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# THE CONCENTRATIONS OF PHTHALIC ACID ESTERS IN A WATER BATH AT SOUS-VIDE HEAT TREATMENT

*Marcela Jandlová, Alžbeta Jarošová, Josef Kameník, Vojtěch Kumbár, Šárka Nedomová*

## ABSTRACT

Esters of phthalic acid are common contaminants of the environment because of their large application in plastics. Phthalic acid esters are used as plasticizers in plastics, and they are also used in plastic intended for contact with food. In our research, we investigated the influence of heating on the migration of phthalic acid esters into the water used as a water bath. The water bath was used to heat the vacuum-wrapped meat, this heating is called the sous-vide method. The plastic thermostable bags containing phthalates were used on the meat packaging. Two esters of phthalic acid dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) have been determined. Three packaged meat samples were heated in a water bath for one hour either at 50 °C or at 60 °C. The water was analyzed always before the heating and after the heating. Average DEHP concentrations in the water dropped after heating at 50 °C in two cases and average DBP concentrations rose in one case and declined in one case. Average DBP concentrations in water declined after heating at 60 °C, while average DEHP concentrations after heating at 60 °C in water increased. The concentrations of phthalic acid esters in the water ranged from 15.2311 µg.L$^{-1}$ to 34.5645 µg.L$^{-1}$ for DEHP and from 0.0433 µg.L$^{-1}$ to 2.6529 µg.L$^{-1}$ for DBP. The heating of vacuum-packed food in plastic phthalate bags in a water bath does not pose a great risk of contamination of water with phthalic acid esters.

**Keywords:** water; dibutyl phthalate; di-2-ethylhexyl phthalate; plasticizer; contaminant

## INTRODUCTION

Sous-vide technology is the heat treatment of vacuum-wrapped food in thermostable bags. Such a processed food has good sensory properties, juicy, fragile, tasty, and it is harmless, it kills microbes and parasites, because the temperature and the time of heating are well controlled in this technology **(Kameník, 2016)**. At the same time, studies have shown that when using the sous-vide method, food has a better nutritional quality – unsaturated fatty acids and vitamins remain in food **(Schellekens, 1996)**. The heat-treated vacuum-packed food is stored in a cold place and reheated before consumption **(Rhodehamel, 1992)**.

Conventional synthetic materials are currently synthetic polymers because of their low weight, softness and transparency, but they are difficult to biodegrade **(Siracusa et al., 2008)**. Various additives are added to the polymers to have the desired properties. However, additives have been found to migrate to water, soil, air, and food, thereby increasing unwanted exposure to humans. Additives can also be released during recycling and from products obtained by recycling plastics **(Hahladakis et al., 2018)**. Additives may be, for example, plasticizers, antioxidants, flame retardants and antistatic additives **(Brimer, 2011)**. Plasticizers provide plastic softness, brittleness, better workability and grip, for example foil. Typical plasticizers are phthalic acid esters **(Robertson, 2013)**. The legislation establishes specific migration limits for both DBP 0.3 mg.kg$^{-1}$ food and DEHP 1.5 mg.kg$^{-1}$ food. A specific migration limit is the amount that can be maximally released from the plastic material into the food. Material meeting specific migration limits should not pose a health hazard to the consumer **(Commission Regulation (EU) No. 10/2011)**. Migration of phthalic acid esters from materials into food accelerates the heater **(Fan et al., 2012)**. Food that was in direct and indirect contact with PVC foil and heated by microwave heating for 3 minutes increased the concentrations of both DBP and DEHP in the food. The control contained an average DBP concentration of 0.056 µg.g$^{-1}$ and DEHP of 0.267 µg.g$^{-1}$, heated food without direct PVC film contact contained an average DBP concentration of 1.850 µg.g$^{-1}$ and DEHP of 2.921 µg.g$^{-1}$, a heated foodstuff with a direct PVC film contact contained an average DBP concentration of 1.769 µg.g$^{-1}$ and DEHP of 4.264 µg.g$^{-1}$ **(Chen et al., 2008)**. The effects of temperature on phthalate migration, for example, have been investigated in the study by **Rose et al. (2012)**, where the transfer of the DEHP plasticizer from a 2 m polyvinyl chloride infusion set to a lipid-based infusion solution investigated for 6 hours at a flow rate of 12 mL.h$^{-1}$ at 24 °C, 32 °C and 37 °C. It was found that at higher temperatures the concentration of phthalates in the infusion solution was higher. And at 32 °C and at 37 °C may exceed the maximum recommended exposure set by EU legislation, which is 20 – 48 μg.kg$^{-1}$ per day, especially in infants and newborns **(Rose et al., 2012)**.

