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OXIDATION AND PHTHALATE CONTENT IN RELATION TO FAT IN FRANKFURTERS

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ABSTRACT

The work analyzes the content of MDA, TVB-N, DBP and DEHP in frankfurters and primary and secondary packaging on the day of purchase and at the end of the expiration date. Frankfurters 1 (F1), fat content of 10.49 g.100g^{-1}, Frankfurters 2 (F2), fat content of 8.01 g.100g^{-1} and Frankfurters 3 (F3) with a fat content of 6.49 g.100g^{-1} are evaluated in the experiment. The MDA content at the beginning of the experiment was 0.07 mg.kg^{-1} in F1, 0.06 in F2 and 0.08 mg.kg^{-1} in F3. During storage for 10 days, the MDA content in all analysed groups of frankfurters increased nonsignificant to the value of 0.08 in F1, 0.07 in F2 and 0.09 mg.kg^{-1} in F3. The increase in TVB-N content was almost the same in all groups. In F1, TVB-N content in frankfurters increased by 1.95 mg.100g^{-1}, in F2 by 2.03 mg.100g^{-1} and in F3 by 2.05 mg.100g^{-1}. From the purchase to the end of the consumption period 10 days), the content of TVB-N in F1 increased by 18.2%, in F2 by 14.17% and in F3 by 14.31%. The content of DBP and DEHP corresponded to the fat content of the frankfurter, at the beginning of the experiment it was the highest in F1, DBP content in F1 was 12.71 µg.g^{-1}, lower in F2 6.62 µg.g^{-1} and lowest in F3 5.46 µg.g^{-1}. DEHP content was 10.15 µg.g^{-1} in F1, 8.14 µg.g^{-1}in F2 and 5.29 µg.g^{-1} in F3. DBP content in sausages increased by 12.35% in F1, by 24.17% in F2 and by 28.58% in F3 during the experiment. The DEHP content increased during the experiment by 12% in F1, by 8.85% in F2 and by 5.45% in F3. However, the increase in DEHP content was consistent with the fat content of the frankfurter, with the highest increase in F1 with the highest fat content and the lowest in F3 with the lowest fat content in secondary packaging, the content of DBP and DEHP decreased by 35-45% during storage at a temperature of 4°C until the expiration date.

Keywords: frankfurters, fat content, malondialdehyde, TVB-N, phthalates DBP, DEHP

INTRODUCTION

Phthalic acid esters are used to improve the properties of plastics such as flexibility, durability and elasticity, and are used in areas such as toys, cosmetics, household materials, medical equipment and food packaging. The release of phthalic acid esters into the environment can cause serious problems for both human and biological life. The greatest migration of phthalates into food occurs from packaging materials that come into the contact with food **(Alp, and Yerlikaya, 2020)**.
From a microbiological point of view, plastic packaging is a safe and convenient way to package food. Different types of plastics are used, each of them has unique properties and applications in the food industry, the most commonly used are polycarbonate, polyethylene, styrene, polypropylene, etc. Plastics for food packaging are made from various polymers and additives that serve to improve elasticity, color or strength. Components of plastic packaging and also additives can, during processing and storage due to inappropriate conditions, e.g. increased temperature or mechanical stress to excessively migrate into the food (**Fasano *et al.*, 2012; Xia *et al.*, 2023).**
The most important source of exposure to phthalates is food, phthalates are mainly in foods with a higher fat content - milk, butter, meat products or meat **(Wang *et al.*, 2015).** Phthalates enter the environment easily by migration or leaching (**Przybylińska and Wyszkwski, 2016**).
Food can be contaminated with phthalates either during primary production, as a result of contamination of water and air, or during processing, transport and handling in the commercial network. Phthalates can migrate into food from plastic packaging, but very often from colors on labels, or from adhesives during the processing of raw materials, due to the use of PVC in the production process, but also from sticking price tags in the business network **(Schecter *et al.*, 2013; Du *et al.*, 2016)**.
According to **Ceballos-Luna *et al.* (2022)** the most used phthalates in food packaging are diethyl phthalate (DEP), diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), di n-octyl phthalate (DOP), phthalic acid (PA), butyl benzyl phthalate (BBzP), dimethyl phthalate (DMP).
Plastic packaging has high heat resistance, which also allows meat products to be sterilized, they are almost impermeable to steam and gas. The following are used in meat production: polyamide (PA), polyester (PES), polyethylene terephthalate (PET), polyethylene (PE), polyvinylidene chloride (PVDC) and polypropylene (PP) packaging (**Budig, 2009**).
Plasticizers in plastic packaging have a low molecular weight and can migrate into packaged foods. Plasticizers, e.g. phthalates are commonly used in PVC and PE. Plasticizers for PE are most often dibutyl phthalate (DBP), dipentyl phthalate (DPP), diethyl phthalate (DEP) (**Bhunia *et al.*, 2013; Ventrice *et al.*, 2013**).
Dibutyl phthalate is not commonly found in nature and is added to hard plastics for softening in packaging that does not come into direct contact with fatty foods **(Nunez *et al.*, 2015).**
Di-(2-ethylhexyl) phthalate (DEHP) s used as plasticizers in medical devices and food packaging. In men, it disrupts the endocrine system as an androgen antagonist, which is related to a lower level of reproductive function in adolescent men. Major metabolite is Mono-(2-ethylhexyl) phthalate (MEHP) **(Luís *et al.*, 2021).**
Phthalates in food packaging are not bound by a chemical bond, they can easily be released from this packaging and transfer to food, especially if the food has a higher fat content **(Yang *et al.*, 2015).**
The migration of phthalates from packaging to food is different, it is influenced by the type of packaging material, the physical and chemical properties of phthalates, the composition of the food, the storage temperature and the length of contact between the packaging and the food. Non-inert materials such as plastics can migrate as chemical impurities both from the outside of the packaging and from the packaging material itself (**Haji Harunarashid *et al.*, 2017**; **Pacyga *et al.*, 2019**; **Pacyga *et al.*,2021).**
Jarošová and Bogdanovičová (2016) found a DBP content of 0.45 mg.kg^{-1} in frankfurters with a fat content of 10% 7 days after production, and in frankfurters with a high fat content of 50% it was up to 3.86 mg.kg^{-1}. Likewise, the content of DEHP was lower in frankfurters with a lower fat content of 10% (3.27 mg.kg^{-1}) compared to the content in frankfurters with a fat content of 50% (5.02 mg.kg^{-1}).
The specific migration limit is therefore the highest allowed amount of a specific migration substance from the material to the food. The specified limit should ensure that the material that is in direct contact with food will not pose a risk to the

