Influence of modified atmosphere and vacuum packaging with and without nanosilver-coated films on different quality parameters of pork

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Abstract Pork is often marketed in packages with high oxygen atmosphere (MAP) or vacuum to improve shelf life and appearance. As silver ions have antibacterial effects, food contact films coated with silver might improve the shelf life of meat. In the present study, pork was wrapped in commercially available films, coated with nanosilver particles, and stored in the two packaging variants MAP and vacuum for 12 days. During storage, samples were analyzed on days 1 (before packaging), 4, 8 and 12 for microbiological contamination, meat quality (e.g., pH, color), and for the percentages of the myoglobin (Mb) redox forms. In addition, the effects of the film were examined after inoculation of the meat with high quantities of methicillin-resistant *Staphylococcus aureus* (MRSA) cells before vacuum storage for 8 days. MAP storage resulted in higher lightness (L^*) values, lower liquid loss and higher Mb oxidation compared to vacuum. Microbiological spoilage was partly affected by the packaging variants with reducing effects of the MAP. The nanosilver-coating only affects the Mb redox form percentages of the pork cutlets and on day 4 the L^* values, whereas microbiological parameters were not influenced. As the nanosilver coating had no influence on the total viable bacteria counts as well as *Pseudomonas* spp.,

Enterobacteriaceae and MRSA counts, an advantage of the nanosilver coating on the shelf life could be excluded.

Keywords Pig meat · Nanosilver film · Meat quality · Total viable counts · Chemical spoilage

Introduction

The consumer demand for sliced meat products (e.g., cutlets), or convenience-related food has increased over the past years (Grunert 2006). As the enlargement of the meat surface during slicing implicates a greater susceptibility to microbial or chemical deterioration, packaging is a frequently used method to preserve quality and sensory characteristics, to extend shelf life, and improve the safety of sliced products (Eilert 2005). Packaging studies have been published using specifically coated food packaging materials which have anti-microbial effects (Realini and Marcos 2014). An interesting compound with antibacterial properties seems to be (nano)silver (Rai et al. 2009; Chernousova and Epple 2013). The effects of (nano)silver on the shelf life of meat have been investigated in different studies using self-coated packaging materials. In some of these studies no effects of nanosilver coated packaging materials on the microbial loads of beef and chicken meat as well as on kiwi and melon but on the drip/juice of these products could be shown (Fernández et al. 2009, 2010; Lloret et al. 2012). In other investigations, antimicrobial effects of nanosilver coated materials on chicken meat or sausages could be found (Azlin-Hasim et al. 2016; Marchiore et al. 2017). As nanosilver coated packaging materials are already commercially available, the effects of these materials on the shelf life of meat have been investigated. However, no antimicrobial effects of these

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nanosilver coated materials on the microbial load of beef, pork and turkey meat during storage have been found (Lee et al. 2011; Gallochio et al. 2016; Deus et al. 2017).

However, only few studies have analysed the effects of commercially available nanosilver coated packaging materials on the microbiological loads of meat in combination with physicochemical parameters (e.g., color, pH, myoglobin redox form percentages). In addition, for the best of our knowledge, no studies have been published that analysed the antimicrobial effects of these material using products previously spiked with foodborne pathogens. Therefore, as a novelty, we analysed in the present study not only the microbiological characteristics of pork cutlets after storage of the meat with and without a commercially available nanosilver coated film, but also the effect of the film on different physicochemical parameters (e.g. pH, myoglobin) and on the microbiological load after spiking the meat with high amounts of methicillin-resistant *Staphylococcus aureus* (MRSA) cells, an important pathogen occurring on pig meat, before storage.

Materials and methods

Commercial nano-silver film, scanning electron microscopy and energy-dispersive X-ray spectroscopy

A transparent film without anti-fog coating (SUEDPACK GmbH & Co. KG, Ochsenhausen, Germany) was coated with the dispersion of nano-silver particles PERLAZID® by Perlen Converting AG (Perlen, Switzerland), a company specialized on the production of films for the cosmetic and pharmaceutical industry. An inspection certificate by the manufacturer confirmed the success of the coating. The coating process caused an increased thickness of the coated film (+3.5 µm) compared to the uncoated. Further analysis by an external accredited laboratory showed a silver concentration of 4.5 mg Ag/kg film. In addition, the film with and without the nano-silver film was analyzed prior to and after meat contact using scanning electron microscopy (Supra 55, Carl Zeiss, Oberkochen, Germany) and energy-dispersive X-ray spectroscopy (EDX) (Ametek, Wiesbaden, Germany).