Esters of phthalic acid are produced in large quantities to produce plastics, phthalates are released from plastics and they are now ubiquitous in the environment where they are worried about their teratogenicity, hepatotoxicity and carcinogenicity **(Liang et al., 2008)**. Esters of phthalic acid also have estrogenic activity, damaging the kidneys and the liver. Acute toxicity is manifested by dizziness, nausea, cough, vision impairment, blood pressure lowering, and others. The widespread phthalate esters are DBP and DEHP. In terms of physical properties, lower phthalates are highly volatile at room temperature, while higher phthalates are non-volatile at room temperature. Esters of phthalic acid have a high boiling point **(Velíšek and Hajšlová, 2009)**. The boiling point for DBP is reported at 340 °C **(ATSDR, 2001)** and the boiling point for DEHP is 384 °C **(ATSDR, 2002)**.

The half-life of DEHP in the sediment was found out about 14 days, while the half-life in river water was found out about 10 hours for DEHP **(Yuwatini, Hata and Taguchi, 2006)**. **Shailaja et al. (2007)** found the shortest half-life of DBP when in the reactor lasted only 0.75 days; other literature reported longer periods. Degradation of DBP was investigated in the study by **Wang et al. (1997)**, when only 3% of DBP disappeared in the abiotic soil within 30 days, up to 66% of DBP concentration disappeared in soil with its own microorganisms, and up to 92% of DBP concentration was degraded in soil with own and added microorganisms. **Zhou et al. (2005)** study reported that degradation of DBP on the soil surface after 30 days was 95.7%. A high concentration above 60 mg.L$^{-1}$ of DEHP slows the degradation of both DBP and DEHP **(Gavala et al., 2003)**.

In wastewater treatment, the use of a combination of more technologies can remove a relatively high percentage of present phthalic acid esters, for example by anaerobic treatment with subsequent processing in the membrane bioreactor can remove about 95% to 97% of phthalates, while only anaerobic treatment without subsequent treatment in the membrane bioreactor can remove about 65% to 71% of phthalates from waste water **(Gao and Wen, 2016)**. Subsequent contamination with water containing phthalic esters, for example, is shown in **Tan et al. (2016)**. The study found that by irrigation of food crops with sewage, the occurrence of phthalic acid esters in crops increased. In the study investigated the effect of sewage irrigation on maize and wheat, they found higher concentrations of phthalic acid esters in wheat grains than in maize grains **(Tan et al., 2016)**.

## Scientific hypothesis

The migration of phthalic acid esters from plastic bags of vacuum-packed meats into water of water bath during one-hour heating at 50 °C and 60 °C is expected.

## MATERIAL AND METHODOLOGY

Vacuum packed meat, which was used for heating in a water bath, was obtained in cooperation with the University of Veterinary and Pharmaceutical Sciences Brno, with the Department of Gastronomy. Vacuum packed meat was heat-treated, so-called sous-vide technology at 50 °C or at 60 °C for 4 or 8 hours. Meat was the pork shoulder purchased in the market of Brno. The wrappings on the meat were the thermostable plastic bags Cryovac® CN300 (Sealed Air Polska Sp. z o.o., Poland).

In the study by **Jandlová, Jarošová and Kameník (2017)** was found in these plastic bags average DBP concentration of 22.47 μg.g$^{-1}$ plastic and average DEHP concentration of 11.76 μg.g$^{-1}$ plastic.

In our experiment, three pieces of vacuum-packed meat were heated at 50 °C or 60 °C per 1 hour. The used temperature corresponded to the previous temperature of the heat treatment of meat pieces. In the sous-vide technology this hourly heating represents a heating before consumption. Water samples were taken before and after heating in three replicates.

