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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 4.021 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 to1.27 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-Carrascal 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 sarcoplasmic protein changes its consistency into a gel and the gel becomes more tender **(Kameník 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, PVDC), 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 **(Kameník 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}. **Fierens 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), 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. **(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 Jarošová, 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 the 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 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 Nicolet 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 cm^2 in size. In an Erlenmeyer flask, the samples were leached for 72 hours in a 1: 1 solution of n-hexane and dichloromethane. Subsequently, the individual samples were extracted 3 times with the solvents n-hexane and dichloromethane. The first extraction took place after 1 hour, the second after 30 minutes and the third after 60 minutes. The extracted solutions were combined and filtered. The filtered solution was evaporated at 40°C on a vacuum evaporator and dried under nitrogen. The extract was rinsed with hexane 3x and transferred to a vial.

If the extract was clear, the entire contents were transferred to a vial, dried under nitrogen and acetonitrile was added. The slightly turbid extract was centrifuged at 1000 rpm/4 °C for 10 minutes. The slightly turbid extract was centrifuged at 1000 rpm/4 °C for 10 minutes. The upper part was removed to the vial, dried to dryness and then the extract was centrifuged again, the upper part was removed to the same vial and dried to dryness with nitrogen. The upper part of the hexane was discarded, the extract in the vial was purified again (2 ml of 65% sulfuric acid and 1 ml of n-hexane). The extract was shaken for 10 minutes and centrifuged at the same time. The upper part of the hexane was removed into a small vial using a Hamilton syringe. The whole procedure was repeated twice, then the hexane phase was evaporated with nitrogen and acetonitrile was added. Samples after the addition of acetonitrile were analyzed by HPLC, where dibutyl phthalate and di (2-ethylhexyl) phthalate were determined **(Jarošová and Bogdanovičová, 2015**).

Determination of PAE (dibutyl phthalate- DBP and di (2-ethylhexyl) phthalate-DEHP) in meat according to **Jarošová et al. (1999).**

Prior to extraction, the samples were homogenized, a portion of the sample was collected in aluminum dishes, and the residue was stored in a freezer at -18 °C. The samples were then lyophilized for 38 hours under reduced pressure below 6 mbar. The lyophilized samples were transferred to Erlenmeyer flasks and extracted 3x with a 1:1 (organic solvents n-hexane and acetone). The first extraction took place after 60 minutes, the second and the third after another 30 minutes. The filtered extracts were evaporated on a rotary evaporator at 40°C and the last residual solvent was dried over nitrogen. The co-extracts were separated from the phthalates by gel permeation chromatography (GPC). An amount of extracted fat (0.05 g) was weighed into the prepared vial and a 1:1 (mobile phase solvent: dichloromethane: cyclohexane) was added. The mixture was vortexed and then 1 ml of sample was injected onto the GPC column with a gas-tight syringe at a flow rate of 1 ml.min^{-1}. The column was injected for approximately 30 minutes, the DBP and DEHP fractions were dissolved in acetonitrile and transferred to a heart flask. Subsequently, the acetonitrile was evaporated at 40 °C on an evaporator and dried under nitrogen. The flask was then washed 3 times with n-hexane to ensure qualitative transfer of the sample to the vials. N-hexane was evaporated from the vial with nitrogen to a volume of 1 ml and concentrated sulfuric acid was added in an amount of 1 ml. The next procedure was repeated as for the colored extracts on the packaging parts.

The content of phthalates with UV detection at 224 nm was determined by HPLC analysis on a Zorbax Eclipse XDB-C8 column (USA). The column wash was 100% acetonitrile and 0.8 ml.min^{-1} after each analysis. The concentration of DBP and DEHP in the samples was evaluated using AgilentChemstationfor LC software based on the calibration curve. DBP and DEHP were identified based on elution time (retention time) and specific spectrum. The sample injection was 10 μl. The detection limit in the fat matrix for phthalates (DBP, DEHP) was 0.1 mg.kg^{-1} in packaging and 0.2 mg.kg^{-1} in meat **(Jarošová and Bogdanovičová, 2015)**.

The obtained results were statistically processed. Mathematical-statistical analysis was performed using the statistical software program SAS (Statistical Analysis System) 9.3 using the application Enterprise Guide 4.2.

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.

