EFFECT OF SOUS VIDE HEAT TREATMENT ON PHTHALIC ACID ESTERS CONTENT IN MEAT

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ABSTRACT
The chemical composition and content of DBP (di-n-butyl phthalate) and DEHP (di-2-ethylhexyl phthalate) in pork shoulder before and after heat treatment in the package by the sous vide method was analyzed. The meat was analyzed raw and after heat treatment at 50 °C and 60 °C. The heat treatment time in the sous vide water bath was 4 hours, 4 hours + 1 hour after 24 hours, 8 hours and 8 hours and 1 hour after 24 hours. The fat content in meat treated at 50 °C was 6.04 in raw meat and after heat treatment after 4 hours. 7.51 and after 8 h. 6.81 g.100g−1, in the shoulder after heat treatment at 60 °C was after 4 hours 6.24 and after 8 h. 6.76 g.100g−1. The content of fatty acids during the sous vide treatment did not significantly change with exception of vaccenic acid, the content of which was statistically significantly reduced at temperatures of 50 °C also at 60 °C. The DBP content in raw shoulder at 50 °C during sous vide heat treatment it increased to 1.91 μg.g−1. The DEHP content during the heat treatment it increased to 23.95 μg.g−1 in the treatment of 4+1 hours. The DBP content in raw shoulder after heat treatment at 60 °C increased to 1.84 μg.g−1 during treatment for 4+1 hours. The DEHP content decreased to 8.72 in the treatment of 4+1 hours and to 4.02 μg.g−1. Based on our results, we can conclude that at both monitored temperatures of sous vide method, the DBP content increased and the DEHP content decreased. The DBP content in raw shoulder at 50°C heat treatment increased to 1.91 μg.g−1 at 4+1 hour heat treatment and to 3.02 at 8+1 hour. The DEHP content increased to 23.95 μg.g−1 in the treatment of 4+1 hours. The content of DBP and DEHP in the packaging material before use was 29.08 μg.g−1; it gradually decreased with the length of the heat treatment, to 15.09 μg.g−1 in the treatment of 8+1 hours. The DEHP content in the unused package decreased to 1.27 μg.g−1 at heat treatment of 8+1 hours. At the heat treatment at of 60°C in the packaging material gradually decreased to 3.18 μg.g−1. The DEHP content decreased to 2.54 μg.g−1.

Keywords: sous vide, heat treatment, pork shoulder, di-n-butyl phthalate, di-2-ethylhexyl phthalate

INTRODUCTION
The sous vide method is a cooking method based on the principle that the food is vacuum packed in a plastic cover and then placed in a water bath to ensure even cooking and a relatively constant temperature of 55-95 °C for 6-48 hours in the meat industry (Baldwin, 2012). This food preparation technique was originally developed for supplying food to customers as a method by which food was prepared after heat treatment without the risk of microbiological contamination (Armstrong, 2000). Currently, this method is used in restaurants for simplicity and adaptability of food preparation (Ruiz-Carrascals et al., 2019).

At lower temperatures, as with conventional heat treatment, the meat retains nutrients and is only minimally affected. During heat treatment, the process of shrinking muscle fibers begins at 35–40°C, during heat treatment of meat, the value of shear force decreases from 50-65°C (the meat softens) and increases up to 80°C. Temperatures over 60°C up to 80°C cause an increase in hardness due to the increasing cohesion of muscle fibers. By raising the temperature of the meat to 65°C, the sarcoplasmatic protein changes its consistency into a gel and the gel becomes more tender (Kamenik et al., 2018).

Co-extruded EVA/PVD/EVA three-layer films are most often used for vacuum packaging in the form of a cover into which the product is placed, and air is sucked out in a chamber packaging machine and the bag is hermetically sealed. Another possibility is to use shrinkable films (PE, PP, PC, PVD), in which the effect of heat tightly wraps around the product around the product and thus reduces the dimensions of the packaged raw material. The package is withdrawn by passing through a tunnel with warm air (about 150 °C) or by immersion in warm water (80-90 ° C) for a few seconds. There is a small space between the foil and the product, which reduces the amount of juice released due to the vacuum (Kamenik et al., 2014; Ceballos-Luna et al., 2022).

