

Effects of freezing temperatures and storage times on the quality and safety of raw turkey meat and sausage products

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ABSTRACT In this study, the effect of frozen storage of turkey meat on the processing properties into raw sausages was investigated. For this purpose, meat from the *Musculus pectoralis* of male turkeys was frozen in 3 independent runs for 12 and 24 wk at -18°C and -80°C . After thawing, the meat was examined physicochemically and microbiologically and processed into raw sausages. The sausages were

examined on d 0, 7, 14, 21, and 28 of storage. The parameters L^* , a^* , b^* , pH-value and a_w -value did not show any relevant significances between the experimental groups. The analysis of TBARS of the sausages made from frozen meat showed significantly higher values on d 14 and 28 compared to the unfrozen control group. Frozen storage also reduced the growth of *Pseudomonas* spp. and *Enterobacteriaceae*.

Key words: turkey meat, meat quality, freezing duration, freezing temperature, raw fermented sausage

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INTRODUCTION

In contrast to the meat production of other slaughter animal species, the production of poultry meat in Germany in 2019 increased by 4.5% compared to the previous year. Turkey meat currently accounts for around 30% of total poultry meat production (BMEL, 2020).

In Germany the volume of foreign trade of meat is clearly higher than that of living animals and this is advantageous, as especially long-distance transports of living animals which are very problematic considering animal welfare could be prevented (BLE, 2020). Besides the Netherlands and Poland, Denmark is the most important importer of poultry meat (BLE, 2020). 10.3% of the in Germany produced poultry meat is marketed outside the EU and 12.2% is imported from third countries (BLE, 2020). Furthermore, the global demand for meat products is increasing and this worldwide trade requires a long shelf life while maintaining the quality of these products (Leygonie et al., 2012a). However, this

implicates that due to hygienic aspects meat can not only be transported unfrozen, but also frozen. Although freezing is a frequently used preservation method of meat, it has been shown that freezing temperature and duration have an influence on physicochemical and microbiological parameters of the meat (Hansen et al., 2004; Vieira et al., 2009; Leygonie et al., 2012a; Kim et al., 2015). While freezing reduces the growth of *Enterobacteriaceae* (Bover-Cid et al., 2006; Medic et al., 2018) and *Pseudomonas* spp. as well as the total plate count of bacteria (Medic et al., 2018), it cannot be prevented that the microorganisms grow again after thawing (Vieira et al., 2009; Dave and Ghaly, 2011).

In recent years, several studies already showed the effect of freezing on physicochemical properties of meat. For example, Utrera et al. (2014a) found increased TBARS concentrations and cooking losses as well as color changes, when raw beef patties were stored for 20 wk at -80°C and -18°C compared to unfrozen patties. Similar changes were also presented by Custodio et al. (2018) for pork and by Lee et al. (2008) and Giampietro-Ganeco et al. (2017) for broiler meat. Hitherto, as far as we know, only few studies have been published that investigated the influence of meat freezing on the processing properties of turkey meat (Palmer et al., 1975; Smith, 1987). Therefore, the aim of this study was to investigate, whether different frozen storage temperatures and frozen storage durations influence the meat quality of turkey meat and its processing characteristics after thawing.

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MATERIALS AND METHODS

Material

For the study in three independent repeats both large breast muscles (*Musculi pectorales* [MP]) of each 6 male turkeys from the same flock of a fast-growing fattening turkey genetic (Aviagen Turkeys Ltd., United Kingdom) were used. The animals with an average age of 22 wk were slaughtered in a conventional slaughterhouse and the carcasses were cooled down to 1°C to 3°C, before they were transported in a refrigerated truck to the Institute for Food Quality and Food Safety on the slaughter day. During husbandry, transport and slaughter all European and German animal welfare regulations were considered. On the following day (24 h postmortem 24 h p.m.), at first an experienced person cut the carcasses into legs, wings, and breast muscles (MP and *M. supracoracoideus*). The carcass, legs, and MP (after removal of the *M. supracoracoideus*) were weighted and the MP were used for further analysis. For turkey carcass characterization, slaughter weight, breast and leg weight and the ratios of the legs and MP were measured before further disassembly. Ratios are calculated from the leg and MP weights and the slaughter weights.

For basic characterization of the unfrozen meat at 24 h p.m. 2 cm thick cutlets (N = 3) per repeat were cut of the MP for physicochemical and microbiological analyses. Furthermore, three 3-cm thick cutlets per repeat (N = 3) were removed from the same MP to determine drip loss, cooking loss and shear force. For analysis of these parameters after freezing and thawing, 2-cm and 3-cm thick cutlets were prepared for each treatment group. These different thicknesses were used for the following reasons: since sample preparation for shear force measurement from 2-cm thin meat pieces would not always be possible because of the direction of the muscle fibers, thicker cutlets were used for this purpose. The remaining samples were thinner in order not to consume an unnecessarily large amount of meat. All samples were frozen at -80°C (Gesellschaft für Labortechnik mbH, Type 6385, Burgwedel, Germany) and then the half of them stored at -80°C in the same freezer and the rest was transferred to a -18°C freezing room until wk 12 and 24. The remaining MP, intended for raw fermented sausage production, were cut into pieces (3 cm × 3 cm × 1 cm) and each 4 kg meat was packed into plastic bags (Dagama eG, Willich, Germany). After sealing the plastic bags, the packaged meat pieces were frozen in a -80°C freezer (Gesellschaft für Labortechnik mbH), or in a -18°C freezing room and again stored until wk 12 and 24 (Figure 1). The differences between the cutlets, used for frozen/thawed analysis, and the meat pieces for sausage production were due to space problems in the -80°C freezer.

Furthermore, in each of the 3 independent repeats, at 24 h p.m., fresh, unfrozen control samples were cut in pieces before processing to raw fermented sausages.

The procedures resulted in the following treatment groups:

- Meat 24 h p.m., frozen storage for 12 wk at -18°C = **12/-18**
- Meat 24 h p.m., frozen storage for 12 wk at -80°C = **12/-80**
- Meat 24 h p.m., frozen storage for 24 wk at -18°C = **24/-18**
- Meat 24 h p.m., frozen storage for 24 wk at -80°C = **24/-80**
- Meat 24 h p.m., no frozen storage = **Control**

Storage and Sausage Production

To produce the raw fermented sausages, the meat of the control group was first briefly frozen due to technological reasons. The frozen stored turkey meat and the meat of the control group was thawed in a cold room at 4°C 24 h before sausage production. From the fresh and frozen-thawed meat, raw fermented sausages were produced considering the following recipe: 68.75 % turkey breast, 28.5 % pork back fat, 2.5% nitrite curing salt, 0.1% glucose, 0.15% sucrose, starter cultures *Lactobacillus sakei*, *Staphylococcus carnosus*, and *Pediococcus acidilactici* (25 g per 100 g meat); Bactoferm F-SC-111 and Safe-Pro B-LC-20, Chr. Hansen Holding GmbH, Hoersholm, Denmark). One batch of sausage was produced per frozen storage temperature and frozen storage duration. A batch of sausage comprised 2 raw sausages per sample day (day of production (d 0), d 7, d 14, d 21 and d 28), of which the test results were combined to form a mean value. Thus, a total of 4 batches of sausage with about 3.36 kg each were produced from the frozen storage meat (12 wk -18°C, 12 wk -80°C, 24 wk -18°C, 24 wk -80°C) and additionally one batch of sausage as a control group. The sausages were directly filled into R2L-D Naturin casings (Naturin Viscofan GmbH, Weinheim, Germany). After weighing, the products were matured in a ripening chamber until d 14 with continuous reduction of humidity (from 96% to 84%) and air temperature (from 22°C to 15°C) at an air circulation between 55 and 65 m/s. On d 3, 6, and 11, the sausages were smoked for 10 min at 18°C to 22°C (T1900 619, Fessmann GmbH und Co., Winnenden, Germany). On d 14 the sausages were transferred to a chilling room and stored hanging at 4°C until d 28.

