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PHTHALATES IN MEAT PRODUCTS IN DEPENDENCE ON THE FAT CONTENT

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ABSTRACT

The content of dibutylphthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in samples of packages intended for thermally processed meat products and release of phthalates from packages into meat products in dependence on the fat content were observed. 80 samples of packages were analyzed, 5 of them wereselected due to exceeding the specific migration limit. The raw meat was prepared, one type with the fat content of 10% and second one with the fat content of 50%. The both types of raw meat were analyzed for the content of DBP and DEHP and packed into chosen packages.The samples of meat products were thermally processed (70 ℃, 10 min in the core), stored until the expiration date at 4 °C and gradually analyzed after 1st, 7th, 14th, 21st and 28th day of storage. Determination of phthalates was carried out by high performance liquid chromatography (HPLC) in the Zorbax Eclipse C8column and by UV detection at a wavelength of 224 mm. The phthalate content in the raw meat was under the limit of detection. According to the EU Commission Regulation no. 10/2011 the specific migration limit of products intended for the contact with food for DEHP (max. 1.5 $mg.kg^{-1}$of food stimulant and DBP max. 0.3 $mg.kg^{-1}$ of food stimulant), wasexceeded already after first day of storage, in case of DBP in two samples with 10% of fat and after 7^{th} day of storage in one sample. In the samples with 50% of fat, SML was exceeded after first day of storage in four samples and in one sample after 14^{th} day of storage. Regarding DEHP in the samples with 10% of fat SML was exceeded after 1^{st} day of storage in one sample and after 7^{th} day of storage also in one sample and after 21^{st} day of storage similarly in one sample. Four samples with 50% of fat had SML exceeded in case of DEHP already after 1^{st} day of storage. By comparison of PAE migration depending on the fat content we concluded that leaching of PAE from a package into food was 2 – 21 times higher in samples with 50% of fat than in samples with 10% of fat.

Keywords: phthalates; DBP; DEHP; package; migration; fat content

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)**.

Regarding the fact that phthalates (PAE) are not tightly bound in polymer matrices, they can easily migrate from products to the hydrosphere, atmosphere and biosphere during production, usage and liquidation. Regarding the wide use and resistance to microorganism they became omnipresent in the environment and they were found almost in all elements of the environment in the whole world such as soil, precipitation, surface water, sea water, underground water, sediment, biota, air, waste water and sewage sludge **(Schiedek et al., 1995, Zhang et al., 2014, Selvaraj et al., 2015, Net et al., 2015, Wang et al., 2014,Wang et al., 2002)**.

Humans are exposed to phthalates through several ways of exposition including breathing, nutrition and dermal absorption **(Das et al., 2014, Meng et al., 2014)**. The main source of exposition for a human is food, therefore various types of food were researched in various countries of the world from the perspective of phthalate contamination **(Das et al., 2014)**. Phthalates may enter into the food chain also through migration from contact materials during growing, production, storage or even during food preparation in a household.

Evaluation of the dietary intake of phthalates in the Belgian adult population revealed that there is the highest intake of DEHP, followed by di-n-butyl phthalate (DNBP), butyl benzyl phthalate (BBP) and diethyl phthalate (DEP). The groups of food, which contribute to the dietary

exposition to the stated group of phthalates include grain and grain products, milk and dairy products, meat and meat products. According to the executed research the intake of DEHP through food was found ($mg.kg^{-1}.d^{-1}$) in Great Britain, Denmark, Germany, France in the values of 3.40 - 4.00; 2.70 - 4.30; 14.0 and 1.46 respectively **(Yang et al., 2015, Fierens et al., 2014a, Ji et al., 2014, Fierens et al., 2014b)**.

Another studies for example dealt with the phthalate content in caps of beer bottles, in olive oil, in infant feeding bottles, yogurt packages and packages intended for microwave ovens. It was also found that with an increased temperature during preparation there is increasing danger of migration of phthalates from packages into food **(Gonzales-Castro et al., 2011, Li et al., 2012, Nanni et al., 2011)**.

The objective of the work was to carry out the analysis of packages used for production of thermally processed meat products. Another objective was to create products with various fat content, fill them into packages and observe if there occurs release of phthalates into a meat product after thermal processing.

MATERIAL AND METHODOLOGY

Packages (n = 80) used for meat products were supplied by a German company. They included plastic and cellulose packages with printing intended for production of thermally processed meat products. 5 packages were used for filling of the products, in which the specific migration limit was exceeded. The samples were analyzed twice. In total, 80 packages were analyzed and 160 analyses were carried out.