Table 1 Basic physicochemical properties of the shoulder of the raw and after sous vide heat treatment at 50 °C (g.100g^{-1})

Parameter	Raw meat	Meat cooked at 50 °C			
		4 hours	4+1 hours	8 hours	8+1 hours
	x̄±SD	x̄±SD	x̄±SD	x̄±SD	x̄±SD
Fat	6.04±0,61^{b}	7.51±0.59^{a}	11.15±0.59^{a}	6.81±0.48^{a}	7.72±0.53^{a}
Proteins	20.87±0,52	19.93±0.31	19.56±0.67	19.79±0.44	19.92±0.09
Minerals	0.91±0,09	0.96±0.12	0.92±0.09	1.06±0.11	1.06±0.11
Water	72.18±0,72	71.60±0.82	68.40±3.12	72.34±0.58	71.30±0.47
pH	5.83±0,06^{b}	5.96±0.04^{a}	5.98±0.07^{a}	5.98±0.04^{a}	5.92±0.12^{a}

Table 2 Basic physicochemical properties of the shoulder of the raw and after sous vide heat treatment at 60 °C (g.100g^{-1})

Parameter	Raw meat	Meat cooked at 60 °C			
		4 hours	4+1 hours	8 hours	8+1 hours
	x̄±SD	x̄±SD	x̄±SD	x̄±SD	x̄±SD
Fat	6.04±0,61	6.24 ±2.74	6.52 ±3.14	6.76 ±1.37	6.66± 1.62
Proteins	20.87±0,52	20.87±0.62	20.58±0.99	21.68±0.79	21.09±0.86
Minerals	0.91±0,09	1.05±0.08	1.04±0.09	1.03±0.08	1.12±0.05
Water	72.18±0,72	71.87±2.17	71.86±2.08	70.53±2.82	71.13±2.79
pH	5.83±0,06^{b}	5,89±0.08^{ab}	6.05±0.06^{a}	6.03b±0.06^{a}	6.04±0.11^{a}

Table 1 presents the basic physicochemical properties of raw and heat-treated meat at 50 °C for different cooking times. Table 2 characterizes the basic composition of raw and heat-treated meat at 60 °C. The protein content in meat prepared at 50 °C before sous vide treatment was 20.87 after heat treatment for 4 hours 19.93 g.100g^{-1} and after 8 hours 19.79 g.100g^{-1}, at 60 °C was after 4 hours 20.87 and after 8 hours 21.68 g.100g^{-1}. 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 water content in the pork shoulder treated at 50 °C was 72.18 g.100g^{-1} in the raw meat and decreased during the heat treatment, after heat treatment for

8+1 hours was 71.30 g.100g^{-1}. After heat treatment at 60 °C for 8+1 h. 71.13 g.100g^{-1}.

Kim et al. (2008) state in agreement with our results, a slightly higher amount of protein content (19.81 g.100g^{-1}), a lower fat content (3.44 g.100g^{-1}) and a higher water content (74.34 g.100g^{-1}). **Dominguez-Hernandez et al. (2018)** report a higher protein content (19.98 g.100g$^{-1)}$ in meat heat treated sous vide at 50 °C, a lower fat content (3.44 g.100g^{-1}) and a higher water content (75.34 g.100g^{-1}) compared to our results. **Latorre et al. (2019)** report different results with the sous vide method, where a temperature of 55 °C was used for 6 hours. Found a protein content of 20.89 g.100g^{-1}, fat 3.07 g.100g^{-1}, minerals 0.98 g.100g^{-1} and water 75.48 g.100g^{-1}.

During sous vide heat treatment at 50 °C, the content of oleic FA increased from 46.62 to 55.35 at 4+1 hours and 51.65 g.100g^{-1} fat at 8+1 hours treatment (Table 3). Also, the α-linolenic FA content increased from 0.23 to 0.31 in the 4+1hour treatment and to 0.27 g.100g^{-1} fat in the 8+1hour treatment. The EPA content decreased from 0.07 to 0.04 in the 4+1hour treatment and 0.04 g.100g^{-1} fat in the 8+1hour treatment. The content of MUFA, PUFA and SFA was not changed by heat treatment.