health of the consumer. For the purposes of the food industry, a specific migration limit of 1.5 mg.kg^{-1} for di (2-ethylhexyl) phthalate (DEHP), 0.3 mg.kg^{-1} for dibutyl phthalate (DBP) and 30 mg.kg^{-1} for benzyl butyl phthalate **(Commission Regulation (EU) No. 10/2011).** DBP and DEHP as plasticizers are allowed to be used according to **Commission Regulation (EU) No. 2023/1442.**
Hot smoke smoking at a temperature of 80-90 °C can also be used for smoking frankfurter. Along with smoking, heat treatment also takes place **(Heinz, 2013; Fellows, 2022).** Phthalates most often enter the body orally and after entry are hydrolyzed to a more bioactive form (monoesters) using enzymes, mainly esterases and lipases present in the liver, intestine and salivary glands **(Benjamin *et al.*, 2015).**
According to **Martínez-Razo *et al.* (2021)** and **Milosevic, *et al.* (2020)** phthalic acid esters (phthalates) are obesogenic and contribute to obesity. Phthalates can significantly alter glucose and lipid metabolism, and consequently the risk of insulin resistance can increase.
According to **Luís *et al.* (2021**) Di-(2-ethylhexyl) phthalate in adolescent males causes a lower level of reproductive functions because it disrupts the endocrine system as an androgen antagonist. In men, the main metabolite is mono-(2-ethylhexyl) phthalate (MEHP).
The aim of the work is to analyze the quality of frankfurters with different fat content and the content of phthalates.

MATERIALS AND METHODS

Characteristics and composition of evaluated commodities.

Frankfurters were purchased in the retail network once a week for two months. Frankfurters with a fat content of 10.5% (Frankfurters 1), 8.0% (Frankfurters 2) and 6.5% (Frankfurters 3) were included in the experiment.
Frankfurters **1** (n=2x9), Chicken delicacy frankfurters, ingredients: mechanically separated chicken meat 58%, drinking water, chicken skins, potato starch, pork fat, salt, stabilizers: triphosphates, guar gum, flavor and aroma enhancer monosodium glutamate, antioxidant erythorbic acid, spice extracts, preservative sodium nitrite. Vacuum packed.
Frankfurters **2** (n=2x9), Chicken frankfurters, ingredients: chicken meat 80%, pork fat, water, salt, sodium nitrite preservative, spices, grape sugar, triphosphate stabilizer, maltodextrin, antioxidant erythorbic acid, spice extracts, garlic, edible collagen tube. Packed in a protective atmosphere.
Frankfurters **3** (n=2x9), frankfurters with turkey meat, ingredients: mechanically separated poultry meat 40%, turkey meat 27%, drinking water, chicken skin, potato starch, salt, spices, grape sugar, maltodextrin, antioxidant erythorbic acid, spice extracts, garlic, sodium nitrite preservative, triphosphate stabilizer, guar gum, edible collagen casing. Vacuum packed.
In the experiment were evaluated quality of frankfurters, and the content of phthalates determined in frankfurters and in their packaging. The content of phthalates in frankfurters was analyzed in relation to the fat content and the length of their storage. The content of fatty acids (FA), malondialdehyde (MDA) and total volatile basic nitrogen (TVB-N) were analyzed. The frankfurters were stored for 10 days at a temperature 4 °C. The content of malondialdehyde, TVB-N and phthalates was analyzed on the day of production and at the end of the shelf life.