Sampling

Physicochemical and Microbiological Analyses at

24 h p.m For the basic characterization of the fresh meat, the 3- and 2-cm thick cutlets (N = 3) were analyzed 24 h p.m. for the following parameters: drip loss, cooking loss, shear force, color, pH-value, *Enterobacteriaceae*, *Pseudomonas* spp., and total plate count. Microbiological analyses were carried out of 2 pooled samples, which were removed from the MP of the 6 carcasses during dissection.

Physicochemical and Microbiological Analyses

After Freezing Before Sausage Production After the samples were stored for 12 and 24 wk at -18°C and

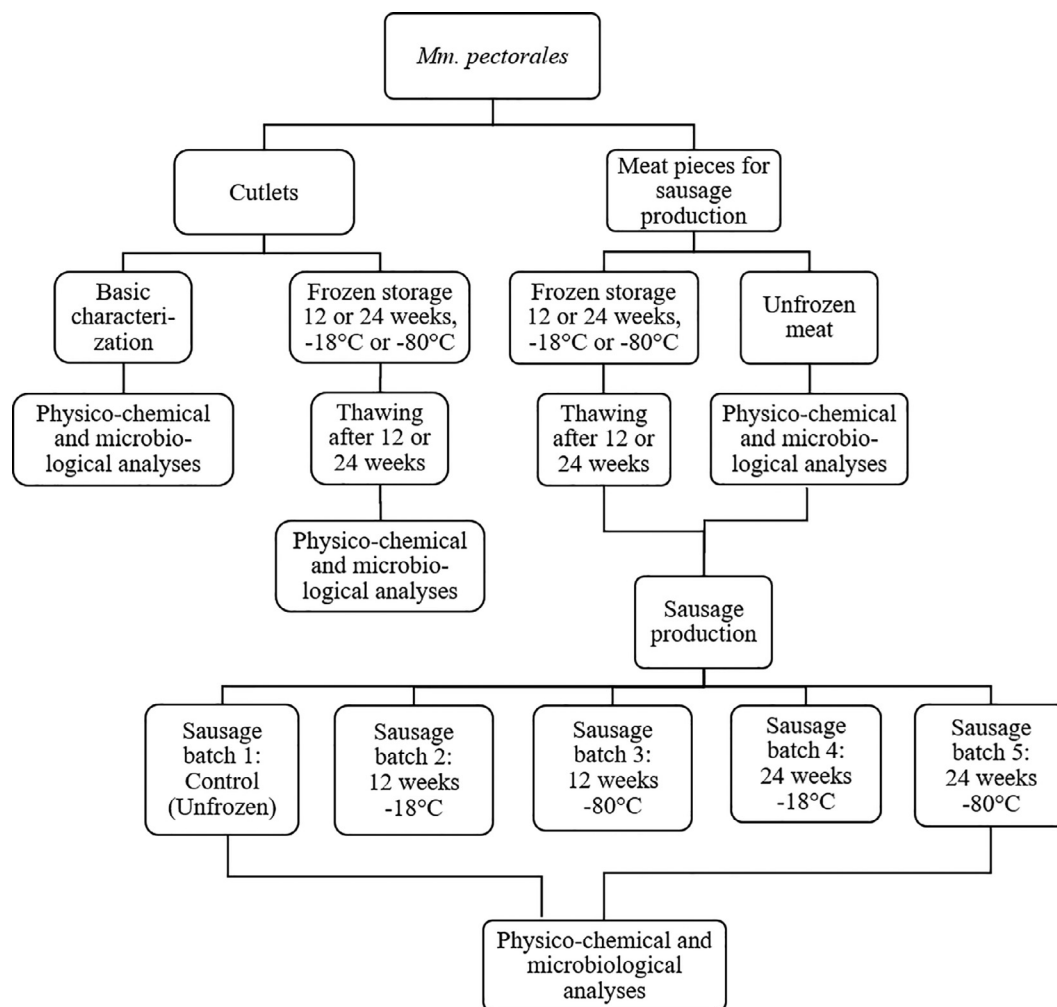


Figure 1. Scheme of the sample collections and analyses.

–80°C, the meat was thawed and the cutlets were analyzed to identify thawing loss, pH-value, color and microbiological changes (*Enterobacteriaceae*, *Pseudomonas* spp., and total plate count) due to the freezing treatment.

Analyses of the Sausages The raw fermented sausages were sampled on the day of production (d 0) and on d 7, 14, 21, and 28. Due to the high sample volume and the small changes expected from experience in previous tests within seven days, the chemical analyses were only carried out on d 0, 14, and 28. Two sausages were sampled on each day of examination.

The following procedures were applied for the control group and the frozen-thawed meat samples.

Physical Analyses

Analyses 24 h p.m. and After Freezing Before Sausage Production The 3-cm thick cutlets were weighed 24 h p.m., then stored at 4°C in a closed plastic box hanging freely from the lid and weighed again 72 h p.m. after dabbing with paper. Relative drip loss is calculated as the percentual difference between weight after 24 h p.m. and weight after 72 h p.m. in relation to the weight at 24 h p.m.

To calculate thawing loss of frozen MP samples, the following formula was applied:

$$\left(\text{Weight}(W)_{24\text{hp.m.}} - W_{24\text{afterthawing}} / W_{24\text{hp.m.}} \right) \times 100.$$

For determination of the cooking loss the cutlets were sealed in plastic bags (Dagama eG) and heated up to a core temperature of 75°C (measured with a digital grill and roast thermometer, TFA Dostmann GmbH & Ca. KG, Wertheim, Germany) in a laboratory water bath (type 1083, Gesellschaft für Labortechnik). They were then cooled down to room temperature (20°C–22°C) and dabbed dry. Cooking loss was calculated as the percentual difference between weight before and after cooking in relation to the weight before cooking.

For modified Warner Bratzler shear force determination (AMSA, 2016; modifications: V-notch blade, slices, only 5 measurements, turkey meat), the meat from the cooking loss analysis was cut into 5 pieces (1 cm × 1 cm × 3 cm) parallel to muscle fiber direction. After that, the meat pieces were sheared with a v-shaped Warner Bratzler blade using the Texture Analyser TA.XT Plus (Stable Micro Systems, Surrey, United Kingdom). The results of 5 measurements per cutlet, expressed in Newton (N), were averaged, and used for further statistical analysis.

Analyses of the Sausages For analysis of the storage loss of the raw sausages the percentual difference between the weight on day of production and the weight on d 1, 7, 14, 21, and 28 in relation to the production day weight was calculated.