Meat products intended for thermal processing were produced in pilot production conditions at the Department of Food Technology at Mendel University in Brno. Two types of raw meat were prepared (the fat content 10% and 50%), which were filled into the packages (n = 5). For every fat content there were 30 samples produced. The samples were stored at 4 °C and they were taken for analysis after 1st, 7th, 14th, 21st and 28th day of storage. The samples were analyzed twice.

In total, there were 12 samples of raw meat analyzed, 300 samples of meat product and there were 624 analyses carried out.

The package samples were leached in the mixture of dissolvent *n*-hexan:dichlormethan (1:1) for 72 hours and subsequently three times extracted (60, 30, 30 minutes). The combined extracts were filtered, evaporated on a rotary vacuum evaporator and dried by nitrogen. Afterwards, the extract was transferred into vials and centrifuged by hexane (5 mL). The top part of the extract (1.5 mL) was isolated into a vial for determination by HPLC (high-performance liquid chromatography) and dried by nitrogen. Vials were again centrifuged, the top part of the extract was isolated (1.5 mL), dried by nitrogen and subsequently the vials were filled up to the volume of 1 mL. If the extracts were colored or bleary, they were cleared by sulfuric acid.

The verified methods for determination of DBP and DEHP in food were used for the analysis of PAE in the samples of raw meat and meat products **(Jarošová et al., 1998, 1999).**

The samples of raw meat and meat products were homogenized, weighed on metal bowls and frozen. Gradually, the frozen samples were lyophilized and subsequently residues of PAE were extracted by n-hexane. PAE was separated from the co-extracts by gel permeation chromatography on the gel Bio beads S-X3. The cleaning procedure with concentrated sulfuric acid was used for completion of cleaning of eluates. Phthalates were determined by the HPLC method with column of Zorbax Eclipse C8 and UV detection at a wavelength of 224 mm. The injection of samples on the column was 10 µl. The final concentrations were calculated based on the calibration curve by the software Agilent Chemstationfor LC and LC/MS systems. The scope of the calibration curves for DBP was from 1.06 $\mu g.mL^{-1}$to106.00 $\mu g.mL^{-1}$ and for DEHP from 1.01 $\mu g.mL^{-1}$ to 100.50 $\mu g.mL^{-1}$. The correlation coefficient for DBP was 0.9999 and for DEHP also 0.9999. The detection limit for DBP was 0.05 $\mu g.mL^{-1}$ and for DEHP 0.11 $\mu g.mL^{-1}$. The results were statistically processed by the program Statistica 12.

The majority of lab glass was flushed by hexane during preparation of samples. At the same time the dry matter and fat content were determined. Concentrations of DEHP and DBP are related to an initial sample.

RESULTS AND DISCUSSION

The concentration of phthalates in the analyzed packages are expressed in$\mu g.dm^2$ and they are stated in Table 1. Every value represents an average from two parallel determinations.

According to the EU Commission Regulation No. 10/2011 for products intended for the contact with food and dishes, a package cannot release its own components into food in the quantity exceeding10 $mg.dm^2$ or 60 $mg.kg^{-1}$ of food or food stimulant. The stated regulation includes also the specific migration limit which is max. 1.5 $mg.kg^{-1}$ of food stimulant for DEHP and max. 0.3 $mg.kg^{-1}$ of food stimulant for DBP.

Tab. 1 Average concentration of DBP and DEHP ($\mu g.dm^{-2}$) in samples of packages used of packing of meat products

Sample	DBP	DEHP
	$\mu g.dm^{-2}$	
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

From all the analyzed packages (n = 80) there were five packages selected, in which the highest concentration (Table 2) of the both phthalates occurred and there was the assumption that they may contaminate final products after thermal processing and storage. The values of DBP in packages moved from 14.12 to 39.13 $\mu g.dm^{-2}$ and in case of DEHP from 64.75 to 134.97 $\mu g.dm^{-2}$.

The phthalate concentrations (DBP and DEHP) in the raw meat and meat products are expressed in $mg.kg^{-1}$ of the initial sample and they are stated in Table 2. Every value represents an average of 12 values (6 parallel samples and every sample analyzed twice).

In the samples separated immediately after mixingthe raw meat, the concentration of phthalates moved under the limit of detection (≤ 0.2 $mg.kg^{-1}$).