Water samples were analyzed at the Department of Food Technology at the Mendel University in Brno. The used method was by **Jarošová et al. (1999)**. Water samples were extracted 3 times with dichloromethane in a separating funnel. Subsequently, the solvent was evaporated on a rotary evaporator, the contents transferred in hexane to the vial, after drying the hexane with nitrogen, the sample in acetonitrile was determined by HPLC (mobile phase acetonitrile, UV detection at 224 nm and used column Zorbax Eclipse C8). The evaluation program was used Data Analysis (Agilent Technology).

## Statistic analysis

The Microsoft Excel (Microsoft Corporation, USA) and Statistics 12 (StatSoft, USA) were used for processing and statistical analysis of data. They were used Shapiro-Wilk's test for the normality test, Grubbs' test to determine outliers and *t*-test to determine sameness of the mean values ($\alpha = 0.05$). *T*-test for dependent samples was used to compare the water sample concentrations before heat treatment with after heat treatment of the same temperature and time. *T*-test for independent samples was used to compare the water sample concentrations after heat treatment of 50 °C with after heat treatment of 60 °C, and to compare the water sample concentrations after heat treatment of the same temperature with the each other.

## RESULTS AND DISCUSSION

Table 1 shows the observed concentrations of the two phthalic acid esters in water of the water bath. The concentrations of phthalic acid esters in water of water bath were for DBP from 15.2311 μg.L$^{-1}$ to 34.5645 μg.L$^{-1}$ for DEHP from 0.0433 μg.L$^{-1}$ to 2.6529 μg.L$^{-1}$. The DBP concentrations were detected higher in the water samples than the DEHP concentrations. The concentrations of DBP in water samples before heat treatment ranged from 16.0477 μg.L$^{-1}$ to 34.5645 μg.L$^{-1}$ and the concentrations of DEHP in water samples before heat treatment ranged from 0.0433 μg.L$^{-1}$ to 2.6529 μg.L$^{-1}$. While the DBP concentrations after heat treatment were in water from 15.2311 μg.L$^{-1}$ to 34.5645 μg.L$^{-1}$ and the DEHP concentrations after heat treatment was in the water from 0.0927 μg.L$^{-1}$ to 2.1658 μg.L$^{-1}$.

Figure 1 and Figure 2, show the average concentrations of DBP and DEHP in μg.L$^{-1}$. The average DBP concentrations dropped in water after heat-treatment at 60 °C in both cases, whereas the average DEHP concentrations grew in water after heat-treatment at 60 °C in both cases. And the average DEHP concentrations dropped in water after heat-treatment at 50 °C in both cases, and the average DBP concentrations in water in one case grew and in one case dropped. The average concentrations of phthalic acid esters are lower before and after heat treatment than the specific migration limits set by legislation, so the amount of phthalates in water by sous-vide heat treatment should not pose a health risk **(Commission Regulation (EU) No. 10/2011)**.

The Table 2 and Table 3 show that first statistically significant difference in mean values was found in the concentrations of DBP in water before and after heat treatment at 50 °C per 1 h of meat heat treatment by 50 °C per 4 h and second statistically significant difference in mean values was found in the concentrations of DEHP in water after heat treatment at 50 °C per 1 h meat heat treatment by 50 °C per 8 h with the concentrations of DEHP after heat treatment at 60 °C per 1 h meat heat treatment by 60 °C per 8 h.

In the study by **Prokůpková et al. (2002)** in which solid phase microextraction (SPME) was used for the determination of phthalic acid esters in water and subsequently determined by GC. In water, two esters of phthalic acid esters: DBP and DEHP were found in the highest concentrations. Drinking tap water, deionized water, water in a glass bottle with a metal lid, which contains the PVC gasket (plasticized by DEHP) and water from a PET bottle were analyzed. Drinking water found DBP of 0.05 μg.L$^{-1}$ and DEHP of 0.66 μg.L$^{-1}$ in deionized water DBP of 0.15 μg.L$^{-1}$ and DEHP of 0.49 μg.L$^{-1}$ in water in a glass bottle DBP of 0.18 μg.L$^{-1}$ and DEHP of 9.78 μg.L$^{-1}$, in water in a PET bottle DBP of 0.20 μg.L$^{-1}$ and DEHP of 2.88 μg.L$^{-1}$.