Meat Quality Parameters

Color parameters (CIE Lab System) L* (lightness), a* (redness), and b* (yellowness) were measured on the meat surfaces of all cutlets 24 h p.m. (before freezing), directly after thawing and on the surfaces of the sausages at d 0, 7, 14, 21, and 28 of storage using a colorimeter (Minolta CR 400, Konica-Minolta GmbH, Langenhagen, Germany, 2° standard observer, D65 illuminant, 8 mm measuring field). The sausages were analyzed after removal of the casing and longitudinal division. For each color determination five points of the meat or sausage surfaces were analyzed and averaged for further statistical analysis. Before measuring, the device was calibrated with a standard white plate (Konica-Minolta GmbH, $y = 84.0$, $X = 0.3226$, $Y = 0.3392$).

The pH-values of all samples to be analyzed were determined using a portable pH-meter (Portamess, Knick GmbH, Berlin, Germany) equipped with a glass electrode (InLab 427, Mettler-Toledo, Urdorf, Switzerland) and a temperature probe. The values were measured at three different points of all cutlets and sausages and the averaged values were used for further statistical analysis. Prior to the analysis, the pH-meter was calibrated using commercially available standard solutions (pH 4.0, pH 7.0, Carl Roth GmbH & Co.KG, Karlsruhe, Germany).

Water activity (a_w -value) was only measured on the into sausages processed meat. Therefore, the sausages were first homogenized (Retsch GM 200, Retsch GmbH, Haan, Germany). Subsequently, a_w -value with corresponding freezing point was determined in triplicates using an a_w -Cryometer (AWK-40, Nagy-Instruments, Gaeufelden, Germany). The results were averaged and used for further statistical analysis.

Chemical Analyses

All chemical analyses were carried out in triplicates from frozen/thawed cutlets and the produced sausages.

At the different collection days, samples were cut into maize grain-sized pieces, frozen in liquid nitrogen (N_2), packaged in vacuum bags and stored at -80°C until analyses.

For the determination of the percentages of the myoglobin redox forms oxymyoglobin (**OxyMb**), deoxymyoglobin (**DeoMb**) and metmyoglobin (**MetMb**) the method, described by Kernberger-Fischer et al. (2017), was used. The meat samples (up to 3 g) were homogenized on ice for 2 utes at 15,000 rpm in 10 mL NaH_2PO_4 (50 mM, pH 7.2) with a Polytron PT 2500 Homogenizer (Kinematica GmbH, Lucerne, Switzerland). After centrifugation for 30 min at $35,000 \times g$ and 4°C (Sorvall RC

5 Plus, Thermo Scientific, Langenselbold, Germany) the supernatant was transferred into 2.5 mL macro cuvettes (Brand GmbH & Co. KG, Wertheim, Germany) and measured at 525 nm, 503 nm, 557 nm, and 582 nm in a spectrophotometer (Evolution 201-UV-VIS-Spectrophotometer, Thermo Scientific). The mean percentages of the different myoglobin redox forms were calculated using the equations according to Tang et al. (2004).

The antioxidant capacity of the samples was determined according to Reichel et al. (2019). The measurement of the concentrations of the thiobarbituric acid reactive substances (**TBARS**) within the samples was performed according to Popp et al. (2013). For this purpose, 10 mL of 20% trichloroacetic acid (**TCA**) and 500 μL butylhydroxytoluene (0.19 M, **BHT**) were added to 1 g of the sample and then homogenized on ice for 1 min at 15,000 rpm (Kinematica GmbH). Afterwards, samples were centrifuged at 6°C and a speed of $2,835 \times g$ for 6 utes (Hermle Labortechnik). The centrifuged samples were filtered through folded qualitative filter paper (303, VWR international GmbH, Darmstadt, Germany) into new tubes. After the addition of 1,000 μL 2-thiobarbituric acid (0.02 M, **TBA**) to 1,000 μL of the filtrate, the suspension was mixed and incubated for 30 min in a water bath (Gesellschaft für Labortechnik GmbH) at 100°C . The measurement of lipid oxidation was performed photometrically (Thermo Scientific) at 532 nm and 570 nm after a 10-minute cooling time on ice. TBARS values were calculated from the results of 570 nm subtracted from 523 nm and compared to calibration curves with values between 0.05 $\mu\text{g}/\text{mL}$ and 0.25 $\mu\text{g}/\text{mL}$ malondialdehyde (**MDA**).

Microbiological Parameters

For microbiological analyses, *Enterobacteriaceae* were tested on d 0, only. The other parameters, such as total plate counts (**TPC**) and *Pseudomonas* spp. were examined on all examination days. At first all samples were diluted 1:10 in sterile NaCl solution with added peptone (0.85 % NaCl, 0.1 % peptone; VWR International) and homogenized in Stomacher bags (Stomacher Strainer Bags, Seward limited, Worthing, United Kingdom) for 2 min at 230 rpm (Stomacher 400 Circulator, Seward). Before application to the petri dishes, appropriate dilution series were prepared (1 mL sample in 9 mL NaCl + peptone).

Enterobacteriaceae were analyzed according to ISO 21528-2:2017 (Violet Red Bile Glucose Agar [**VRBG**], CM 1082, Oxoid GmbH; 37°C , 48 h). In this case, the detection limit was 1.0 \log_{10} cfu/g.

The determination of total aerobic mesophilic count (TPC) was carried out according to ISO 4833-1:2013. 1 mL of diluted sample material was pipetted into petri dishes, filled with Plate Count Agar (PC, CM 0325, Oxoid GmbH, Wesel, Germany) and incubated at 30°C for 48 h. The detection limit was 1.0 \log_{10} cfu/g.

Pseudomonas spp. were grown at 30°C for 48 h after spreading 0.1 mL of the certain dilutions on

Cephaloridine Fucidin Cetrimide (CFC) selective agar (CM559, Oxoid GmbH) (ISO 13720:2010-12). The detection limit was 2.0 log₁₀ cfu/g.

If no colonies could be counted on the plates after the respective incubation time, half of the detection limit was used for further statistical calculation.

Statistical Analyses

For analysis of the data the software SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC) was used, considering the following model:

$$Y_{ijk} = \mu + D_i + T_j + DT_{ij} + R_k + \varepsilon_{ijk}$$

with Y_{ijk} = observation value; μ = overall mean, D_i = fixed effect of freezing duration, T_j = fixed effect of freezing temperature; DT_{ij} = fixed effect of the interaction of T and D, R_k = random effect of repeat; ε_{ijk} = random error. At first, normal distribution was determined using the Shapiro-Wilks-Test. Data sets showing normal distribution were analyzed using two-factorial ANOVA, the other (non-parametric) results with the Kruskal-Wallis-Test. Non-parametric parameters of the meat were shear force and *Enterobacteriaceae* number and of the sausages storage loss, b^* , a_w , TBARS, *Pseudomonas* spp. and *Enterobacteriaceae* number. If on one of the storage days of the sausages the parameter was nonparametric, the parameter was generally considered as nonparametric.

The Tukey multiple comparison test was performed to compare the different treatment groups. All values were presented as means \pm standard deviation (SD). Means were significant if the P value was lower than 0.05. All experiments were independently replicated at least 3 times.

RESULTS

Physicochemical and Microbiological Parameters After Slaughter at 24 h p.m

The slaughter parameter results and the results of the physicochemical and microbiological characterization of the meat at 24 h p.m. are shown in Table 1. The yield of Turkey breast averaged by 35.2% during the three repeats with 6 turkey carcasses and was calculated from the turkey slaughter weight (14.6–15.9 kg) and breast

Table 1. Mean and standard deviation values of slaughter and basic characterization 24 h p.m. of turkey meat before freezing treatment.