In the first sample of product with 10% of fat, DBP gradually increased from the value 0.21 (after first day of storage) up to 0.68 $mg.kg^{-1}$of the initial sample. In the sample with 50% of fat it reached the values from 3.64 up to 4.7 $mg.kg^{-1}$ of the initial sample. Based on the measured values we can state that at the end of expiration period (28th day) the DBP content was approximately 7x higher in products with 50% of fat than in products with 10% of fat. DEHP reached in the sample with 10% of fat values from 2.19 to 3.57 $mg.kg^{-1}$ of the initial sample and in the sample with 50% of fat from 4.96 to 6.48 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period, there was 2x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the second sample of product with 10% of fat there was DBP detected only at the end of the expiration period (0.22 $mg.kg^{-1}$ of the initial sample), but it did not exceed SML (0.3 $mg.kg^{-1}$). In the sample with 50% of fat, DBP reached values from 1.18 to 3.61 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period there was 16x higher content of DBP in the product with 50% of fat than in the product with 10% of fat. DEHP in samples with 10% of fat was, similarly as in case of DBP, detected only at the end of the expiration period (0.40 $mg.kg^{-1}$ of the initial sample) but it did not exceed SML (1.5 $mg.kg^{-1}$). In the sample with 50% of fat its content ranged from 4.81 to 8.34 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period, there was 21x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the third sample of product, DBP was not detected in any sample of both fat contents (10% and 50%) during the whole time of expiration period. DEHP was detected in the sample with 10% of fat only at the end of the expiration period (0.20 $mg.kg^{-1}$ of the initial sample) but it did not exceed SML (1.5 $mg.kg^{-1}$). In the sample with 50% of fat its content ranged from 1.26 to 2.5 $mg.kg^{-1}$ of the initial sample, but it exceeded SML only after 7th day of storage. At the end of expiration period, there was 13x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the fourth sample of product with 10% of fat DBP gradually increased from the value 0.72 (after first day of storage) up to 1.30 $mg.kg^{-1}$ of the initial sample. In the sample with 50% of fat it reached values from 0.85 up to 1.63 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period (28th day), the content of DBP in both products (10% and 50%) did not differ too much. DEHP in the sample with 10% of fat reached values from 0.77 to 2.01 $mg.kg^{-1}$ of the initial sample and in the sample with 50% of fat from 3.87 to 11.67 $mg.kg^{-1}$ of the initial sample. At the end of the expiration period (28th day) there was almost 6x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat.

In the fifth sample of product with 10% of fat, DBP gradually increased from the value of 0.68 (after first day of storage) up to 1.19 $mg.kg^{-1}$of the initial sample. In the sample with 50% of fat it reached values from 3.60 up to 7.95 $mg.kg^{-1}$of the initial sample. Based on the measured values we can state that at the end of expiration period (28th day) there was 7x higher content of DBP in the product with 50% of fat than in the product with 10% of fat. DEHP in the sample with 10% of fat reached values from 1.44 to 2.89 $mg.kg^{-1}$of the initial sample and in the sample with 50% of fat from 7.12 to 8.54 $mg.kg^{-1}$of the initial sample. At the end of expiration period there was 3x higher content of DEHP in the product with 50% of fat than in the product with 10% of fat. There was confirmed highly statistically significant evidence of migration of DBP and DEHP depending on the fat content (p=0,000**) and depending on the period of storage (p=0,000**).

According to the EU Commission Regulation No. 10/2011 there was exceeded the specific migration limit already after 1st day of storage in case of DBP in two samples with 10% of fat (4 and 5) and after 7th day of storage in one sample (1). In samples with 50% of fat there was SML exceed already after first day of storage in four samples (1, 2, 4, 5) and in one sample (3) after 14th day of storage.

In case of DEHP in the sample with 10% of fat there was SML exceeded after 1st day of storage in one sample (1), after 7th day of storage also in one sample (5) and after 21st day of storage in one sample (4). The samples (1, 2, 4, 5) with 50% of fat in case of DEHP exceeded SML already after 1st day of storage.

Based on the results (Table 1) we can state that DBP content in packages contributed by 20 % and in case of DEHP by 80% to the overall content of PAE. This finding corresponds with results of cumulation in meat products, higher concentration in meat products were found in case of DEHP (Table 2). By the comparison of PAE depending on the fat content we concluded that leaching of PAE was 2 – 21 times higher in samples with 50% of fat than in samples with 10% of fat.