**Morita, Nakamura and Mimura (1974)** examined the content of two phthalates dibutyl phthalate esters and di-2-ethylhexyl phthalate in Tokyo waters. They found higher concentrations of phthalic acid esters further downstream than the upper reaches of the same river. The concentrations of phthalic acid esters were detected in river water from 0.4 μg.L$^{-1}$ to 6.8 μg.L$^{-1}$, in untreated water from 1.9 μg.L$^{-1}$ to 8.2 μg.L$^{-1}$ and in tap water from 1.2 μg.L$^{-1}$ to 3.3 μg.L$^{-1}$.

**Rastkari et al. (2017)** examined the transfer of phthalic acid esters into acidic liquids (vinegar, lemon juice, verjuice) at different storage temperatures (4 °C, 25 °C, 50 °C). The analyses were carried out after 0, 2, 4, 6 month of storage. The concentrations of DEHP in acidic liquids stored in HDPE and PET bottles grew with higher storage temperatures, even with increasing storage times. The concentration of DBP in acidic liquids stored in HDPE and PET bottles grew with higher temperature, but only to 25 °C, and with increasing storage time but only for 2 months. After 2 months and at 50 °C the concentrations of DBP decreased.

**Mousa, Basheer and Al-Arfaj (2013)** measured the concentrations of phthalates in mineral bottled water exposed to direct sunlight for up to 222 hours. The concentration of DBP and DEHP rose in the water up to 36 hours of exposure to sunlight when a sample temperature of 43 °C was measured, the average concentrations of DBP in water were ranged from 14.69 μg.L$^{-1}$ to 22.34 μg.L$^{-1}$ and the average concentrations of DEHP in the water were ranged from 42.77 μg.L$^{-1}$ to 75.71 μg.L$^{-1}$.

**Wang et al. (2015)** investigated the concentrations of sixteen phthalic acid esters in drinking water and source water from 19 places of Zhejiang Province in China. The water samples were taken during wet and dry season. They found that phthalate concentrations were lower in the dry season than in the wet season, and lower concentrations were found in source water than in drinking water. The concentrations of DBP were ranged from 0.06 μg.L$^{-1}$ to 0.22 μg.L$^{-1}$ in source water in the wet season, and from ND to 0.08 μg.L$^{-1}$ in source water in dry season. The concentrations of DBP in drinking water ranged from 0.05 μg.L$^{-1}$ to 0.64 μg.L$^{-1}$ in the wet season and from 0.01 μg.L$^{-1}$ to 0.08 μg.L$^{-1}$ in the dry season. The concentrations of DEHP in source water were detected from ND to 1.68 μg.L$^{-1}$ in the wet season and from ND to 1.11 μg.L$^{-1}$ in dry season. The concentrations of DEHP in drinking water were measured from 0.12 μg.L$^{-1}$ to 4.58 μg.L$^{-1}$ in the wet season and from 0.02 μg.L$^{-1}$ to 1.33 μg.L$^{-1}$ in the dry season.

**Liu, Chen and Shen (2013)** examined the concentrations of phthalic acid esters in a water source in northeastern China. Most of the phthalic acid esters were detected DBP and DEHP. The concentrations of DBP were found out from
52.5 ng.L$^{-1}$ to 4 498.2 ng.L$^{-1}$ and DEHP from 128.9 ng.L$^{-1}$ to 6570.9 ng.L$^{-1}$.

In our study, we found higher DBP concentrations in water samples than DEHP, as opposed to **Prokůpková et al. (2002)**, **Mous, Basheer and Al-Arfaj (2013)**, **Wang et al. (2015)**, and **Liu, Chen and Shen (2013)**, which detected higher concentrations of DEHP than DBP. However, in our study used plastic bags also had higher DBP concentrations than DEHP **(Jandlová, Jarošová and Kameník, 2017)**.