Parameter	Mean value	SD	Minimum	Maximum
Slaughter weight (kg)	15.2	0.7	14.6	15.9
Breast weight (kg)	5.4	0.4	4.9	5.6
Breast ratio ¹ (%)	35.2	2.7	32.6	38.0
Leg weight (kg)	4.5	0.02	4.5	4.5
Leg ratio ¹ (%)	29.7	1.3	28.3	30.7
Drip loss ² (%)	0.9	0.2	0.8	1.2

¹Ratios calculated in relation to the slaughter weight.

²Drip loss determined between 24 h and 72 h postmortem.

weight (4.9–5.6 kg). The average drip loss was 0.9%, the cooking loss 19.1% and the shear force result was 16.6 N. L^* value of 52.3, a^* value of 5.2 and b^* value of 1.8 were measured in the unfrozen meat with an averaged pH of 5.7. With regard to the microbiological analyses, TPC values between 3.2 and 3.7 log₁₀ cfu/g and *Pseudomonas* spp. results between 3.6 and 4.0 log₁₀ cfu/g were obtained. The mean value of *Enterobacteriaceae* was 2.9 log₁₀ cfu/g.

Results of the Turkey Meat Analyses Before Processing (Fresh, Frozen-Thawed)

Physical Parameters Table 2 shows the results of the physical parameters of the unfrozen and frozen-thawed meat before processing to sausages. Significant effects of the time for the parameters thawing loss and b^* were found. Thawing loss and b^* values were additionally influenced by the freezing temperature, whereas an impact of the interaction was only obtained for the thawing loss results. Frozen meat shows significantly higher thawing loss values than the unfrozen control group. Furthermore, 12/-80 cutlets had lower thawing loss values ($P < 0.05$) compared to those of the 12/-18 samples, whereas after 24 wk no effect of the freezing temperature was obtained. For b^* values, samples of unfrozen control group showed significantly lower values than 12/-18 cutlets, while the samples of the other treatment groups were comparable with all other groups.

Chemical Parameters The relative amount of OxyMb after 24 wk of frozen storage was lower than in the control group ($P < 0.05$), while the relative amount of MetMb after 24 wk of storage was significantly higher compared to the unfrozen control group and the 12 wk frozen samples. In contrast to the MetMb results, the OxyMb percentages of the 12/-18 and 12/-80 samples were comparable with the other groups. No significant influence of the time, temperature and their interaction on the TBARS and antioxidant capacity results were found (Table 2).

Microbiological Parameters TPC results did not show significant differences neither in terms of storage time, nor storage temperature.

Growth of *Pseudomonas* spp. was reduced ($P < 0.05$) in meat stored for 24 wk, regardless of storage temperature, compared to the control samples. The same result could be obtained for the *Enterobacteriaceae* with the exception that the 12-wk samples also showed a significant reduction in comparison to the unfrozen control group (Figure 2).

Results of the Raw Fermented Sausages

Physical Parameters The weight losses of the sausages were between 18.7 and 21.5% on d 7 of storage increasing up to d 28 with values between 35.5 and 37%. On d 14 significantly higher storage loss results of the 24/-18 and 24/-80 sausages compared to the 12/-18 and 12/-80 samples could be found, whereas on the other

Table 2. Mean and standard deviation values of physicochemical meat quality parameters of turkey meat before and after freezing and thawing depending on the frozen storage time and temperature.

Storage time in weeks	0	12		24		<i>P</i> -value time	<i>P</i> -value temp.	<i>P</i> -value time × temp.
		Storage temperature in °C	−18	−80	−18			
Parameter	4							
Thawing loss (%)	0.0 ^c ± 0.0	2.6 ^a ± 0.5	1.2 ^b ± 0.4	1.9 ^{ab} ± 0.2	1.3 ^b ± 0.5	0.0001	0.0030	0.0028
Cooking loss (%)	19.1 ± 0.9	17.6 ± 7.1	20.6 ± 4.1	22.3 ± 3.2	24.1 ± 4.7	0.28	0.43	0.66
Shear force (N)*	16.6 ± 2.2	17.0 ± 0.9	16.9 ± 1.8	19.3 ± 7.7	17.7 ± 2.1	0.99	0.74	0.96
pH	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.8 ± 0.0	5.7 ± 0.1	0.69	0.98	0.61
L*	52.3 ± 1.3	54.1 ± 3.2	51.5 ± 0.6	51.7 ± 1.9	52.4 ± 1.7	0.78	0.44	0.30
a*	5.2 ± 1.0	5.0 ± 1.7	5.6 ± 0.7	4.5 ± 0.5	5.5 ± 0.7	0.90	0.19	0.44
b*	1.8 ^b ± 0.4	4.1 ^a ± 0.6	2.7 ^{ab} ± 0.3	3.1 ^{ab} ± 0.6	3.1 ^{ab} ± 0.6	0.006	0.08	0.04
OxyMb ¹ (%)	31.8 ^a ± 0.6	24.1 ^{ab} ± 4.4	28.1 ^{ab} ± 2.1	20.5 ^b ± 3.0	23.1 ^b ± 3.4	0.0025	0.17	0.19
MetMb ² (%)	49.3 ^b ± 0.4	55.2 ^b ± 3.4	49.9 ^b ± 3.1	57.9 ^a ± 2.3	56.3 ^a ± 2.6	0.004	0.14	0.08
TBARS ³	0.07 ± 0.03	0.10 ± 0.06	0.08 ± 0.05	0.08 ± 0.01	0.07 ± 0.03	0.86	0.59	0.83
Antiox. capacity ⁴	4.9 ± 0.5	4.7 ± 0.3	4.8 ± 1.1	5.1 ± 0.4	6.0 ± 0.7	0.14	0.26	0.25

Boldface *P*-values indicate a significant effect ($P \leq 0.05$) of freezing time, freezing temperature and their interaction.

*Nonparametric parameter (Kruskal-Wallis-Test).

^{abc}Mean values in a row within the same parameter followed by a different letter differ significantly ($P \leq 0.05$) by Tukey's test.

¹OxyMb = Oxymyoglobin in %.

²MetMb = Metmyoglobin in %.

³TBARS = Thiobarbituric Acid Reactive Substances in μg malondialdehyde/g meat.

⁴Antiox. Capacity = Antioxidant capacity in μM Trolox equivalent /g meat.

days no effect on the storage losses of the sausages could be found.

The pH-value were 5.36 ± 0.20 and the a_w -values 0.91 ± 0.04 . Both parameters were not influenced by the freezing-thawing treatment at all storage days of the sausages (data not shown).

Irrespective of the storage temperature, sausages from meat, stored for 12 wk, showed higher L* values on the day of production ($P < 0.05$) than those, manufactured from unfrozen meat. This effect could not be detected

during further storage of the sausages. With regard to the a* results, sausages, stored at -80°C , showed higher a* values on the day of production ($P < 0.05$) than 12/-18 and 24/-18 sausages. The b*-values were comparable between the experimental groups at all storage days (Table 3).