Our experiment pointed at the fact that the content of plasticizers leached from packages increases with temperature, period of storage and fat content and this finding corresponds also with results of another authors. There was found increasing average concentration of DEHP in pasteurized skimmed milk (20 $\mu g.kg^{-1}$), in comparison to full fat milk (35 $\mu g.kg^{-1}$) and cream (1400 $\mu g.kg^{-1}$) **(Castle et al., 1990)**. Values of DEHP correlating with the fat content in milk were also confirmed in the study of Sharman et al. (1994). In milk with up to 1% of fat there was detected DEHP in the range of 0.02 – 0.04 $mg.kg^{-1}$, 0.05 $mg.kg^{-1}$of DEHP in milk with 1% of fat, 0.10 – 0.38 $mg.kg^{-1}$ in milk with 3% of fat and 1.06 – 1.67 $mg.kg^{-1}$ in cream with 35% of fat **(Sharman et al., 1994)**. **Shuangling and Kangquan (2009)** found that migration of DEHP from a PVC foil into meat increased with rising temperature and time.

The maximum migration was recorded at 90 ˚C and 30 minutes of effecting (75.12 mg.dm^{-2}).
The overall migration limit (10 mg.dm^{-2}) was exceeded in all observed combinations of time and temperature, except for the combination of 10 ˚C and <41 hours when migration was not observed.

Barros et al. (2011) in his study executed the analysis of food that might be contaminated by DEHP and di-etylhexyladipátem (DEHA). There were observed 18 different types of food with less than 3% of fat with possibility to be packed in plastic foils. The study proved that all food was contaminated by DEHP and DEHA while the content of observed phthalates was increasing with the period of storage.

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.g^{-1} in the packaging film and 206.5 ng.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.kg^{-1} in vegetables and from 0.4 to 6.20 mg.kg^{-1} in soils with an average concentration of 2.56 and 2.23 mg.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.kg^{-1} but <2.50 mg.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.

Tsumara et al. (2001) observed the content of phthalates in ten samples of lunch half-products packed in plastic packages. The quantity of DEHP in the samples moved from 45 to 517 ng.g^{-1}, with the average value of 198 ng.g^{-1}. DBP was not detected in any sample.

The main source of phthalates in food, especially in those with a high fat content, is their direct contact with surfaces of production equipment and package materials. **Tsumara et al. (2001)** demonstrated increase of DEHP in chicken. From the initial value 0.080 mg.kg^{-1} before thermal processing the content of DEHP increased to 13.10 mg.kg^{-1}after frying at a nonstick pan and further to 16.90 mg.kg^{-1} after packaging.

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.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

Table 2 Average concentrations of DBP and DEHP (mg.kg^{-1}) in the samples of raw meat and meat products (n = 5) with the fat content of 10 % and 50 % after 1st, 7th, 14th, 21st and 28th days of storage at 4 °C.

Sample	FAT (%)	Product		1st day		7th day		14th day		21st day		28th day	
		DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP	DBP	DEHP
1	10	ND		0.21	2.19	0.45	3.27	0.45	3.39	0.47	3.42	0.68	3.57
	50			3.64	4.96	3.86	5.02	4.64	6.33	4.66	6.4	4.7	6.48
2	10			ND	ND	ND	ND	ND	ND	ND	ND	0.22	0.4
	50			1.18	4.81	2.37	6.45	2.42	6.65	3.08	8.21	3.61	8.34
3	10			ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	50			ND	1.26	ND	1.66	ND	1.8	ND	1.84	ND	2.5
4	10			0.72	0.77	0.8	0.98	0.83	1	1.22	1.75	1.3	2.01
	50			0.85	3.87	1.08	8.53	1.17	9.55	1.56	11.3	1.63	11.67
5	10			0.68	1.44	0.69	1.47	0.72	1.5	0.91	2.34	1.19	2.89
	50			3.6	7.12	4.92	7.94	6.31	8.04	6.68	8.5	7.95	8.54

Note: ND – the limit of detection of DBP and DEHP in fat matrices – 0.2 mg.kg^{-1}.

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.

In dairy products, more than 80% of the total concentration of phthalates ranging from 50 to 200 $\mu g.kg^{-1}$ in ordinary milk came from suction machines. Further processing and packaging may lead to increase of the DEHP concentration in cream and cheese products **(Casajuan a Lacorte, 2004).**

CONCLUSION

The objective of the work was to observe the content of phthalates (DBP and DEHP) in packages intended for packing of meat products and observation of potential migration of phthalates from packages into products after thermal processing and storage until the expiration time depending on the fat content.

According to the EU Commission Regulation no. 10/2011 in comparison to the specific migration limits for DBP (0.3 $mg.kg^{-1}$) and DEHP (1.5 $mg.kg^{-1}$), 3 samples of packages stated in Table 1 (1, 4, 5) would not meet the regulation with respect to the specific migration limit (Table 2) after 1st day of storage. The samples of packages 2 and 3 would be suitable during the whole time of storage if it would be filled with theraw meat with the fat content of 10% (Table 2).

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