration of 2.56 and 2.23 $mg \cdot kg^{-1}$. DnBP, DEHP and DnOP contributed to the overall phthalate content in vegetables and soils in more than 90%, but the ratios of DnOP and DnBP in vegetables were significantly ($p<0.05$) higher than in soils. The average concentration of phthalates in mustard, celery and lettuce was > 3.00 $mg \cdot kg^{-1}$ but < 2.50 $mg \cdot kg^{-1}$ in the corresponding soil. Stems and leaves of the vegetables accumulated larger amounts of phthalates. No mutual relationship was detected between the phthalate content in vegetables and in the soils.

In a study by Moreira et al. (2015), the content of 8 plasticisers in spices and in roast chicken meat stored in plastic bags was monitored. The values detected ranged between 0.01 and 0.18 $g \cdot kg^{-1}$. The samples showed presence of diisobutyl phthalate and dibutyl phthalate. The highest concentration of plasticisers was detected in spice used for roasting chicken meat.

A study by Wang et al. (2013) discussed the migration behaviour of 9 phthalate plasticizers in food with higher fat content, and the influence of temperature on the migration amount of these substances. The studied substances were: dimethyl phthalate (DMP), diethyl phthalate (DEP), diallyl

phthalate (DAP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), diisononyl ortho-phthalate (DINP) and diisodecyl ortho-phthalate (DIDP). The results have shown that the thickness of the plastic film is an essential factor in the process of phthalate migration. Another important condition in the study of the migration behaviour was temperature. Measurements have proven that higher temperature accelerates the transfer and the migration of phthalate plasticisers increases. Each of the studied substances was affected differently by the increasing temperature. For instance, DINP and DIDP were affected minimally, since equilibrium was established and increasing the temperature did not change the migration amount. The migration mount measured in the temperature range of 5°C to 70°C ranged between 80 and 350 mg · kg^{-1} for DMP, 75 to 375 mg · kg^{-1} for DEP, 75 to 350 mg · kg^{-1} for DAP, 50 to 350 mg · kg^{-1} for DIBP, 75 to 325 for DBP mg · kg^{-1}, 100 to 275 mg · kg^{-1} for BBP and 110 to 170 mg · kg^{-1} for DEHP. The migration amount for DINP and DIDP reached equilibrium. This equilibrium migration amount for DINP was 140 mg · kg^{-1} and for DIDP 160 mg · kg^{-1}. The migration values of phthalate plasticisers differ.

The toxic effects of phthalates have been observed by a number of authors (for example Wang et al. 2013, Guo et al. 2010). Due to their potential toxic effects, phthalates are being replaced by alternative substances in plastic products (Barros et al. 2011).

CONCLUSION

The aim of the study was to monitor the content of phthalic acid esters in packaging used for meat products, and to observe the level of PAE migration into meat products after heat processing depending on the fat content of the meat products.

On the basis of specific migration limits, monitoring of the PAE migration from packaging into food was performed with the intent to determine whether phthalate migration does not exceed the specific migration limit. With regard to the specific migration limit for a 10% fat meat product, 2 of the samples analysed (packaging no. 4, 5) for DBP and 1 sample analysed for DEHP (packaging no. 1) already exceeded the legislative limits after heat processing (70°C for 10 minutes in the core). In a 30% fat meat product, 4 of the samples analysed (packaging no. 1, 2, 4, 5) for DBP and 3 samples analysed for DEHP (packaging no. 1, 4, 5) did not meet the legislative limits, and in the 50% fat meat product, 4 of the samples analysed did not meet the specific migration limit (packaging no. 1, 2, 4, 5) for DBP as did 4 samples analysed for DEHP (packaging no. 1, 2, 4, 5). The analysis performed has confirmed that the migration of phthalates is influenced by heat processing and grows depending on the fat content in the food.

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