Chemical Parameters Regarding the TBARS results on d 14 and 28 of storage, the values were significantly lower, if frozen-thawed meat was used for sausage production. On examination d 0, 14, and 28, sausages,

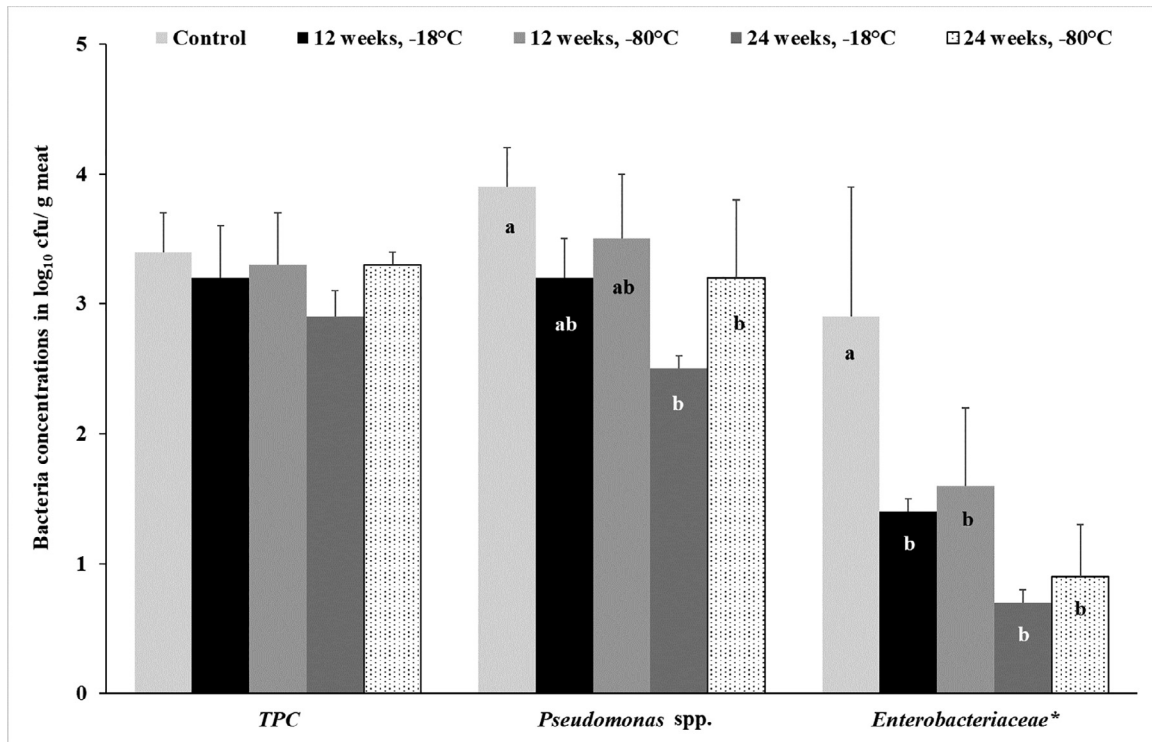


Figure 2. Mean and standard deviation values of microbial meat quality parameters of turkey meat before and after freezing and thawing depending on the frozen storage time and temperature; cfu = colony forming units; if no colonies were detected on the agar the half value of the detection limit of 1.0 (Total plate count (TPC), *Enterobacteriaceae*, 0.7 log₁₀ cfu/g) or 2.0 log₁₀ cfu/g meat (1.7 log₁₀ cfu/g) was considered for (statistical) analysis and result presentation; *Nonparametric parameter (Kruskal-Wallis-Test); ^{abc}Mean values in a column within the same parameter followed by a different letter differ significantly ($P \leq 0.05$) by Tukey's test.

Table 3. Mean and standard deviation values of color parameters of turkey sausages depending on the frozen storage time and temperature and the storage day after production of the sausages from the unfrozen and frozen/ thawed turkey meat.

Storage time in weeks	Storage temperature in °C	12		24		<i>P</i> -value time	<i>P</i> -value temp.	<i>P</i> -value time × temp.	
		0	4	-18	-80				-18
Parameter	Day								
L*	0	66.1 ^b ± 1.9	69.0 ^a ± 0.6	69.8 ^a ± 1.6	67.2 ^{ab} ± 2.1	67.2 ^{ab} ± 0.9	0.03	0.76	0.85
	7	64.8 ± 1.7	65.3 ± 0.9	65.0 ± 0.2	65.8 ± 0.3	66.6 ± 0.5	0.07	0.61	0.50
	14	62.5 ± 2.2	63.3 ± 1.1	62.6 ± 0.7	64. ± 0.7	63.9 ± 0.4	0.18	0.32	0.70
	21	63.4 ± 0.2	64.8 ± 1.4	64.5 ± 0.5	63.8 ± 0.9	63.7 ± 0.8	0.09	0.70	0.89
	28	64.0 ± 1.2	63.8 ± 1.9	63.5 ± 0.9	65.1 ± 0.6	64.9 ± 0.6	0.19	0.73	0.93
a*	0	5.6 ± 1.0	4.9 ^b ± 0.2	6.1 ^a ± 0.6	4.6 ^b ± 0.7	5.6 ^a ± 0.3	0.43	0.0029	0.03
	7	7.3 ± 0.5	7.01 ± 0.7	7.6 ± 0.4	7.1 ± 0.4	7.5 ± 0.9	0.09	0.18	0.44
	14	7.2 ± 0.8	7.7 ± 0.7	8.0 ± 0.4	7.3 ± 0.6	8.0 ± 0.5	0.37	0.14	0.38
	21	8.2 ± 0.2	8.5 ± 0.2	8.8 ± 0.2	8.5 ± 0.5	8.9 ± 0.5	0.15	0.11	0.27
	28	8.6 ± 0.1	8.6 ± 0.2	8.9 ± 0.2	8.7 ± 0.6	9.3 ± 0.4	0.23	0.06	0.10
b* ⁺	0	10.9 ± 1.0	11.7 ± 0.3	12.2 ± 1.1	11.7 ± 0.2	11.7 ± 0.1	0.18	1.00	0.40
	7	8.6 ± 2.1	8.0 ± 0.1	7.9 ± 0.1	8.1 ± 0.7	8.7 ± 1.0	0.61	0.93	0.84
	14	8.6 ± 2.4	8.0 ± 0.3	7.8 ± 0.4	7.9 ± 0.3	8.2 ± 0.3	0.87	1.00	0.85
	21	9.2 ± 2.0	8.8 ± 0.5	8.6 ± 0.4	9.0 ± 0.3	8.9 ± 0.2	0.49	0.42	0.76
	28	10.0 ± 2.0	8.9 ± 0.5	8.7 ± 0.7	9.7 ± 0.2	9.4 ± 0.2	0.08	0.37	0.25

Boldface *P*-values indicate a significant effect ($P \leq 0.05$) of freezing time, freezing temperature and their interaction.

⁺Nonparametric parameter (Kruskal-Wallis-Test).

^{ab}Mean values in a row within the same parameter and day followed by a different lower case differ significantly ($P \leq 0.05$) by Tukey's test.

produced from meat stored for 24 wk, showed higher antioxidant capacity values ($P < 0.05$) than sausages, made from meat stored frozen for 12 wk. Control sausages showed comparable results with those of 12/-18 and 12/-80 stored sausages on d 14 (Figure 3).