Study 1

Meat storage and sampling

On three different dates, hams of 36 German crossbreed pigs, which were slaughtered 24 h before, were obtained from an industrial slaughterhouse in Germany and transported at 3 °C to the Institute of Food Quality and Food

Safety, Hannover, Germany. On every date twelve hams were cut in our meat hygiene room into four cutlets (diameter: 1.5 cm; Ø weight: 171.9 ± 82.6 g). Before packaging, samples for microbial examination were taken and weight, electric conductivity, pH, lightness (L^*) and redness (a^*) values were determined. For analysis of myoglobin (Mb) redox form percentages up to eight circular meat pieces (1.27 cm) were stamped from the cutlets, immediately frozen in liquid nitrogen and stored at -80 °C until further analysis.

One half of the cutlets were transferred to polypropylene (PP) trays (227 × 178 mm, ES Plastic GmbH & Co. KG, Passau, Germany) and wrapped with a polyethylene-ethylene vinyl alcohol-polypropylene (PE-EVOH-PP) transparent film (Suedpack GmbH & CO KG, Ochsenhausen, Germany) (MAP 1, V 1). The other half of the cutlets was wrapped in the same film commercially coated with nanosilver particles (Perlen Converting AG, Switzerland) (MAP 2, V 2). During wrapping, direct contact of the silver-coated side with the meat surface was ensured. Afterwards each tray was transferred into a transparent vacuum bag (Dagama eG, Willich, Germany). The thickness of the PE/PA bags was 90 µm with permeability values of $50 \text{ cm}^3/\text{m}^2 \text{ d}$ [day] bar at 23 °C and 75% relative humidity [RH] for O_2 and $150 \text{ cm}^3/\text{m}^2 \text{ d}$ bar at 23 °C and 75% RH for CO_2 . The bags were either evacuated (V 1, V 2), or evacuated and subsequently flushed with a mixture of 80% O_2 and 20% CO_2 (MAP 1, MAP 2) (VC 999, Inauen Maschinen AG, Herisau, Switzerland). Every tray contained two cutlets and was stored at 7 °C. On days 4, 8 and 12 L^* and a^* values were determined through the transparent vacuum bag and the film wrapped around the cutlets. On days 4, 8 and 12 two packages per variant were opened, weighed for analysis of liquid loss and analyses from day 1 were repeated. All opened packages were excluded from further storage.

Microbiological parameters

On day 1 (before packaging), 5 g of each cutlet were sampled and two samples were pooled. On days 4, 8 and 12, from each of the cutlets of the opened trays 10 g were collected, which made a total of 16 samples per sampling day. The samples were homogenized (Seward Stomacher 400 circulator, Seward Ltd., West Sussex, UK) in a dilution of 1:10 with sterile saline solution with peptone (0.85% NaCl, 0.1% peptone). The suspensions were diluted $1:10^1$ – $1:10^4$ dependent on expected bacterial growth results and the serial dilutions were spread in duplicate on the following media:

- Standard I nutrient agar (Merck KGaA, Darmstadt, Germany) for cultivation of total viable bacteria counts (TVC, 72 h, 30 °C)

- *Pseudomonas* species agar base CM 559 with selective cetrimidine-fucidin-cephalosporin supplement (SR0151, Oxoid Deutschland GmbH, Wesel, Germany) for analysis of *Pseudomonas* species (44 h, 30 °C)
- Violet Red Bile Glucose Agar (VRBGA) (Oxoid Deutschland GmbH, Wesel, Germany) for analysis of *Enterobacteriaceae* (48 h, 37 °C)

All data are expressed in log₁₀ colony forming units (CFU)/g meat.

Meat quality parameters

The pH values were measured with a portable pH meter (Knick Portamess, Knick GmbH, Berlin, Germany) equipped with a glass electrode (InLab 427, Mettler-Toledo, Urdorf, Switzerland). The pH meter was calibrated with two pH standard solutions (pH 7.0, pH 4.0; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) before pH determination and adjusted to the mean temperature of the meat samples (7 °C). For pH determination the electrode was inserted in the centre of the muscle, the measurement was taken after waiting at least thirty-seconds until the pH values were nearly stable. The accuracy of the instrument was regularly controlled using the pH standard solutions. Each value was an average of two determinations.