The vacuum film for sous vide is compatible with all common vacuum cleaners. The foil is suitable for refrigerators, freezers, microwaves and for dry cooking. Of the additives added in the production of plastics, plasticizers - plasticizers - pose the greatest risk. In some cases, they make up 40% of the total packaging material and are highly lipophilic, making them easy to extract with the fatty components of the food. The most common plasticizers used in packaging are phthalic acid esters, especially di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) (Lahimer et al., 2017). The content of phthalates in food is not set directly but only as a specific migration limit. According to the Commission Regulation no. 10/2011/EC in comparison to the specific migration limits for DBP is 0.3 mg.kg−1, and for DEHP is 1.5 mg.kg−1. Fiorens et al. (2014) found 1.4 μg.kg−1 DEP and 90 μg.kg−1 DEHP in fresh pork meat.

Mean dietary intakes of DEHP in the general population is 2.34, children 4.51, and adults 2.03 μg.kg−1 bw per day. The main food sources of DEHP dietary intake are cereals (39.44%), drinking water (16.94%) and meat (15.81%) in children, and cereals (44.57%), meat (15.70%) and drinking water (12.28%) for adults (Sui et al., 2014; Da Silva et al., 2017).

According to the EU Commission Regulation no. 10/2011 the specific migration limit (SML) 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, was exceeded already after first day of storage, in case of DBP in two samples with 10% of fat and after 7 days 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 14th day of storage. Regarding DEHP in the samples with 10% of fat SML was exceeded after 1st day of storage in one sample and after 7th day of storage also in one sample and after 21th day of storage similarly in one sample. Four samples with 50% of fat had SML exceeded in case of DEHP already after 1st 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. (Jarošová, and Bogdanovičová, 2015).
In foods the lowest average concentration of dibutyl phthalate (4.13 μg·g⁻¹), was found in Old Bohemian salami and the lowest concentration of di(2-ethylhexyl) phthalate - DEHP (2.86 μg·g⁻¹) was measured in milk. The highest average concentrations of dibutyl phthalate (23.91 μg·g⁻¹) and di(2-ethylhexyl) phthalate (50.80 μg·g⁻¹) were determined in the meat spread (Jandlová and Jarováová, 2019).

The aim of the work was the chemical analysis of meat in the raw state, after heat treatment and after homogenization of pork shoulder samples. The content of phthalates and migration of DBP and DEHP in meat after heat treatment at 50 and 60 °C were also monitored using the "sous vide" method and in packages in which the meat was vacuum-packed.

**MATERIAL AND METHODS**

Pork shoulder (n = 40) was used for chemical analysis. The meat was cut into slices with a thickness of 1.8 - 2 cm and a weight of 150-200 g into packages, then it was vacuum-packed and heat-treated.

For vacuum packaging of meat, the most used coextruded three-layer foils in a form of bags, into which the meat is placed, and air is sucked out in a chamber packaging machine and the plastic bag is hermetically sealed. It is also possible to use shrinkable foils, which, after hermetic sealing and heat, wrap around the packaged raw material and thus reduce the size of the packaged meat (Kameník and Chomát, 2013). The cooking bags used for the analysis were with a thickness of 60 μm. A total of 40 samples of packaging parts were analyzed for DBP and DEHP phthalates, 5 samples of unused packaging, 2 x 20 samples of packaging used for vacuum packaging of meat during heat treatment: 50 °C, 4 hours (n = 5), 4 + 1 hours (n = 5), 8 hours (n = 5) and 8 + 1 hours (n = 5), 60 °C, 4 hours (n = 5), 4 + 1 hours (n = 5), 8 hours (n = 5) and 8 + 1 hours (n = 5). Preparation of meat samples and heat treatment by the sous vide method, pork shoulder (n=40) was cut into slices 18-20 mm thick and immediately vacuum-packed in cooking bags with a thickness of 60 μm at room temperature 20 °C. The meat slices were packed individually. The sous vide heat treatment was performed in a water bath at 50 °C and 60 °C in a SoftcOOKER Y09. The heat treatment lasted 4 hours or 8 hours, after each heat treatment the demineralized water was changed. Samples of meat and packaging were analyzed in triplicate, a total of 40 meat samples were prepared, of which 5 samples of raw meat, 20 samples heat-treated at 50 °C and 20 samples at 60 °C in individual time variations. After heat treatment, the meat was cooled to 20 °C and then stored in a refrigerator at 6 °C. The meat samples were homogenized and subsequently the chemical composition was analyzed using a Nicotek 5700. Phthalic acid esters in packages were determined according to the method of Gajdůšková et al. (1996). From each sample we took a suitable sample part, which was cut into small pieces, 10 cm² in size. In an Erlenmeyer flask, the samples were leached for 72 hours in a 1:1 solution of n-hexane and dichloromethane. The first extraction took place after 24 hours, 7.51 and after 8 h. 6.81 g.100g