Microbiological Parameters The TPC was not influenced by the freezing and thawing on all storage days (Figure 4). However, the number of *Pseudomonas* spp. were between 1.8 and 2.9 log₁₀ cfu/ g sausage. On d 0 the results within the 24/-18 and 24/-80 sausages were significantly higher compared to the sausages, produced with meat, frozen for 12 wk, on the day of production. However, there was no significant difference to the control group. On all other storage days, the results for *Pseudomonas* spp. were below the detection limit of 2.0 log₁₀ cfu/ g sausage (Data not shown).

For the *Enterobacteriaceae* results a significant effect of the freezing could be detected. The samples, frozen for 12 and 24 wk, showed lower *Enterobacteriaceae* results of approximately 0.8 ± 0.4 log₁₀ cfu/ g sausage in comparison to the unfrozen control samples with 1.7 ± 0.9 log₁₀ cfu/ g sausage (Data not shown).

DISCUSSION

The physicochemical parameters after slaughter mainly agree with other publications that investigated turkey meat samples (Werner et al., 2008; Werner et al., 2009; Popp et al., 2013) and the reference values of the BIG 6 Turkey Performance Goals (Aviagen, 2020) with slight deviations of individual parameters. Werner et al. (2008) found slaughter weights averaging 15.5 kg and breast muscle percentages of 36.8% in turkey genetics of different weights. The mean pH 24 h p. m. in turkey meat was about 5.85 while the lightness value was 48.29 (El Rammouz et al. 2004). The results of our own study showed a higher L* value which,

according to Owens et al. (2000), does not yet indicate quality losses such as pale soft and exudative meat. The maximum shear force of the present study did not reach the values of previous studies (El Rammouz et al. 2004; Werner et al. 2009; Popp et al. 2013). However, it should be noted, that the results were obtained using different methods, making it difficult to compare these results. The results of the microbiological investigations agree with the results of Fraqueza et al. (2008). This research group determined a value of 4.5 log₁₀ cfu/g for the colonization of fresh turkey meat with *Pseudomonas* spp. and a value of 2.9 log₁₀ cfu/g for *Enterobacteriaceae*. The value determined for TPC in the present study was also below the value determined for this parameter by Fraqueza et al. (2008). Therefore, the meat used for the freezing experiments can be considered representative.

Frozen/Thawed Meat Before Processing

According to Fennema (1996), the water holding capacity (WHC) of meat is a term often used to describe molecules that are able to trap water physically in a way that inhibits exudation. This WHC can be measured by the determination of drip loss, thawing loss and cooking loss. The present results show that freezing significantly influences the amount of exudative loss and thus also the WHC of turkey meat. Increased exudative loss can be caused by ice crystal formation (Leygonie et al., 2012b). Higher freezing temperatures result in slower freezing rates (Grujić et al., 1993) and these lead to the formation of large intercellular crystals, whereas fast freezing rates at low temperatures lead to many small intracellular ice crystals in muscle tissue (Grujić et al., 1993; Dave and Ghaly, 2011). This process destroys the structure of muscle tissue and results in quality losses, such as increased exudation (Leygonie et al., 2012a). Denaturation of proteins during freezing leads to reduced WHC

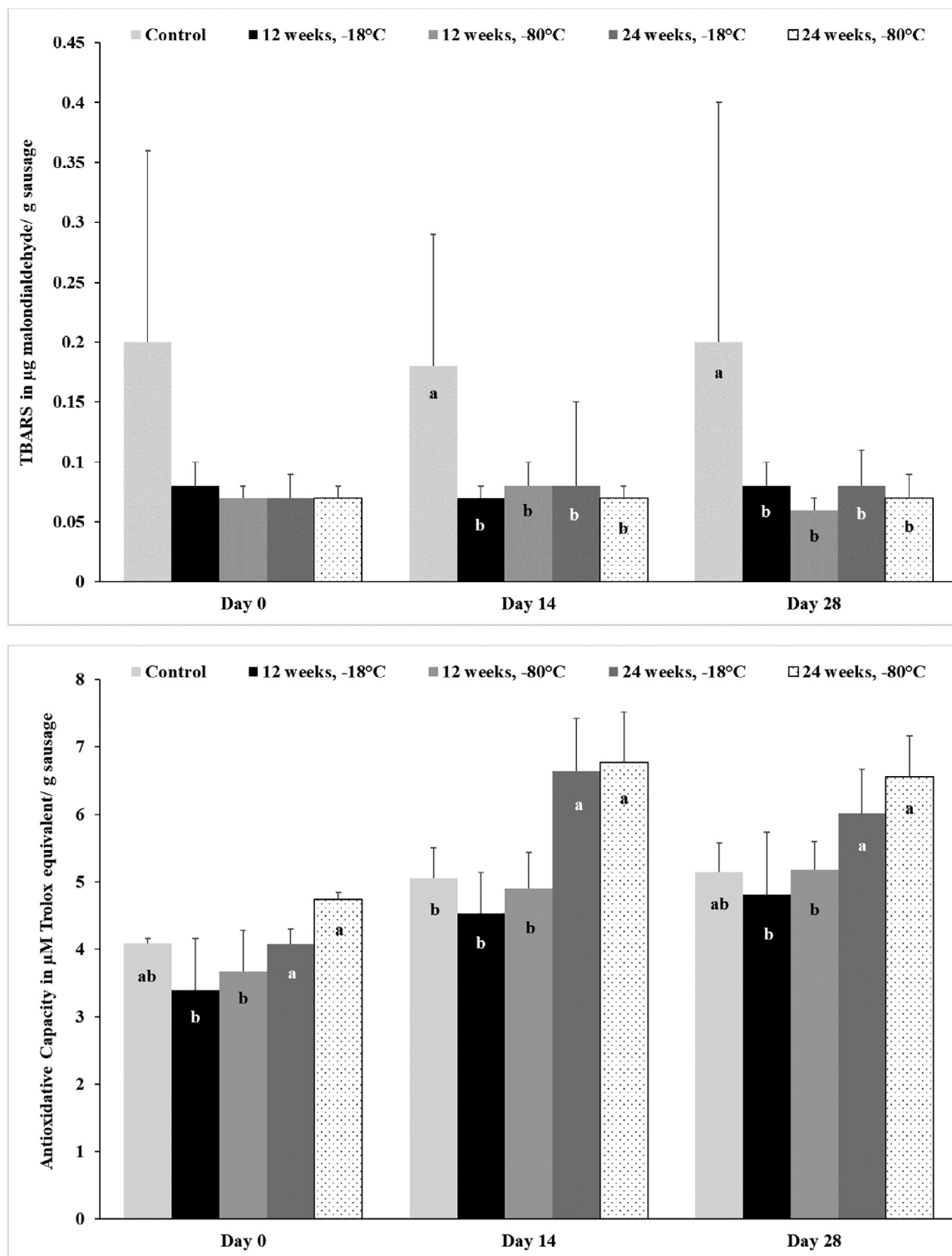


Figure 3. Mean and standard deviation values of the thiobarbituric acid reactive substances (TBARS) concentrations (upper figure, nonparametric parameter [Kruskal-Wallis-Test]) and antioxidative capacity values (lower figure) of turkey sausages depending on the frozen storage time and temperature and the storage day after production of the sausages from the unfrozen and frozen/ thawed turkey meat; ^{ab}Mean values in a row within the same parameter and day followed by a different lower case differ significantly ($P \leq 0.05$) by Tukey's test.