Table 3 Fatty acid content in the shoulder during heat treatment 50 °C (g.100g^{-1} fat)

Parameter	Raw meat	Meat cooked at 50 °C			
		4 hours	4+1 hours	8 hours	8+1 hours
	x̄±SD	x̄±SD	x̄±SD	x̄±SD	x̄±SD
Fat	6.04±0,61^{b}	7.51±0.59^{a}	11.15±0.59^{a}	6.81±0.48^{a}	7.72±0.53^{a}
Lauric FA	0.094±0.02	0.05±0.01	0.04±0.02	0.08±0.01	0.07±0.01
Myristic FA	1.34±0.76	1.31±0.01	1.29±0.88	1.31±0.01	1.29±0.01
Palmitic FA	24.25±0.91	24.25±0.09	24.22±4.1	24.31±0.0	24.15±0.21
Stearic FA	10.85±0.12	10.87±0.06	10.91±0.14	11.09±0.11	10.87±0.11
Vaccenic FA	4.76±0.05^{a}	4.58±0.04^{bc}	4.56±0.09^{c}	4.51±0.04^{b}	4.56±0.03^{b}
Oleic FA	46.62±0.62	47.63±3.66	55.35±0.08	49.04±0.8	51.65±0.17
Linoleic FA	4.61±0.48	6.23±0.26	4.51±0.21	5.79±0.61	4.48±0.4
Conjugated linoleic FA	0.12±0.03	0.11±0.02	0.08±0.01	0.09±0.01	0.10±0.01
α-linolenic FA	0.23±0.29	0.32±0.21	0.31±0.22	0.28±0.2	0.27±0.08
Eicosenoic FA	0.69±0.07	0.68±0.12	0.87±0.01	0.48±0.04	0.61±0.05
Arachidonic FA	1.15±0.11	0.93±0.24	0.76±0.26	0.79±0.12	1.07±0.11
EPA	0.07±0.22	0.04±0.02	0.04±0.30	0.04±0.01	0.04±0.01
DPA	0.14±0.01	0.12±0.02	0.13±0.02	0.12±0.01	0.12±0.01
DHA	0.05±0.02	0.04±0.01	0.04±0.01	0.05±0.01	0.04±0.01
Omega-3 FA	0.61±0.75	0.77±0.01	0.83±0.03	0.73±0.02	0.72±0.02
Omega-6 FA	5.12±1.35	4.81±0.24	4.88±2.34	5.42±0.75	4.11±0.37
MUFA	56.39±1.06	57.27±0.86	57.43±2.17	56.85±0.43	56.27±0.71
PUFA	9.58±0.38	8.54±0.16	7.95±0.79	8.91±0.22	8.75±0.56
SFA	33.55±0.68	33.42±0.28	32.95±1.28	33.62±0.61	33.48±0.21

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+1hour treatment and to 0.07 g.100g^{-1} in the 8+1hour 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 eicosenoic FA decreased during the treatment of 4+1 hours from 0. 61 to 0.41 and in the 8+1hour 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+1hour treatment. Omega-6 FA content increased from 5.12 in raw meat to 6.85 g.100g^{-1} in the 4+1hour treatment and to 7.35 g.100g^{-1} in the 8+1hour 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.

Table 4 Fatty acid content in the shoulder during heat treatment 60 °C (g.100g^{-1} fat)

Parameter	Raw meat	Meat cooked at 60 °C			
		4 hours	4+1 hours	8 hours	8+1 hours
	x̄±SD	x̄±SD	x̄±SD	x̄±SD	x̄±SD
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±0.26	24.22±0.14	24.37±2.0^{a}	24.18±2.02
Stearic FA	10.84±0.12	10.97±0.17	10.97±0.0	11.08±0.08	10.97±0.01
Vaccenic FA	4.76±0.05^{a}	4.55±0.06^{b}	4.52±0.05^{b}	4.45±0.11^{b}	4.52±0.09^{b}
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±0.0	0.09±0.0^{a}	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
Eicosenoic FA	0.61±0.05	0.39±0.01	0.41±0.25	0.39±0.01	0.32±0.01
Arachidonic FA	1.15±0.13	0.88±0.14	0.81±0.21	0.91±0.14	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±1.15	55.78±1.32	56.59±1.67	54.62±1.81	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±0,9^{a}

Cho et al. (2005) analyzed the content of fatty acids in *m. longissimus dorsi* (LD), *m. triceps brachii* (TB) and *m. semimembranosus* (SM). Statistically significant differences were found in oleic acid, which was demonstrably higher in the triceps brachii than in LD and SM. **Wood et al. (2008)** also found out the highest amount of oleic acid, namely 32.8 g.100g^{-1} in *m. semimembranosus*, which is similar in composition to *m. triceps brachii*. The content of stearic acid (2.2 g.100g^{-1}) is higher than in our results, the content of palmitic acid, namely 23.2 g.100g^{-1}, is slightly lower than the average content in our results, where found out at 50°C it is content 24.22 g.100g^{-1}, at 60 °C found out 20.94 g.100g^{-1}.