| Parameter | Raw meat | Meats cooked at 50 °C | Meats cooked at 60 °C |
|-----------|----------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |

**RESULTS AND DISCUSSION**

The chemical composition and content of DBP (di-n-butyl phthalate) and DEHP (di-2-ethylhexyl phthalate) in pork shoulder before and after heat treatment in the package by the sous vide method was analyzed. The meat was analyzed raw and after heat treatment at 50 °C and 60 °C. The heat treatment time in the sous vide water bath was 4 hours, 4 hours + 1 hour after 24 hours, 8 hours and 8 + 1 hour after 24 hours.

| Parameter | Raw meat | Meats cooked at 60 °C | Meats cooked at 50 °C | Meats cooked at 50 °C |
|-----------|----------|----------------------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |

**Table 1**

| Parameter | Raw meat | Meats cooked at 50 °C | Meats cooked at 60 °C | Meats cooked at 60 °C |
|-----------|----------|----------------------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |

**Table 2**

| Parameter | Raw meat | Meats cooked at 50 °C | Meats cooked at 60 °C | Meats cooked at 60 °C |
|-----------|----------|----------------------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |

**Table 3**

| Parameter | Raw meat | Meats cooked at 50 °C | Meats cooked at 60 °C | Meats cooked at 60 °C |
|-----------|----------|----------------------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |

**Table 4**

| Parameter | Raw meat | Meats cooked at 50 °C | Meats cooked at 60 °C | Meats cooked at 60 °C |
|-----------|----------|----------------------|----------------------|----------------------|
|           | 4 hours  | 4+1 hours            | 8 hours              | 8+1 hours           |
During sous vide heat treatment at 60 °C, the lauric FA content decreased from 0.09 in raw meat to 0.04 in the 4+1 hour treatment and to 0.07 g.100g⁻¹ in the 8+1 hour treatment. The content of Oleic FA did not change during the treatment of 4+1 hours, but after the treatment of 60 °C for 8+1 hours, it decreased from 46.61 in raw meat to 44.62 g.100g⁻¹. The content of eicosanoic FA decreased during the treatment of 4+1 hours from 0.61 to 0.41 and in the 8+1 hour treatment to 0.32 g.100g⁻¹. The EPA content decreased from a value of 0.07 in raw meat to 0.04 g.100g⁻¹ in the 4+1 hour, and as well at 8+1 hour treatment. Omega-6 FA content increased from 5.12 in raw meat to 6.85 g.100g⁻¹ in the 4+1 hour treatment and to 7.35 g.100g⁻¹ in the 8+1 hour treatment. The content of fatty acids during the sous vide treatment did not significantly change with the exception of vaccenic acid, the content of which was statistically significantly reduced at temperatures of 50 °C and also at 60 °C.