of these proteins (Augustynska-Prejsnar et al., 2019; Utrera et al., 2014b). Huff-Lonergan and Lonergan (2005) described that fluid in muscles is normally bound in and between myofibrils. As rigor progresses, the space available for water binding is reduced and the fluid leaks into the extramyofibrillar space, as the myofibril decreases. The present results show that

frozen turkey meat has a higher exudative loss than unfrozen meat and are therefore consistent with results from previous studies with beef (Farouk et al., 2003), pork (Estevez et al., 2011), and chicken meat (Augustynska-Prejsnar et al., 2019). If we added storage temperature as a factor in the present study, meat, stored at -80°C , has fewer liquid losses after thawing than

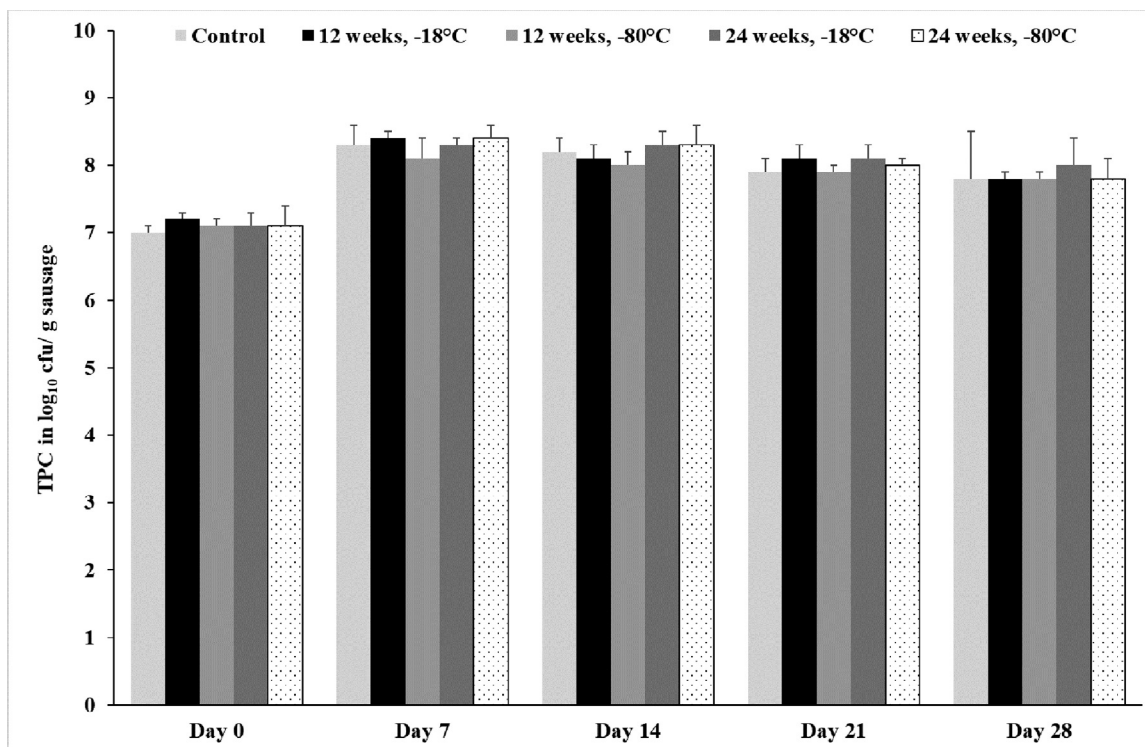


Figure 4. Mean and standard deviation values of the total plate count (TPC) results of the turkey sausages depending on the frozen storage time and temperature and the storage day after production of the sausages from the unfrozen and frozen/ thawed turkey meat; cfu = colony forming units.

turkey meat stored at -18°C . This is probably due to reduced structural changes caused by ice crystal formation. [Utrera et al. \(2014a\)](#) also found that temperature is crucial for ice crystallization and thus also affects meat quality ([Sakata et al., 1994](#)). The lower the freezing temperature, the higher the proportion of frozen water ([Grujić et al., 1993](#)).

The parameters cooking loss and shear force did not show any significant differences between the treatment groups. Although changes in shear force are discussed in broilers ([Lee et al., 2008](#); [Giampietro-Ganeco et al., 2017](#)) and cattle ([Aroeira et al., 2016](#)), there are also publications that are consistent with existing results. For example, [Vieira et al. \(2009\)](#) found no effects on beef tenderness and [Muela et al. \(2012\)](#) showed no differences between fresh and frozen lamb in sensory tests. [Vieira et al. \(2009\)](#) also did not find an influence of freezing/thawing on cooking loss, suggesting that the water, that leaks during cooking, is mainly composed of chemically bound water, and melting fat. These factors are not influenced by freezing and thawing.

The pH-values of the turkey meat of the present study does not show any differences between the unfrozen and frozen samples. [Alonso et al. \(2016\)](#) stored pork at -20°C for up to 2 yr and found higher pH-values in frozen compared to unfrozen samples. In contrast, [Ali et al. \(2015\)](#) showed a decrease of pH-values in chicken breast meat after some freezing-thawing cycles within 6 wk. The effects on the pH values might be due to loss of minerals and peptides as exudates during thawing ([Leygonie et al., 2012b](#)) or changes of the

isoelectrical points of the proteins (by denaturation) ([Alonso et al., 2016](#)).

In the present study, there were no significant alterations of the L^* and a^* -values after freezing turkey meat but the b^* -values increased. According to [Mancini and Hunt \(2005\)](#) meat color is one of the most important factors in the consumers' choice of buying a product and an indicator of freshness. Color stability is mainly determined by Mb and its redox forms and is affected by the formation of ice crystals during freezing ([Anon and Calvelo, 1980](#)). OxyMb makes the product appear bright red, while DeoMb and MetMb are related to a paler appearance of the meat ([Mancini and Hunt, 2005](#)). According to the latter publication, many different factors, such as heme concentration and oxidation status, influence the color of meat. It is therefore not surprising that there are different statements about how frozen storage influences L^* . [Ali et al. \(2015\)](#) investigated the effect of different freeze-thaw cycles on the quality of broiler meat and found increased lightness values after freezing due to protein denaturation, while beef became darker after freezing ([Vieira et al., 2009](#)). [Lee et al. \(2008\)](#) found a decrease in lightness in broiler breast meat after long-term freezing.

Freezing processes decrease MetMb reducing enzymes through muscle fiber and protein denaturation accompanied with higher MetMb and lower OxyMb percentages ([Farouk and Swan, 1998](#); [Farouk et al., 2003](#)). In addition, [Ben Abdallah et al. \(1999\)](#) stated that globin denaturation in beef increases the susceptibility of Mb to autoxidation and production of MetMb. The

significantly higher MetMb levels in frozen/thawed meat compared to control samples and meat, stored for 12 wk, principally agree with this assumption. Also, for pork, stored at -20°C , the MetMb content increases with storage time (Alonso et al., 2016). In this context it is logical that with the higher MetMb ratios at 24 wk due to (auto) oxidation and reduced MetMb reducing activity OxyMb percentages decreased in the present study.