The electric conductivity (EC) was measured by using the measurement device from Matthäus GmbH & Co. KG, Nobitz, Germany. The EC meter was calibrated with a specific calibration block (10 mS/cm). For EC determination the electrode was inserted in the center of the muscle. Each value was an average of two determinations.

The lightness (L*) and redness (a*) values were determined using a colorimeter (Minolta CR-400, Konica-Minolta GmbH, Langenhagen, Germany) through the vacuum bag and transparent film. For the determination, MAP were turned before measurement to assure direct contact of the meat to the film and returned again for further storage. The aperture size of the apparatus was 8 mm and the illuminant D65. The colorimeter was calibrated before every experiment with a standard white plate (Konica-Minolta GmbH) having L*, a* and b* values of 97.38, 0.02 and 1.55, respectively. For direct determination the surface of the meat was exposed to air for at least 15 min at room temperature before determining the color of the muscle. Each L* and a* value was an average of six determinations.

Chemical parameters

Relative proportions of the myoglobin (Mb) redox forms deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb) and metmyoglobin (MetMb) were estimated according to Tang et al. (2004). Up to 5 g of the circular meat pieces

were homogenized on ice in 10 ml NaHPO₄ (50 mM, pH 7.2) for 2 min at 15,000 U/min using a Polytron PT 2500 homogenizer (Kinematica GmbH, Luzern, Switzerland). The suspension was centrifuged at 4 °C for 30 min at 35,000×g (Sorvall RC 5 C Plus, Thermo Scientific Langensfeld, Germany), the supernatant was transferred to 2.5 ml cuvettes and subsequently measured photometrically (Evolution 201-UV-VIS-Spectrophotometer, Thermo Scientific) at 525, 503, 557 and 582 nm. The extinction results were used for Tang's equations and percentage proportions of the different redox forms were calculated.

Study 2

Inoculation, meat storage and sample collection

On five different dates, five pork hams of crossbreed pigs, which were slaughtered 24 h before, were obtained from an industrial slaughterhouse and transported at 3 °C to the Institute of Food Quality and Food Safety. For the inoculation, a methicillin-resistant *Staphylococcus* (*S.*) *aureus* strain (ST398, t011) was used, since ST398 *S. aureus* are highly prevalent among pig herds in Europe and are frequently isolated from pork meat. The strain was stored at −80 °C and was grown at 35 ± 1 °C overnight on Brain Heart Infusion (BHI) agar plates containing 5% defibrinated horse blood (Oxoid, Wesel, Germany) prior to the inoculation study. On the test day [24 h post mortem (p.m.)] ten cutlets were chopped out of the hams and each cutlet were halved. On each surface of the twenty halves a square of 3 × 3 cm, defining the inoculation area, was sliced with a disposable scalpel. Before inoculation always two halves were put into a PP tray (ES Plastic GmbH & Co. KG, Passau, Germany), to receive ten packages. The inoculum suspension was prepared by selecting a single colony of the *S. aureus* strain from the fresh agar plate and transferring it into a tube containing 5 ml of sterile sodium chloride (0.9%). In brief, the inoculum density was adjusted to a final concentration of approximately 5 × 10⁷ *S. aureus* CFU/ml. Inoculation was performed by pipetting 100 µl of the freshly prepared suspension homogeneously on the indicated area of the meat surfaces. After a drying phase of 10 min four of the cutlet halves were immediately sampled to determine the recovery rate. The determination of the *S. aureus* concentrations was performed by using a disposable dry swab. The drip was dabbed off the inoculation area. Afterwards the swab was vortexed in 2 ml sterile sodium chloride solution (0.9%) until the solution becomes turbid. Subsequently, serial dilutions were performed and 100 µl of each dilution step were streaked out in a dual approach on BHI agar plates containing 5% defibrinated horse

blood and incubated 24 h at 35 °C. The other eight trays containing sixteen cutlet halves were prepared for storage as follows: within each tray one cutlet was covered with the PE-EVOH-PP film (SUEDPACK) and the with the PE-EVOH-PP film coated with nano-silver (Perlen). The film was pressed on the meat to ensure close contact with the inoculation area. Afterwards each tray was sealed in a plastic bag (ALLPAX®, ALLPAX GmbH & Co. KG, Papenburg, Germany) and stored at 7 °C up to 8 days in atmospheric air. On the days 4 and 8 of storage four cutlet halves of each packaging variant were randomly selected and sampled as described before and excluded from further storage.

Chemicals

All chemicals were purchased from Applichem GmbH, Darmstadt, Germany, unless otherwise indicated.