In our study, we have not come to the conclusion that the heating cause migration of phthalic acid esters into water, since the increase of the concentrations after heating was only by DBP concentrations in one case at 50 °C and in two cases by DEHP concentrations at 60 °C. For example, **Chen et al. (2008)** reported that thermal microwave heating has resulted in the migration of phthalic acid esters into foodstuff, when the foodstuff had close or free contact with PVC film. Similarly, **Fan et al. (2012)** stated, the heating accelerated the migration of phthalates into food.

Why did not only the increase in phthalic acid ester concentrations during heating? It can be also caused by possible heat degradation, or it could occur **Velíšek and Hajšlová (2009)** vaporization to the air.

**Table 1** Concentrations of phthalic acid esters, dibutyl phthalate (DBP) and di (2-ethylhexyl) phthalate (DEHP) in water samples heat-treated at 50 °C and 60 °C per 1 hour each with three samples of vacuum packed sous-vide meat.

| Descriptions of water samples | DBP [μg.L$^{-1}$] | DEHP [μg.L$^{-1}$] |
|---|---|---|
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 20.0267 | 1.3175 |
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 27.2050 | 1.5955 |
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 18.6800 | 0.5643 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 30.1417 | 0.3564 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 34.5645 | 0.9186 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h | 25.5281 | 0.4505 |
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 27.3995 | 2.6529 |
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 23.5183 | 1.1866 |
| before the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 25.9074 | 0.0433 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 26.4121 | 1.2342 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 20.1415 | 0.5508 |
| after the heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | 16.0477 | 0.0927 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 30.1417 | 0.3564 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 34.5645 | 0.9186 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 25.5281 | 0.4505 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 25.5632 | 2.1658 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 19.6026 | 0.1539 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | 32.4342 | 0.8114 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 26.4121 | 1.2342 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 20.1415 | 0.5508 |
| before the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 16.0477 | 0.0927 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 17.2808 | 1.7364 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 15.2311 | 1.9161 |
| after the heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | 16.2460 | 1.8160 |

**Table 2** The results of T-test for dependent samples; phthalic acid ester concentrations in water before heat treatment are compared with concentrations in water after heat treatment.

| Descriptions of campared water samples | Statistically significant difference |
|---|---|
| DBP before and after heat treatment at 50 °C per 1 h of meats treated by 50 °C per 4 h | $p$ <0.05 |
| DEHP before and after heat treatment at 50 °C per 1 h of meats treated by 50 °C per 4 h | $p$ >0.05 |
| DBP before and after heat treatment at 50 °C per 1 h of meats treated by 50 °C per 8 h | $p$ >0.05 |
| DEHP before and after heat treatment at 50 °C per 1 h of meats treated by 50 °C per 8 h | $p$ >0.05 |
| DBP before and after heat treatment at 60 °C per 1 h of meats treated by 60 °C per 4 h | $p$ >0.05 |
| DEHP before and after heat treatment at 60 °C per 1 h of meats treated by 60 °C per 4 h | $p$ >0.05 |
| DBP before and after heat treatment at 60 °C per 1 h of meats treated by 60 °C per 8 h | $p$ >0.05 |
| DEHP before and after heat treatment at 60 °C per 1 h of meats treated by 60 °C per 8 h | $p$ >0.05 |