Analysis of chemical composition and fatty acid content

Frankfurters samples were analyzed by FT-IR analysis (Nicolet 6700) of chemical and fatty acids **Čuboň *et al.* (2021).**

Determination of phthalates in packaging

Determination of dibutyl phthalate and di (2-ethylhexyl) phthalate in packaging materials according to **Gajdůšková *et al.* (1996).** A representative sample was taken from each sample into an Erlenmeyer flask. The samples were leached in a solvent mixture of n-hexane: dichloromethane 1:1 for 72 hours and subsequently extracted 3 times with the same solvents. The first extraction was after 60 minutes, the second and third after 30 minutes. The extracted solutions were mixed, filtrated and evaporated on a vacuum evaporator at a temperature of 40 °C and dried with nitrogen. The remaining extract was transferred to vials by rinsing with hexane (2 + 2 + 1 ml). The obtained extract was colored differently depending on the analyzed samples. The entire clear extract was transferred to a vial, dried to dryness and acetonitrile was added. If the obtained extract was slightly colored, it was centrifuged for 10 minutes at 1000 rpm/4°C. The upper part of the extract was collected in a vial and dried. The extract was centrifuged again, the upper part was taken into the same vial, dried, and then the vial was filled with acetonitrile to a volume of 1 ml. Extracts that were colored or cloudy were evaporated with nitrogen to 1 ml and purified with sulfuric acid. 1 ml of concentrated sulfuric acid was added to a vial with 1 ml of extract. The vial was shaken for 10 minutes, then centrifuged for 1 minute. (1000 rpm/4°C). We removed the top of the hexane with a Hamilton syringe into the waste. We added 2 ml of 65% sulfuric acid and 1 ml of n-hexane to the vial. It was then transferred to a shaker for 10 minutes and centrifuged for 10 minutes. The upper hexane phase was collected with a Hamilton syringe into a small vial. The procedure was repeated two more times. The collected hexane phase was evaporated with nitrogen and acetonitrile was added and the sample was prepared for HPLC analysis.

Determination of phthalates in frankfurters

Determination of phthalates in frankfurters Analysis of dibutyl phthalate and di (2-ethylhexyl) phthalate in frankfurters according to **Jarošová (2006).** Whole frankfurters were homogenized and a representative sample taken was stored at -18°C. The successively frozen samples were lyophilized for 38 hours. Lyophilized samples were transferred to Erlenmeyer flasks and extracted three times with a mixture of n-hexane and acetone in a ratio of 1:1. The combined filtered extracts were evaporated with a rotary evaporator at a temperature of 40 °C and dried with nitrogen. 0.05 g of extracted fat was weighed into the vials and supplemented with a mobile phase solution (dichloromethane: cyclohexane 1:1). The mixture was vortexed and a gas-tight syringe was used to inject 1 ml of the sample onto the column. A fraction containing DEHP and DBP was collected in the flask. The captured fraction containing phthalates was concentrated on a vacuum rotary evaporator at 40 °C and dried with nitrogen. By washing the flask three times (2+2+1 ml) with hexane, the transfer of the sample to the vials was ensured. Hexane was evaporated from the vial with nitrogen to a volume of 1 ml and 1 ml of concentrated sulfuric acid was added. The procedure was repeated as for colored extracts from packaging. Phthalates were determined using the HPLC method with UV detection at a wavelength of 224 nm, column Zorbax Eclipse XDB-C8. 100% acetonitrile was used as the mobile phase. Quantitative evaluation of the concentration of DEHP and DBP was performed on the basis of the calibration curve by AgilentChemstationfor LC software. Sample injection onto the column was in the amount of 10 µl.
The detection limit for phthalates in the fat matrix was 0.2 mg.kg^{-1} in frankfurters and 0.1 mg.kg^{-1} in packaging.

Determination of malondialdehyde

Content of malondialdehyde (MDA) was determined by spectrophotometric method. The secondary lipid oxidation in the meat and meat products is determined as the thiobarbiturnumber malondialdehyde (MDA) contents in mg.kg^{-1}. Sample was preparate according to **(Marcinčák *et al.*, 2004).** To a centrifuge tube (50 cm^3) was weighed 1.5 g of the frankfurters and 1 cm^3 of ethylenediaminetetraacetic acid (EDTA) and 5 cm^3 of 0.8% butylated hydroxytoluene (BHT) were added and gentle mixing. Nearly before homogenization (30 seconds at 10,000 rpm) was added 8 cm^3 of 5% trichloroacetic acid (TCA).
The Diax 900 (Heidolph, Germany - laboratory homogenizer) was used and then centrifuged for 5 min. (3500 x g, 4 °C) on a Universal 320 centrifuge (Hettich, Germany). The top hexane layer was removed after centrifugation, the sample was filtered (Watahatman 4 filter paper). The filtered sample was made up to 10 cm^3 of 5% TCA. Total 4 ml of samples were collected and 1 ml of TBA was added in a tube. Malondialdehyde stock solution was prepared by acid hydrolysis of 1,1,3,3-tetramethoxy propane (TMP). MDA standard was prepared from stock solution.
Both standards and samples were in a water bath tempered at 70 °C for 90 min. After cooling in an ice bath and tempering the samples for 45 min. at room temperature, was absorbance measured at 532 nm on a UV-spectrophotometer UVmini-1240 (Shimazu, Japan). The obtained data were recalculated and the concentration of MDA was expressed in mg.kg^{-1} of frankfurters.

Determination of total volatile bases nitrogenous

Determination of total volatile bases nitrogenous (TVB-N) was analysed by Commission Regulation (EC) No 2074/2005 (vapor deposition and subsequent titration). Volatile nitrogenous bases were extracted from the homogenized frankfurters by a solution of 0.6 mol.L^{-1} of perchloric acid., The extract was distilled after alkalization with water vapour. The volatile alkaline components were absorbed by the acid absorbent collection tank. The concentration of TVB-N was analysed by titration of absorbed basic substances using a Tashiro indicator. To calculate the concentration of TVB-N, the titration of the solution in the collection vessel for hydrochloric acid was used **(Cviková, 2019**).