Statistical analysis

The data were analyzed with the software Statistica 10.0 (StatSoft, Hamburg, Germany). The statistical model included in study 1 the factors “packaging variant” (P, vacuum, MAP), “silver coating” (S, non-coated, coated) and the interaction of the factors ($P \times S$) and in study 2 the factor “silver coating” (S, non-coated, coated). The Shapiro–Wilks-test was used to ensure that the data were normally distributed. Normally distributed data were analyzed using two-way-ANOVA and the TUKEY-post hoc-test, if F-value was lower than 0.05. A probability error of 0.05 was taken into account.

Results and discussion

Commercial nano-silver film

Visual inspection revealed a homogeneous distribution of the coating material on the film. The presence of nano-silver particles on the coated film compared to the uncoated film was confirmed by scanning electron microscopy (Fig. 1b). EDX analysis showed in addition to silver (Ag), other elements such as copper (Cu), phosphate (P) and calcium (Ca) on the film (Fig. 1d). Probably the dispersion used for the silver coating also contains these substances. The electron microscopic picture of the nano-silver coated films after meat contact shows lower concentration and another arrangement of nano-silver particles than the picture of the film before meat contact (Fig. 2). The latter indicates a reaction of nano-silver particles caused by the meat surface or the drip.

Quality of unpacked meat

To characterize the raw material (ham) different parameters were determined 24 h post mortem (p.m.) prior to packaging (Table 1). As the ham consists of different muscles (e.g., *M. semimembranosus*, *M. biceps femoris*), we considered mainly studies investigating the ham or *M. semimembranosus* of the pigs. Although the already published data are very inconsistent, the pH results (5.49 ± 0.23) mainly correspond with data of Mörlein et al. (2007), Blacha et al. (2013), and Scheier et al. (2014) and the EC values (9.69 ± 2.96 mS/cm) with those of Cilla et al. (2006), and Blacha et al. (2013). The meat L* data (48.94 ± 3.40) mainly agree with results of Mörlein et al. (2007), and Tomovic et al. (2008) and the a* values (9.62 ± 1.61) with those of Cilla et al. (2006), and Tomovic et al. (2008). Concerning the low pH values (<5.5) and high EC results (>9 mS/cm) Honikel and Fischer (1977) and Mörlein et al. (2007), respectively, would categorize the meat as PSE (pale, soft, exudative) meat. Following the classification of Garrido and Honikel (1995) the L* values (<56) and the b* values (<5 , data not shown) do not indicate the analyzed cutlets as PSE meat.

Packaging experiments

The total viable count (TVC) concentrations increased from 2.20 to 2.71 log₁₀ CFU/g meat on day 4 to 5.60–6.34 log₁₀ CFU/g meat on day 12, whereas the *Pseudomonas* spp. and *Enterobacteriaceae* values rose from 0.91 to 1.81 on day 4 to 4.11–4.39 log₁₀ CFU/g meat on day 12 and from 0.00 to 0.08 on day 4 to 0.30–2.08 log₁₀ CFU/g meat on day 12, respectively (Table 2). As shown in Table 2 we could only detect a difference of the TVC results on day 12, the cutlets in MAP 1 and MAP 2 showed significantly ($P < 0.05$) higher TVC results compared to the vacuum packaged meat. Considering the *Enterobacteriaceae* and *Pseudomonas* spp., the vacuum packed cutlets showed significantly ($P < 0.05$) higher concentrations than the MAP 1 and MAP 2 stored products on day 4 (only *Enterobacteriaceae*) and day 8. However, no significant effect of the nano-silver film on the TVC, *Enterobacteriaceae* and *Pseudomonas* spp. counts during the 12 day storage period could be found. The little effects of the factor “packaging variant” are difficult to discuss and shouldn’t be overestimated. However, the TVC result partly agree with the data of Taylor et al. (1990) or Blacha et al. (2014) who found no TVC differences between pork meat stored in vacuum and high oxygen packages up to day 20 or 12, respectively. Sheridan et al. (1997) or Hayes et al. (2010) stored raw pork sausages or lamb meat, respectively, in 80% O₂ and vacuum and couldn’t find any differences of the TVC values, too. The *Pseudomonas* spp. results partly agree