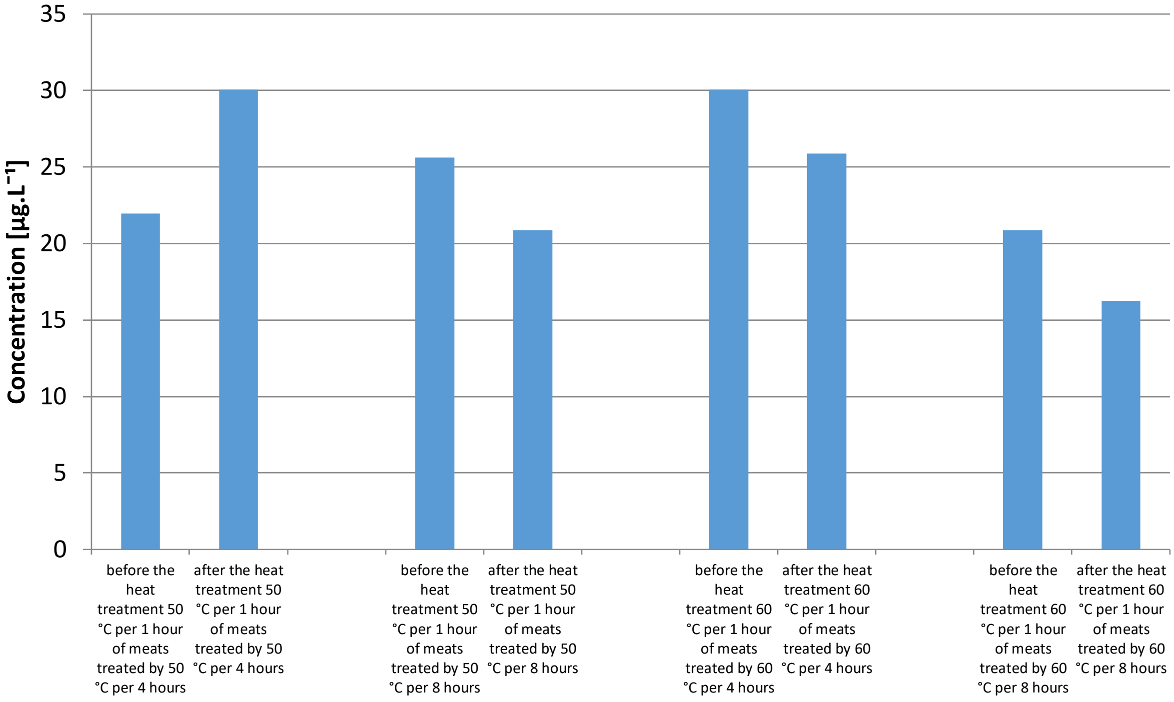


**Figure 1** The average concentrations of DBP in water of water bath before and after heating.

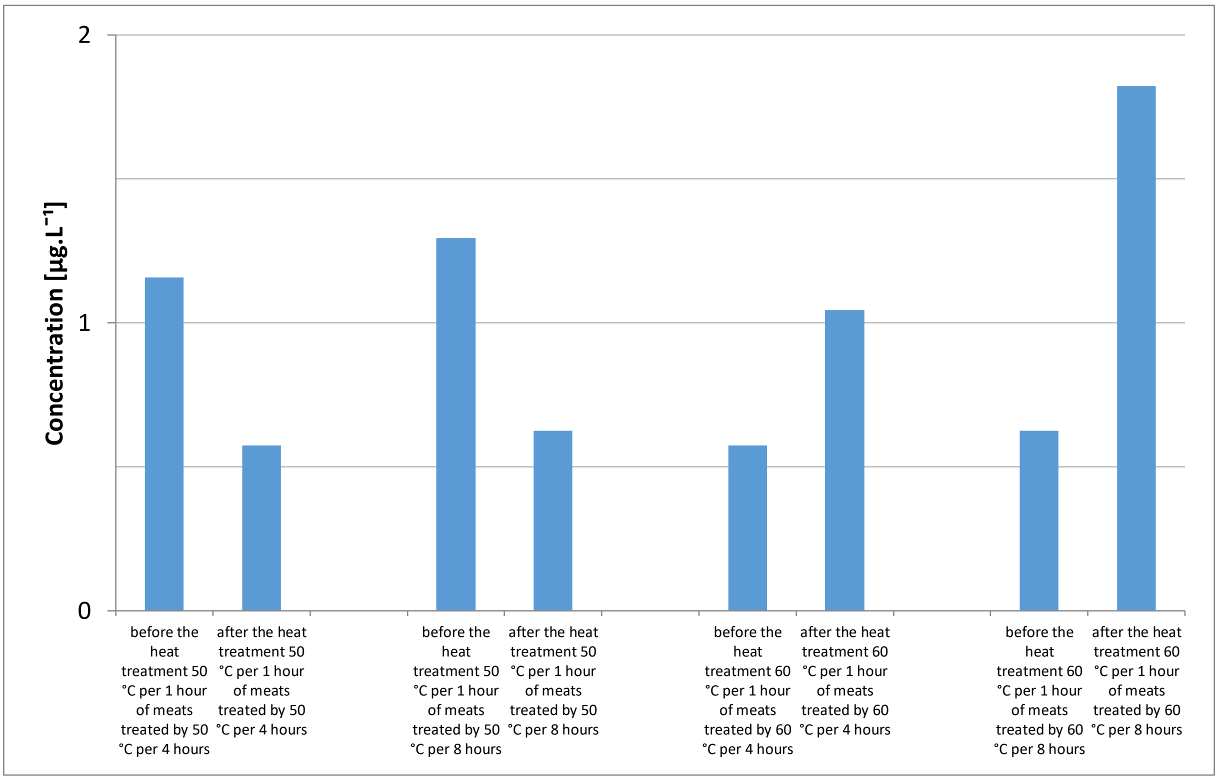


**Figure 2** The average concentrations of DEHP in water of water bath before and after heating.

**Table 3** The results of T-test for independent samples; comparison of phthalic acid ester concentrations in water samples after heat treatment.

| **Descriptions of campared water samples** | **Statistically significant difference** |
|---|---|
| DBP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h with DBP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | $p > 0.05$ |
| DEHP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h with DEHP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h | $p > 0.05$ |
| DBP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h with DBP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | $p > 0.05$ |
| DEHP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h with DEHP after heat treatment 60 °C per 1 h of meats treated by 60°C per 8 h | $p > 0.05$ |
| DBP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h with DBP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | $p > 0.05$ |
| DEHP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 4 h with DEHP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 4 h | $p > 0.05$ |
| DBP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h with DBP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | $p > 0.05$ |
| DEHP after heat treatment 50 °C per 1 h of meats treated by 50 °C per 8 h with DEHP after heat treatment 60 °C per 1 h of meats treated by 60 °C per 8 h | $p < 0.05$ |