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

RESULTS AND DISCUSSION

For the purposes of the work, three market types of frankfurters from the commercial network with different fat content were selected. Frankfurters were purchased on the day of delivery to the retail network directly from the manufacturer. The frankfurters were labeled as Frankfurters 1 with a fat content of 10.49 g.100g^{-1}, Frankfurters 2 with a fat content of 8.01 g.100g^{-1} and Frankfurters 3 with a fat content of 6.49 g.100g^{-1}. The differences between individual groups were statistically significant at the 0.05 significance level (Table 1). Statistically, the lowest protein content was in frankfurters with the highest fat content 17.97

g.100g^{-1}, but in Frankfurters 3 the protein content was similar as the sausage group 2, because in Frankfurters 3 was the highest water content 70.48 g.100g^{-1}.
Opposite our results **Vilar *et al.* (2020)** found out higher contents of fat 11.92 similar content of protein 18.61 and lower water content in frankfurters made from pork meat purchased in local market in Ireland. **Tobin *et al.* (2012**) analyzed the effect of salt and fat content on the sensory properties of frankfurters.
Frankfurters with fat contents from 10% to 15% with salt contents from 2.5 to 3% were significantly the most acceptable preferred by consumers.

Kang *et al.* (2020) analyzed the influence of fat content on the sensory quality of frankfurters.
The protein content was from 16.69 to 17.06% and the fat content from 3.26 to 18.32%, frankfurters with fat content 3,26% were the best sensorially evaluated (5.23 points) and it increased with increasing fat content. Frankfurters with a fat content of 18.32%, the best sensory evaluation was 7.08 points.

Table 1 Basic chemical composition of frankfurters (g.100g^{-1})

Parameter	Frankfurters **1**			Frankfurters **2**			Frankfurters **3**		
	x	s	v %	x	s	v %	x	s	v %
Proteins	17,97 ^{b}	0,31	1,82	20,61^{a}	0,45	2,31	20,52 ^{a}	0,33	1,53
Fat	10,49 ^{a}	0,48	4,57	8,01 ^{b}	0,54	7,19	6,49 ^{c}	0,49	7,55
Water	67,03 ^{b}	0,41	0,61	68,84 ^{b}	0,65	1,01	70,48 ^{a}	0,52	0,74
Minerals	0,87	0,05	6,66	0,86	0,06	6,66	0,88	0,06	11,11
NaCl	1,66 ^{a}	0,13	6,92	1,68 ^{a}	0,06	3,46	1,63 ^{a}	0,08	3,54

Delgado-Pando *et al.* (2014) compared to our results in frankfurters found a higher proportion of PUFA 10.6, a lower proportion of MUFA 49.5 and a higher proportion of SFA 39.4%, approximately the same content of palmitic acid 23.4% and a higher proportion of stearic acid 13.8%.

Table 2 Fatty acid content (g.100g^{-1}) of fat

Parameter	Frankfurters **1**			Frankfurters **2**			Frankfurters **3**		
	x	s	v %	x	s	v %	x	s	v %
Fat (g.100g^{-1})	10,49 ^{a}	0,48	4,57	8,01 ^{b}	0,54	7,19	6,49 ^{c}	0,49	7,55
Lauric acid	0,05 ^{a}	0,01	3,01	0,05 ^{a}	0,01	8,08	0,06 ^{a}	0,01	7,31
Myristic acid	1,29 ^{a}	0,01	1,05	1,28 ^{a}	0,01	0,70	1,31 ^{a}	0,02	1,14
Palmitic acid	24,21 ^{a}	0,15	0,64	24,08 ^{a}	0,11	0,41	24,23 ^{a}	0,05	0,26
Heptadecanoic acid	0,23 ^{a}	0,02	6,62	0,26 ^{a}	0,02	7,49	0,26 ^{a}	0,02	7,77
Stearic acid	11,05 ^{a}	0,15	1,41	11,14 ^{a}	0,15	1,41	11,05 ^{a}	0,08	0,68
Vaccenic acid	4,51 ^{b}	0,02	0,65	4,52 ^{b}	0,07	1,61	4,61 ^{a}	0,02	0,83
Oleic acid	55,06 ^{a}	0,06	0,12	50,92 ^{b}	1,73	3,34	50,07 ^{b}	0,66	1,39
Linoleic acid	3,81 ^{b}	0,65	16,85	5,52 ^{a}	0,12	2,26	5,70 ^{a}	0,25	4,55
Conjugated linoleic acid	0,11 ^{a}	0,01	4,51	0,12 ^{a}	0,01	6,45	0,12 ^{a}	0,01	8,31
α-linolenic acid	0,21 ^{a}	0,01	6,02	0,20 ^{a}	0,02	7,40	0,20 ^{a}	0,01	4,11
Eicosenoic acid	0,76 ^{a}	0,07	8,42	0,82 ^{a}	0,11	13,15	0,68 ^{a}	0,13	18,02
Arachidonic acid	0,95 ^{a}	0,16	15,05	1,27 ^{a}	0,19	13,69	1,16 ^{a}	0,19	15,65
Eicosapentaenoic acid	0,07 ^{a}	0,01	3,09	0,08 ^{a}	0,01	3,82	0,08 ^{a}	0,01	6,51
Docosapentanoic acid	0,13 ^{a}	0,01	4,76	0,13 ^{a}	0,01	5,36	0,13 ^{a}	0,01	3,16
Docosahexaenoic acid	0,04 ^{a}	0,02	5,39	0,04 ^{a}	0,01	5,86	0,04 ^{a}	0,01	6,85
Omega 3 fatty acids	0,69 ^{a}	0,03	3,72	0,63 ^{a}	0,09	13,07	0,62 ^{a}	0,02	3,31
Omega 6 fatty acids	3,86 ^{c}	0,42	11,17	6,37 ^{b}	0,31	5,02	7,12 ^{a}	0,45	6,81
SFA	30,47 ^{b}	0,42	1,42	32,83 ^{a}	1,03	3,12	33,41 ^{a}	0,53	1,58
MUFA	59,62 ^{a}	0,78	1,28	57,74 ^{b}	0,44	0,77	57,12 ^{b}	1,11	1,94
PUFA	5,11 ^{b}	0,55	10,95	7,55 ^{a}	0,56	7,66	8,06 ^{a}	0,54	6,57
Cholesterol	1,29 ^{a}	0,03	2,35	1,02 ^{b}	0,04	4,35	0,93 ^{b}	0,09	9,55