In the present study, TBARS and antioxidant capacity did not show any significances between the treatment groups. Lipid oxidation also takes place in meat, which is, according to Gray et al. (1996), a major factor impairing the quality of meat. The reason for this is an imbalance between pro-oxidative factors and the antioxidant capacity. However, the TBARS results in the present study are contradictory to other studies that found increased formation of secondary lipid oxidation products as shown by Custodio et al. (2018) and Zhang et al. (2019) in pork or lower results of frozen pork compared to unfrozen meat (Sakata et al., 1994). It can be assumed that the frozen and thawed turkey meat in the present study would not be perceived as altered/rancid by the consumer as a whole, since the threshold value for rancidity is 0.5 mg MDA/kg meat (Diaz et al., 2008) and the maximum values of 0.08 μg MDA/g meat in the present study are clearly lower.

Basic mechanisms for spoilage of foods include autolytic/oxidative processes and microbial spoilage (Dave and Ghaly, 2011). The diversity of microorganisms that grow on meat are responsible for spoilage of this products depends on many factors along the food chain (Dave and Ghaly, 2011) like rearing, fattening, slaughtering, cutting, packaging, transport, and storage as well as consumer's handling. The spoilage of meat and meat products is thus also determined by the initial bacterial flora (Doulgeraki et al., 2012), but *Pseudomonas* spp. are nevertheless one of the most important bacteria groups on meat (Lin et al., 2004). The microbiological results in the present study show that the growth of *Pseudomonas* spp. and *Enterobacteriaceae* are inhibited after freezing, although the TPC results were not significantly influenced by the freezing procedure. Manios and Skandamis (2015) also found a reduction of bacterial growth of microorganisms by freezing on beef, stored at -22°C for 5 d. A similar growth inhibition by freezing was found by Medic et al. (2018) in pork. However, these authors showed a reduction up to 1.0 \log_{10} cfu/g of the TPC results during an 18-mo frozen storage period. The reason for this reduced microbiological result could be that up to 60% of the microorganisms die during the freezing process (Rahman and Valez-Ruiz, 2007). Rahman and Valez-Ruiz (2007) described that the cell membrane of microorganisms is damaged by ice crystal formation when the temperature is lowered to the freezing range. As a result, important cell-internal substances, such as potassium ions or RNA, leak out and the viability of the microorganisms decreases. In addition, the cells may die due to osmotic dehydration. In contrast

to the present study, Vieira et al. (2009) presented an increasing bacterial growth on beef with higher duration of frozen storage up to 90 d. The microorganisms that survive the freezing process are able to continue growing after thawing (Vieira et al., 2009). Furthermore, due to structural damage of the muscle cells, caused by ice crystals, microbiological growth may even be accelerated after thawing (Vieira et al., 2009; Leygonie et al., 2012a).

Raw Fermented Sausages

For technological reasons, sausages are often produced from frozen meat to prevent excessive heating during the manufacturing process (Popp et al., 2013). The frozen raw material may have an influence on the quality of the product. Therefore, the impact of duration of frozen storage or storage temperature on the processing properties of this frozen meat producing raw sausages were analyzed in the present study. According to Smith (1987) freezing causes protein insolubility and changes myofibrillar microstructures of turkey meat. The myofibrillar gel matrix changes from filamentous to spherical, which, in turn, leads to a reduced WHC. However, the present results show no significant differences of the storage losses ($P > 0.05$) between the control samples and samples, frozen stored for up to 24 wk indicating no impact on the WHC. Only on d 14, the 24 weeks stored samples showed higher storage losses than 12 wk samples. However, these values should not be overestimated, as this difference was only found on d 14.

A reduction in weight during ripening process, among other things, is due to the water loss/ drying of the sausage. During this process chemically-unbound active water leaks out of the sausage and this liquid loss could be analyzed by determination of the a_w -value. As the weight loss in the present study was mainly comparable in all treatment groups, the similar a_w -values of the sausages, produced from frozen and unfrozen meat, are understandable.

For the production of raw sausages, curing salt containing sodium nitrite is added to the sausage to redden it. The color change is due to the reduction of the nitrite to nitric oxide (NO) and formation of nitroso-myoglobin (NO-Mb) (Kabisch, 2013). As far as we know, no studies have been published that analyzed the effect of freezing of turkey meat on its processing properties. For example, there are no studies that investigated the influence of freezing on the subsequent color of the sausage. Popp et al. (2013) presented that sausages, produced with turkey meat with varying color, show similar color values indicating no impact of the raw meat color, if curing salt is added during processing but the impact of freezing on the color was not considered. Beside this, the similar color results during storage of the raw sausages in the present study agree with the comparable pH and color values of the turkey meat before processing as well as with the similar pH results of the sausages after production. Due to the isolated occurrence, the significant

color changes (L^* day 0, a^* day 0), should not be overinterpreted. Therefore, it seems reasonable to produce raw fermented sausages from frozen turkey meat without any complications in consumer's acceptance.

Lipid oxidation, analyzed by TBARS determination, also occurs in meat products (Bruna et al., 2001; Popp et al., 2013). The present results indicate that lipid oxidation can be reduced by freezing before processing to raw fermented sausage. So far, no studies have been published that analyzed this parameter in relation to frozen raw material. In general, changed lipid oxidation is due to altered balance between pro-oxidative and antioxidant factors within the products, meaning that higher antioxidant capacity is probably related to lower (lipid) oxidation (Gray et al., 1996). This relation could be partly seen in the present study. For example, on d 14 and 28 the sausage samples, produced with 24 weeks frozen meat, showed lower TBARS and higher antioxidant capacity results. However, as the relations are quite inconsistent, especially considering the 12/-18 and 12/-80 sausages, further investigations might be useful including other methods to analyze antioxidant capacities like the DPPH or FRAP tests (Ahn et al., 2004; Fernandes et al., 2016). Basically, 2 different approaches are used to measure the antioxidant potential: hydrogen atom transfer and electron transfer. The FRAP test, which measures the extent to which the sample is able to reduce iron, is subject to the latter mechanism. The DPPH test reduces free radicals and combines both measurement approaches. Since there can be interactions in meat regarding the antioxidant mode of action, it may be advisable to combine several test methods for a more accurate determination of antioxidant capacity.

Survival and growth of microorganisms are influenced by the a_w - and pH-values of the sausages. Each microorganism has its own optima for certain parameters, such as a_w - and pH-values, within the best possible growth is given. With some exceptions, the a_w - and pH-value results were comparable between the treatment groups. This indicates a similar bacterial growth between the groups. It has to be considered that the generally higher TPC results in all groups at all storage days were due to addition of the starter culture bacteria. Whereas the *Enterobacteriaceae* results, analyzed only at d 0, were comparable with the values directly after thawing, the *Pseudomonas* spp. numbers after 24 wk of freezing were quite inconsistent. After thawing the results were significantly lower compared to the unfrozen samples, but at d 0 of sausage storage the values in the sausages were comparable. At all other ripening days the number of *Pseudomonas* spp. were below the detection limit of $2.0 \log_{10}$ cfu/g sausage, which can be explained by a reduced redox value caused by the multiplication of the starter cultures (Kabisch, 2013). The differences at d 0 should not be overestimated.

In conclusion, frozen storage of turkey meat for up to 24 wk at -18°C and at -80°C has small effects on the processing properties into raw sausages. The thawed meat showed higher exudative losses, but the weight losses and drying of the sausages produced from it period

were comparable for all experimental groups at the end of the storage time. Based on the evaluation of the TBARS results and the antioxidant capacity, it can be assumed that there are no taste impairments of the meat to be processed due to frozen storage. Microbiological safety can even be improved by freezing prior to sausage production.

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DISCLOSURES

All authors declare that they have no conflict of interest.

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