## CONCLUSION

In the study, we expected the migration of phthalic acid esters by heating from plastic bags to water of water bath. The increase of the average DEHP concentrations in the water of water bath was found after heat treatment at 60 °C. The increase of the average DBP concentrations was found at 50 °C, where a statistically significant difference was found. In other cases, the average concentrations of phthalic acid esters after heat treatment decreased. This can be justified by the potential volatility and degradation of phthalic acid esters during heating. The heating of vacuum-packed food in plastic phthalate bags does not pose a great risk of contamination of water with esters of phthalic acid.

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**Contact address:**

*Marcela Jandlová, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemedelska 1, 613 00 Brno, Czech Republic, Tel.: +420545133338,
E-mail: marcela.jandlova@mendelu.cz
ORCID: https://orcid.org/0000-0002-4364-4696

Alžbeta Jarošová, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemedelska 1, 613 00 Brno, Czech Republic, Tel.: +420545133191, +420545133563,
E-mail: alzbeta.jarosova@mendelu.cz
ORCID: https://orcid.org/0000-0002-9809-3529

Josef Kameník, University of Veterinary and Pharmaceutical Sciences Brno, Faculty of Veterinary Hygiene and Ecology, Department of Gastronomy, Palackeho tr. 1946/1, 612 42 Brno, Czech Republic, Tel.: +420541562600,
E-mail: kamenikj@vfu.cz
ORCID: https://orcid.org/0000-0002-0615-0638

Vojtěch Kumbár, Mendel University in Brno, Faculty of AgriSciences, Department of Technology and Automobile Transport, Zemědělska 1, 613 00 Brno, Czech Republic, Tel.: +420545132128,
E-mail: vojtech.kumbar@mendelu.cz
ORCID: https://orcid.org/0000-0003-3987-4613

Šárka Nedomová, Mendel University in Brno, Faculty of AgriSciences, Department of Food Technology, Zemedelska 1, 613 00 Brno, Czech Republic, Tel.: +420545133193,
E-mail: sarka.nedomova@mendelu.cz
ORCID: https://orcid.org/0000-0001-8840-7849

Corresponding author: *