The content of fatty acids is shown in Table 2. We found a significant difference in the content of oleic acid (F1 55.06, F2 50.92 and F3 50.07 11g.100g^{-1} of fat). The content of linoleic acid was the lowest in F1 3.81, and the highest in F3 5.70 11g.100g^{-1} of fat. The content of omega 6 fatty acids was significantly lowest in F1 3.86, in F2 it was 6.37 and the highest in F3 7.12 11g.100g^{-1} of fat. Significantly lowest SFA content was in F1 (30.47) and the highest in F3 (33.4111g.100g^{-1} of fat). The highest MUFA content was in F1 (59.62) and the lowest in F3 (57.1211g.100g^{-1} of fat). Statistically, the lowest PUFA content was in F1 (5.11) and the highest in F3 (8.0611g.100g^{-1} of fat).
Estévez *et al.* (2007) found out higher content of lauric acid (0,11g.100g^{-1} of fat), bat lower content of palmitic acid 20,1, stearic acid 9,02 and arachidonic acid 0,23 g.100g^{-1} of fat, they also found a higher proportion of PUFA 11.1 g.100g^{-1} of fat compared to our results.
Compared to our results **Kılıç, and Özer (2019)** found a higher content of lauric acid (1.85), myristic acid (3.86), stearic acid 28.787 and SFA (57.82) and, on the contrary, less MUFA (38.61 g.100g^{-1} of fat), but frankfurters were made from beef.

Protein degradation on the day of purchase evaluated as TVB-N was the lowest in F1 10.71 mg.100g^{-1} and the highest in F3 14.32 (Tab. 3). In all sausages, we found a statistically significant increase in TVB-N at the end of the consumption period. In F1, the TVB-N content increased to 12.66 mg.100g^{-1}, in F2 to 15.39 and in F3 to 16.37 mg.100g^{-1}. The increase in TVB-N content was almost the same in all groups. In F1, TVB-N content increased by 1.95 mg.100g^{-1}, in F2 by 2.03 mg.100g^{-1} and in F3 by 2.05 mg.100g^{-1}. From the purchase to the expiration date 10 days), the content of TVB-N in F1 increased by 18.2%, in F2 by 14.17% and in F3 by 14.31%.
In agreement with our results, **İncili *et al.* (2022)** found a statistically significant increase in TVB-N content in frankfurters within 14 days of production. **Elewa *et al.* (2016)** found, in agreement with our results, 9.74 mg.100g^{-1} at the beginning of the experiment, but after 14 days they found a more significant increase in TVB-N up to 21.84 mg.100g^{-1}.

Table 3 Content of malondialdehyde (mg.kg^{-1}) and TVB-N (mg.100g^{-1}) in Frankfurters

Parameter		Frankfurters after purchase			Frankfurters at the expiration date		
		x	s	v %	x	s	v %
Frankfurters 1 **(F1)**	**Proteins** (g.100g^{-1})	17,97 ^{a}	0,31	1,82	18,08 ^{a}	0,18	0,79
	TVB-N (mg.100g^{-1})	10,71 ^{a}	0,25	0,49	12,66 ^{a}	0,62	5,24
	Fat (g.100g^{-1})	10,49 ^{a}	0,48	4,57	10,29 ^{a}	0,35	3,53
	MDA (mg.kg^{-1})	0,07 ^{a}	0,01	3,51	0,08 ^{a}	0,01	1,81
Frankfurters 2 **(F2)**	**Proteins** (g.100g^{-1})	20,61 ^{a}	0,45	2,31	20,71 ^{a}	0,25	1,16
	TVB-N (mg.100g^{-1})	13,36 ^{b}	0,35	2,55	15,39 ^{a}	0,63	4,08
	Fat (g.100g^{-1})	8,01 ^{a}	0,54	7,19	8,18 ^{a}	0,24	2,65
	MDA (mg.kg^{-1})	0,06 ^{a}	0,01	0,46	0,07 ^{a}	0,01	2,93
Frankfurters 3 **(F3)**	**Proteins** (g.100g^{-1})	20,52 ^{a}	0,33	1,53	20,48 ^{a}	0,11	0,55
	TVB-N (mg.100g^{-1})	14,32 ^{b}	0,52	3,83	16,37 ^{a}	0,45	2,67
	Fat (g.100g^{-1})	6,49 ^{a}	0,49	7,55	6,41 ^{a}	0,37	5,77
	MDA (mg.kg^{-1})	0,08 ^{a}	0,01	6,76	0,09 ^{a}	0,01	6,32

The MDA content in frankfurters at the beginning of the experiment was 0.07 mg.kg^{-1} in F1, 0.06 in F2 and 0.08 mg.kg^{-1} in F3. During storage for 10 days, the MDA content in all treated groups increased nonsignificant to the value of 0.08 in F1, 0.07 in F2 and 0.09 mg.kg^{-1} in F3 (Tab. 3).