| Parameter | Raw meat | 4 hours | 4+1 hours | 8 hours | 8+1 hours |
|-----------|----------|---------|-----------|---------|-----------|
| Fat       | 6.04±0.61 | 6.24±2.74 | 6.52±3.14 | 6.76±1.37 | 6.66±1.62 |
| Lauric FA | 0.09±0.03 | 0.07±0.02 | 0.06±0.03 | 0.07±0.02 | 0.07±0.01 |
| Myristic FA | 1.35±0.67 | 1.31±0.12 | 1.30±0.11 | 1.31±0.59 | 1.30±0.37 |
| Palmitic FA | 24.46±1.79 | 24.46±2.61 | 24.22±1.14 | 24.37±2.0 | 24.18±2.02 |
| Stearic FA | 10.84±0.12 | 10.97±0.17 | 10.97±0.0 | 10.18±0.08 | 10.97±0.01 |
| Vaccumic FA | 4.76±0.05 | 4.55±0.06 | 4.52±0.05 | 4.50±0.11 | 4.52±0.09 |
| Oleic FA | 46.61±1.61 | 47.45±3.49 | 46.32±5.31 | 44.13±3.01 | 44.62±4.0 |
| Linoleic FA | 4.61±0.47 | 6.55±1.51 | 6.54±1.52 | 6.26±0.83 | 6.92±0.72 |
| Conjugated linoleic FA | 0.12±0.02 | 0.10±0.01 | 0.09±0.01 | 0.09±0.0 | 0.10±0.01 |
| α-linolenic FA | 0.25±0.19 | 0.28±0.06 | 0.29±0.04 | 0.31±0.03 | 0.27±0.03 |
| Eicosanoic FA | 0.61±0.05 | 0.39±0.01 | 0.41±0.25 | 0.39±0.01 | 0.32±0.01 |
| Arachadonic FA | 1.15±0.13 | 0.88±0.14 | 0.81±0.21 | 0.91±0.19 | 0.97±0.15 |
| EPA | 0.07±0.02 | 0.05±0.01 | 0.04±0.01 | 0.05±0.02 | 0.04±0.01 |
| DPA | 0.14±0.03 | 0.13±0.01 | 0.12±0.01 | 0.12±0.01 | 0.11±0.01 |
| DHA | 0.05±0.02 | 0.04±0.01 | 0.03±0.01 | 0.06±0.02 | 0.07±0.01 |
| Omega-3 FA | 0.61±0.04 | 0.71±0.03 | 0.73±0.03 | 0.71±0.03 | 0.65±0.04 |
| Omega-6 FA | 5.12±1.36 | 6.81±1.63 | 6.85±1.89 | 9.42±1.16 | 7.35±0.45 |
| MUFA | 55.41±1.36 | 55.78±1.32 | 56.59±1.67 | 56.42±1.61 | 54.56±1.16 |
| PUFA | 9.55±0.66 | 9.33±1.37 | 9.57±1.62 | 9.96±1.28 | 9.59±0.63 |
| SFA | 33.66±1.59 | 33.98±0.91 | 33.16±0.74 | 33.62±0.92 | 34.71±0.99 |

Table 3. Fatty acid content in the shoulder during heat treatment 50 °C (g.100g⁻¹ fat)
The DBP content in raw shoulder after heat treatment at 60 °C during heat treatment by the sous vide method increased to 1.84 µg.g⁻¹ during treatment for 4+1 hours and to 1.05 µg.g⁻¹ at a temperature of 60 °C during treatment for 8+1 hours. The DEHP content during the heat treatment of 50 °C was 5.14 µg.g⁻¹. The content of DBP (53 °C, 18 hours) was 4.09 µg.g⁻¹. DEHP content (53 °C, 18 hours) was 3.14 µg.g⁻¹. The DBP content (70 °C, 2 hours) was 2.45 µg.g⁻¹. Thus, this study confirms the decrease in the concentration of phthalates after heat treatment, in agreement with our results.

Moreira et al. (2014) analyzed the content of phthalic acid esters in meat prepared by the sous vide method at a temperature of 60 °C for 4 hours and 65 °C for 5 hours. The highest concentration of phthalates (DBP, DEHP) was in raw meat samples and the lowest at 65 °C for 5 hours. The authors found that the concentration of phthalic acid esters in heat-treated meat decreases with higher temperature. In contrast to the findings of the mentioned authors, in our results, the DBP content increased, but the DEHP content decreased, in agreement with the mentioned authors, by heat treatment.

The content of DBP and DEHP in the packaging before use and after heat treatment of the meat was also analyzed. The content of DBP in the unused package was 29.08 µg.g⁻¹; it gradually decreased with the length of the heat treatment, and at a temperature of 50 °C we found the lowest content of 15.09 µg.g⁻¹ in the treatment of 8+1 hours (Table 7). The DEHP content in the unused package was 5.04 µg.g⁻¹ and the lowest content was 1.27 µg.g⁻¹ at heat treatment of 8+1 hours.