The DBP (di-n-butyl phthalate) content in raw shoulder at 50 °C heat treatment was 1.85, and during sous vide heat treatment it increased to 1.91 μg.g^{-1} at 4+1 hour heat treatment and to 3.02 at 8+1 hour heat treatment (Table 5) . The DEHP (di-2-ethylhexyl phthalate) content in the raw shoulder was 10.02 μg.g^{-1} and during the heat treatment it increased to 23.95 in the treatment of 4+1 hours and to 13.51 μg.g^{-1} in the treatment of 8+1 hours. Initial values of DBP and DEHP were affected by packaging during transport.

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	x̄	SD
DBP	1.85	0.33	2.52	0.67	1.91	41.13	2.41	0.72	3.02	0.34
DEHP	10.02^{b}	1.41	19.35^{a}	7.49	23.95^{a}	1.05	7.48^{b}	1.04	13.51^{b}	9.97

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 1.06 during heat treatment for 8+1 hours (Table 6). The DEHP content decreased to 8.72 in the treatment of 4+1 hours and to 4.021 μg.g^{-1} in the treatment of 8+1 hours.

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.

Bogdanovičová (2015) reports the content of DBP 0.01 to 1.31 mg.kg^{-1} and DEHP 0.01 to 1.92 mg.kg^{-1} in non-heat-treated foods. In duck meat packed in consumer packaging, it found a DEHP content above 0.01 to 1.92 mg.kg^{-1}.

Jandlová et al. (2017) analyzed the content of phthalic acid esters in meat (*m. semimembranosus*) without heat treatment and after heat treatment at 53 °C, 18 hours and with subsequent heat treatment 70°C for 2 hours. The content of both DBP and DEHP in raw meat was higher compared to our results (DBP: 36.08 μg.g^{-1}, DEHP: 65.95 μg.g^{-1}). The content of DBP (53 °C, 18 hours) was 4.09 μg.g^{-1}. DEHP content (53 °C, 18 hours) was 5.14 μg.g^{-1}. The DBP content (70 °C, 2 hours) was 3.24 μg.g^{-1}. DEHP content (70 °C, 2 hours) was 4.25 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 foundthat 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.

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	x̄	SD
DBP	0.85	0.33	1,08	0,19	1,84	1,82	2,46	12,43	1,06	6,08
DEHP	10.02^{a}	1.41	12,81^{a}	6,24	8,72^{a}	2,54	7,08^{b}	1,11	4,02^{b}	0,45

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.

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	x̄	SD
DBP (μg.g^{-1})	29.08	4.15	25.99	12.32	22.68	14.01	18.75	5.93	15.09	16.33
DEHP (μg.g^{-1})	5.04	1.58	4.51	0.72	4.16	1.15	3.30	0.34	1.27	17.58

At the heat treatment at of 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.

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	x̄	SD
DBP (μg.g^{-1})	29.08^{a}	4.15	16.49^{b}	4.36	11.33^{b}	1.20	4.86^{b}	2.37	3.18^{c}	0.98
DEHP (μg.g^{-1})	5.04	1.58	4.02	2.02	2.79	0.83	4.01	2.37	2.54	0.59

Bogdanovičová and Jarošová (2015) analyzed the effect of heat treatment on the migration of phthalates from packaging to meat during heat treatment sous vide in technological packaging. The resulting concentration of DBP after heat treatment varied in the samples from 4.35 to 20.95 μg.dm^{-2} and DEHP ranged from 0.3 to 103.33 μg.dm^{-2}. The content of DBP and DEHP in the packaging after heat treatment is higher compared to our results. This study also confirms that the migration of phthalates is affected by the heat treatment of the meat sample.

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.

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

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