Estévez *et al.* (2007) found out gradually increase MDA content in frankfurters for 60 days experiment, frankfurters were storage at 4 °C. Significant increasing of MDA content found out between day 0 and day 60 from 0.37 to 0.94 mg.kg^{-1}.

Vilar, *et al.* (2020) found out MDA content 0,459 mg.kg^{-1} at the 63 days of experiment.

So *et al.* (2020) found TBARS values ranged from 0.18 to 0.20 MDA (mg.kg^{-1}) in beef frankfurters.

Table 4 Content of phthalates in Frankfurters (µg.g^{-1}) of the original mass

Parameter		Frankfurters after purchase			Frankfurters at the expiration date		
		x	s	v %	x	s	v %
Frankfurter 1 (F1)	**DBP** (µg.g^{-1})	12,71 ^{b}	0,52	4,11	14,28 ^{a}	0,54	3,87
	DEHP (µg.g^{-1})	10,15 ^{b}	0,87	8,48	12,18 ^{a}	1,96	16,28
	Fat (g.100g^{-1})	10,49 ^{a}	0,48	4,57	10,29 ^{a}	0,35	3,53
Frankfurter 2 (F2)	**DBP** (µg.g^{-1})	6,62 ^{b}	1,89	28,50	8,22 ^{a}	0,61	7,42
	DEHP (µg.g^{-1})	8,14 ^{b}	2,42	30,31	8,86 ^{a}	1,95	22,31
	Fat (g.100g^{-1})	8,01 ^{a}	0,54	7,19	8,18 ^{a}	0,24	2,65
Frankfurter 3 (F3)	**DBP** (µg.g^{-1})	5,46 ^{b}	0,81	15,21	7,02 ^{a}	0,60	8,67
	DEHP (µg.g^{-1})	5,29 ^{a}	0,44	8,91	5,58 ^{a}	0,41	8,03
	Fat (g.100g^{-1})	6,49 ^{a}	0,49	7,55	6,41 ^{a}	0,37	5,77

The content of dibutyl phthalate (DBP)) at the beginning of the experiment was 12.71 µg.g^{-1} in F1, 6.62 µg.g^{-1} in F2 and 5.46 µg.g^{-1}in F3 (Table 4). During the storage period, the DBP content increased in F1 to 14.28 µg.g^{-1} (by 12.35%), in F2 to 8.22 µg.g^{-1} (by 27.17%), and in F3 to 7.02 µg.g^{-1} (by 28.57%).

The content of diethylhexyl phthalate (DEHP) at the beginning of the experiment was 10.15 µg.g^{-1} in F1, 8.14 µg.g^{-1} in F2 and 5.29 µg.g^{-1} in F3. During storage (10 days), the DEHP content increased in F1 to 12.18 µg.g^{-1}, in F2 to 8.86 µg.g^{-1} and in F3 to 5.58 µg.g^{-1}. In relative terms, the DEHP content in F1 increased by 16.66%, in F2 by 8.84% and in F3 by 5.48%.

The content of DBP and DEHP corresponded to the fat content of the frankfurter, at the beginning of the experiment it was the highest in F1, where the fat content was the highest 10.49 g.100g^{-1}, the lower content was in F2, where the fat content was 8.01 g.100g^{-1} and the lowest was in F3 with a fat content of 6 ,49 g.100g^{-1}. During storage for 10 days, however, the increase in DBP content was opposite to the fat content of the sausages, the DBP content increased the most in F3 frankfurter with the lowest fat content. However, the increase in DEHP content was consistent with the fat content of the frankfurter, with the highest increase in F1 with the highest fat content and the lowest in F3 with the lowest fat content.

Migration of DBP from packaging to product was according to **Ceballos-Luna *et al.* (2022**) 2.05 mg kg^{-1}. In raw unpackaged meat, **Schecter *et al.* (2013)** found the DBP content in poultry 0.7 and beef 0.7 ng.g^{-1}. They found a DEHP content of 1.9 in unpackaged beef and 50 in packaged beef. The aforementioned authors found a DEHP content of 18.6 ng.g^{-1} in packaged chicken meat.