At the heat treatment at 60 °C, we found the DBP content in the unused packaging to be 29.08 µg.g⁻¹; and during the treatment of the meat with the sous vide method, its content gradually decreased (Table 8), with a heat treatment of 8+1 hours, it was statistically significantly lower at 3.18 µg.g⁻¹. The DEHP content was 5.04 µg.g⁻¹ in the unused package and after 8+1 hours of treatment it was reduced to 2.54 µg.g⁻¹. With both methods of sous vide heat treatment, the content of DBP and DEHP decreased, but at a temperature of 60 °C it was more significantly, and the reduction of DEP was statistically significant.

CONCLUSION

The aim of experiment was analyzed chemical composition and content of DBP (di-n-butyl phthalate) and DEHP (di-2-ethylhexyl phthalate) in pork shoulder before and after heat treatment in the package by the sous vide method was analyzed. The meat was analyzed raw and after heat treatment at 50 °C and 60 °C. The heat treatment time in the sous vide water bath was 4 hours, 4 hours + 1 hour after 24 hours, 8 hours and 8 hours + 1 hour after 24 hours.

The fat content in meat treated of sous vide at 50 °C increased. The content of fatty acids during the sous vide treatment did not significantly change with exception of vaccenic acid. The vaccenic acid statistically significantly reduced at temperatures of 50 °C and also at 60 °C.

The DBP content in raw shoulder at 50 °C and 60 °C during sous vide heat treatment increased. The DEHP content during the heat treatment 50 °C increased but at 60 °C decreased.

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### Table 5 Phthalate contents in raw meat and heat-treated meat at 50 °C (µg.g⁻¹)

| Parameter | Raw shoulder | Sous vide at 50 °C |
|-----------|--------------|-------------------|
|           | 4 hours      | 4+1 hours         | 8 hours  | 8+1 hours |
|           | x̄ SD        | x̄ SD             | x̄ SD    | x̄ SD     |
| DBP       | 1.85 0.33    | 2.52 0.67         | 1.91 0.13| 2.41 0.72 |
| DEHP      | 10.02 1.41   | 19.35 7.49        | 23.95 1.05| 7.48 1.04 |

### Table 6 Phthalate contents in raw meat and heat-treated meat at 60 °C (µg.g⁻¹)

| Parameter | Raw shoulder | Sous vide at 60 °C |
|-----------|--------------|-------------------|
|           | 4 hours      | 4+1 hours         | 8 hours  | 8+1 hours |
|           | x̄ SD        | x̄ SD             | x̄ SD    | x̄ SD     |
| DBP       | 0.85 0.33    | 1.08 0.19         | 1.84 0.24| 12.43 0.06|
| DEHP      | 10.02 1.41   | 12.81 6.24        | 2.02 0.25| 0.11 0.03 |

### Table 7 Phthalate contents in unused and used technological packaging during heat treatment of meat at 50 °C (µg.g⁻¹)

| Parameter | Unused packaging | Packaging after cooking at 50 °C |
|-----------|------------------|---------------------------------|
|           | 4 hours          | 4+1 hours                       | 8 hours  | 8+1 hours |
|           | x̄ SD            | x̄ SD                           | x̄ SD    | x̄ SD     |
| DBP (µg.g⁻¹) | 29.08 4.15      | 25.99 12.32                    | 22.68 14.01| 18.75 5.93 |
| DEHP (µg.g⁻¹) | 5.04 1.58      | 4.51 0.72                      | 4.16 1.15 | 3.30 0.34 |

### Table 8 Phthalate contents in unused and used technological packaging during heat treatment of meat at 60 °C (µg.g⁻¹)

| Parameter | Unused packaging | Packaging after cooking at 60 °C |
|-----------|------------------|---------------------------------|
|           | 4 hours          | 4+1 hours                       | 8 hours  | 8+1 hours |
|           | x̄ SD            | x̄ SD                           | x̄ SD    | x̄ SD     |
| DBP (µg.g⁻¹) | 29.08 4.15      | 16.49 4.36                     | 11.33 1.20| 4.86 2.37 |
| DEHP (µg.g⁻¹) | 5.04 1.58      | 4.02 2.02                      | 2.79 0.83 | 4.01 2.37 |

The content of DBP and DEHP in the packaging gradually decreased with the length of the heat treatment.

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