Table 5 Content of phthalates in packages of Frankfurters 1 primary packaging cellophane casing

Parameter	Frankfurters 1 after purchase			Frankfurters 1 at the expiration date		
	x	s	v %	x	s	v %
DBP (µg.dm^{-2}) cellophane casing	7,01 ^{a}	0,83	12,01	2,31 ^{b}	1,34	57,86
DEHP (µg.dm^{-2}) cellophane casing	6,72 ^{a}	3,36	50,25	1,67 ^{b}	0,42	26,02
DBP (µg.dm^{-2}) upper packaging	29,33 ^{a}	4,01	13,64	17,62 ^{b}	5,01	28,44
DEHP (µg.dm^{-2}) upper packaging	4,34 ^{a}	2,05	47,40	2,35 ^{b}	0,45	19,85
DBP (µg.dm^{-2}) upper packaging with label	50,65 ^{a}	1,59	3,57	24,26 ^{b}	2,29	9,57
DEHP (µg.dm^{-2}) upper packaging with label	26,55 ^{a}	9,22	34,76	19,89 ^{a}	7,81	39,39

The DBP content of the cellophane casing, after purchase the DBP concentration was 7.01 µg.dm^{-2} and at the expiration date it decreased to 2.31 µg.dm^{-2} in Frankfurters 1 (Table 5). The DEHP content in the cellophane casing after purchase was 6.72 µg.dm^{-2} and at the expiration date it decreased to 1.67 µg.dm^{-2}. The consumer package consisted of 2 identical foils (vacuum packaging) made of the same material, but on one layer there was a label with product information. For that were analyzed the mentioned packages separately, the top packaging (without label) there was

DBP content of 29.33 µg.dm^{-2} after purchase and 17.62 µg.dm^{-2} at the expiration date of the consumption period.

The packaging with the label (top packaging) contained a significantly higher DBP at the beginning 50.65 µg.dm^{-2} and at the expiration date it decreased to 24.26 µg.dm^{-2}. The top packaging without a label contained of 4.34 µg.dm^{-2} DEHP after purchase and 2.35 µg.dm^{-2} at the expiration date. The DEHP content in the packaging with the label (top packaging) after purchase was 26.55 µg.dm^{-2} and decreased to 19.89 µg.dm^{-2} during storage (expiration date). Based on our results, we can conclude that the content of monitored phthalates was significantly higher in labeled packages (top packaging). The content of phthalates decreased during storage in the packaging. Phthalates in packaging materials are not chemically bound and can easily be released from these materials. They migrate phthalates from packaging materials to food, especially if the food has a higher fat content **(Yang *et al.*, 2015).**

The migration of phthalates, from packaging to food varies depending on the type of packaging material, the physical and chemical properties of the migrating

substance, the food content, the storage temperature and the length of contact between the packaging and the food. For non-inert materials such as plastics, elastomers, chemicals can migrate from the outside of the packaging or from the packaging material **(Haji Harunarashid *et al.*, 2017).**

Table 6 Content of phthalates in packages of Frankfurters 2 **primary packaging collagen casing**

Parameter	Frankfurters 2 after purchase			Frankfurters 2 at the end of the expiration date		
	x	s	v %	x	s	v %
DBP (μg.dm^{-2}) upper packaging	21,01 ^{a}	12,36	58,78	6,05 ^{b}	2,76	45,87
DEHP (μg.dm^{-2}) upper packaging	4,55 ^{a}	1,58	34,62	2,85 ^{b}	1,75	61,35
DBP (μg.dm^{-2}) upper packaging with label	42,45 ^{a}	13,70	32,18	32,37 ^{b}	10,91	33,66
DEHP (μg.dm^{-2}) upper packaging with label	37,08 ^{a}	5,61	15,21	26,84 ^{b}	10,31	38,62
DBP (μg.dm^{-2}) lower hard packaging	7,51 ^{a}	0,67	8,77	4,15 ^{b}	3,14	75,24
DEHP (μg.dm^{-2}) lower hard packaging	7,81 ^{a}	4,74	60,80	2,96 ^{b}	0,52	18,78
DBP (μg.dm^{-2}) lower hard packaging with label	50,59 ^{a}	22,71	45,06	36,54 ^{b}	15,05	40,02
DEHP (μg.dm^{-2}) lower hard packaging with label	88,85 ^{a}	15,15	17,18	47,23 ^{b}	4,01	8,07

The contents of phthalates in the packaging of Frankfurters 2 are in Table 6. The Frankfurters 2 were in collagen (consumable casing) and directly packed in secondary packaging. Secondary packaging consisted of a lower harder packaging (tray) and an upper foil (in a protective atmosphere). There was a label on both layers. On the top foil was the name of the product and the manufacturer, and on the bottom part of the packaging (tray) was a label with data on the composition of the product and a barcode. The DBP content of the top film without a label after purchase was 21.01 μg.dm^{-2} and decreased to 6.05 μg.dm^{-2} at the expiration date. The DEHP content in the top film without a label was 4.55 μg.dm^{-2} after purchase and decreased to 2.85 μg.dm^{-2} at the expiration date. The DBP content of the top foil with the label after purchase was 42.45 μg.dm^{-2} and at the expiration date decreased to 32.37 μg.dm^{-2}. The DEHP content in the top foil with the label after purchase was 37.08 μg.dm^{-2} and at the expiration date it was reduced to 26.84 μg.dm^{-2}. The bottom package without a label had a DBP content of 7.51 μg.dm^{-2} after purchase and 4.15 μg.dm^{-2} at the expiration date. The lower packaging without a label had a DEHP content of 7.81 μg.dm^{-2} after purchase and 2.96 μg.dm^{-2} at the expiration date. The bottom package with the label had a DBP content of 50.59 μg.dm^{-2} after purchase and 36.54 μg.dm^{-2} at the expiration date. The bottom package with the label had a DEHP content of 88.85 μg.dm^{-2} after purchase and 47.23 μg.dm^{-2} at the expiration date. Based on the analysis, we can conclude that the content of monitored phthalates was significantly higher in labeled packages. The phthalate content decreased during storage.

In general, we can state that the higher content of phthalates is in thicker secondary packaging, which are marked and have a higher proportion of dark color.

Harmon and Otter, (2022) report the migration of phthalates in general from packaging to sausages 1.7 mg.dm^{-2}.

Ceballos-Luna *et al.* (2022) found a DEP content of 32 μg.g^{-1} in frankfurters packaging.

According to **Luís *et al.* (2021),** phthalates are used as plasticizers for hard plastics that do not come into the contact with fatty foods, but in the case of the products we monitored, they were used to package frankfurters in collagen casings, where the collagen casings do not represent a barrier for the penetration of phthalates into the frankfurters, which contains fat.

Table 7 Content of phthalates in packages of Frankfurters 3 primary packaging collagen casing

Parameter	Frankfurters 3 after purchase			Frankfurters 3 at the end of the expiration date		
	x	s	v %	x	s	v %
DBP (μg.dm^{-2}) upper packaging	9,31 ^{a}	5,41	58,27	4,51 ^{b}	4,03	85,12
DEHP (μg.dm^{-2}) upper packaging	3,61 ^{a}	1,30	38,06	2,12 ^{b}	0,60	30,32
DBP (μg.dm^{-2}) upper packaging with label	43,85 ^{a}	12,10	26,97	36,83 ^{a}	12,36	34,14
DEHP (μg.dm^{-2}) upper packaging with label	45,09 ^{a}	4,34	9,52	28,11 ^{b}	0,65	2,22

Table 7 show the content of phthalates in the packaging of Frankfurters 3. The primary package (gut) was made of collagen (consumable). The secondary package consisted of 2 identical foils (vacuum packaging), where the top side contained information about the product and the manufacturer, and the bottom side was unmarked. With this type of packaging of frankfurters in collagen casings, it is not required to separate the casing from the product, for that reason we did not analyze the content of phthalates in the collagen casing. The DBP content of the unlabeled secondary packaging was 9.31 μg.dm^{-2} after purchase and decreased to 4.51 μg.dm^{-2} at the expiration date. The DEHP content was 3.61 μg.dm^{-2} after purchase and decreased to 2.12 μg.dm^{-2} during storage. The DBP content of the labeled package was 43.85 μg.dm^{-2} after purchase and decreased to 36.83 μg.dm^{-2} at the expiration date. DEHP content was 45.09 μg.dm^{-2} after purchase and decreased to 28.11 μg.dm^{-2} at the expiration date. When analyzing all packaging for individual types of products, we can state that the content of both DBP and DEHP was significantly higher after purchase than at the end of consumption. It is likely that phthalates as volatile substances were released during storage and their concentration in the products increased. From our analysis, we can conclude that the packaging with a paper-glued label had a many times higher content of monitored phthalates than the packaging without a label. Consistent with our results, **Xue *et al.* (2010)** found a higher content of phthalates in parts of the package with a label. For this reason, the printing inks used in food labeling were also tested and it was confirmed that the inks are also a source of phthalates**. Van Holderbeke *et al.* (2014)** found content of DEHP in the packages ranged from 1.1 to 482 ng.cm^{-2}. The content of DEHP in the mentioned study corresponds to our results of its content in packages without a label.

Also, **Fang *et al.* (2017)** monitored the migration of DEHP and DBP from polypropylene packaging to food simulants depending on the heating time. The DEHP concentration in the heated samples ranged from 33.3 μg.l^{-1} to 159.8 μg.l^{-1}. DBP was present in the tested samples from 10.2 μg.l^{-1} to 104.9 μg.l^{-1}. The concentration of DEHP and DBP increased with increasing heating time (1 and 5 minutes). **Bogdanovičová (2016)** reports the concentration of DBP in packaging ranging from undetectable values to 89.3 μg.dm^{-2} and the concentration of DEHP from undetectable values up to 188.0 μg.dm^{-2}. **Jarošová and Bogdanovičová (2015)** analyzed the effect of DBP and DEHP content in meat products packed in unlabeled packaging heated to a temperature of 70 °C for 10 minutes. They found a DBP content of 11.38 μg.dm^{-2} and a DEHP content of 33.21 μg.dm^{-2} in the packaging. The content of DBP in meat products after heat treatment was 0.242 μg.g^{-1} and the content of DEHP 1.59 μg.g^{-1}.

CONCLUSION

The work analyzes the content of phthalates and MDA in sausages and packaging during storage. The frankfurters were labeled as Frankfurters 1 with a fat content of 10.49 g.100g^{-1}, Frankfurters 2 with a fat content of 8.01 g.100g^{-1} and Frankfurters 3 with a fat content of 6.49 g.100g^{-1}. Based on our results, we can conclude that the content of DBP and DEHP in the frankfurters at the expiration date was about 50 % lower than after purchase. Based on our results, we can conclude that the content of monitored phthalates was significantly higher in labeled packages (top packaging). The content of phthalates decreased during storage in the packaging. From our analysis, we can conclude that the packaging with a paper-glued label had a many times higher content of monitored phthalates than the packaging without a label.

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