Fig. 1 Electron microscopic pictures and energy-dispersive X-ray spectroscopic results of the uncoated (**a**, **c**) and nanosilver-coated PE-EVOH-PP layers (**b**, **d**); **a** bar = 2 μ m, **b** bar = 100 nm; **d** arrows showing the detection of silver (Ag)

with the results of Mastromatteo et al. (2009) and Sheridan et al. (1997) who also found no differences between *Pseudomonas* spp. counts stored in high oxygen and vacuum stored poultry patties and pork sausages. The higher quantities of *Enterobacteriaceae* in vacuum packages agree with the results of Li et al. (2014) who also found these effects in vacuum compared to MAP packed pork tenderloins during storage for 18 days. With regard to the effect of the silver coating, the results of other studies published are quite inconsistent. Some of them showed an antimicrobial effect on the drip or juice but not on the products. Fernández et al. (2009) found a significant reduction of the total aerobic and lactic acid bacteria counts in meat drip absorbed by the pad after contact of chicken meat with nanosilver loaded absorbent pads for 3 days. In another study Fernández et al. (2010) detected after storage of beef for up to 11 days in modified atmosphere packages (MAP) in contact with nanosilver loaded absorbent pads, significantly lower total aerobic bacteria and *Pseudomonas* spp. counts in the meat drip absorbed by pads, but not on the meat. Lloret et al. (2012) presented a reduction of bacteria counts in beef and poultry exudates (drip) and kiwi and melon juice after storage with nanosilver loaded

absorbent pads for 10 (kiwi, melon), or 11 days (beef, poultry). Azlin-Hasim et al. (2016) presented a reduction of the total aerobic and *Pseudomonas* spp. counts on the surface of chicken breast fillets wrapped in nanosilver coated films, whereas Marchiore et al. (2017) showed a reduction of the lactic acid bacteria content on chicken sausages immersed in nanosilver solution and stored for up to 30 days. Lee et al. (2011) found after contact of beef, pork and turkey meat with silver-zeolite loaded meat wrapping papers for 3 days no reduction of the *Pseudomonas putida* counts, whereas Gallochio et al. (2016) found no effect of commercial nanosilver coated plastic bags on the total viable and *Pseudomonas* spp. counts of chicken meat balls stored for 7 days. Similar results were shown by Deus et al. (2017) after storage of turkey breast meat in commercial nanosilver coated films for 12 days.

As in the microbiological investigation of study 1 only the “natural” contamination of the pork cutlets was considered, we additionally analyzed the effects of the different films using pork cutlets spiked with higher concentrations of *S. aureus* (Study 2). In this study, the number of *S. aureus* on the surface of the inoculation area, 10 min after spiking, ranged from 6.12 log₁₀ CFU/ml to

Fig. 2 Electron microscopic pictures of the nanosilver-coated PE-EVOH-PP layers before (a) and after (b) food contact; a bar = 400 nm, b bar = 1 μ m

Table 1 Least square means (LSM), standard deviation (SD), minimum and maximum values of different parameters of the pork cutlets before packaging

Parameter	LSM	SD	Minimum	Maximum
pH _{24 h p.m.}	5.49	0.23	5.00	6.38
EC _{24 h p.m.} (mS/cm)	9.69	2.96	4.25	13.25
L* _{24 h p.m.}	48.94	3.40	41.86	59.32
a* _{24 h p.m.}	9.62	1.61	6.03	13.30

n = 144

EC electrical conductivity, L* lightness, a* redness

6.96 log₁₀ CFU/ml (6.53 ± 0.24 log₁₀ CFU/ml). As the initial inoculation suspension contained a concentration of approximately 7.7 log₁₀ CFU/ml, we had a 1.0–1.5 log reduction of the bacteria concentration after spiking probably due to the dry swab method. However, as the reduction was comparable in all experiments, a systematic effect of the swab method could be considered. During storage no significant differences between the storage of *S. aureus*-spiked cutlets under nano-silver film or films without this

coating could be found (data not shown). On day 4, non-nano-silver coated cutlets contained 6.19 ± 0.27 log₁₀ CFU/ml and nano-silver-coated meat pieces 6.08 ± 0.55 log₁₀ CFU/ml, whereas on day 8 the non-silver packaged meat had 6.06 ± 0.64 log₁₀ CFU/ml and the nano-silver wrapped cutlets 6.16 ± 0.67 log₁₀ CFU/ml. We stopped the study at day 8, as longer stored cutlets halves showed advanced deterioration. The data indicate that the concentrations of silver ions migrating to the meat surface were too low to impact bacterial counts. It could be assumed that the majority of silver migrates to the drip reducing an antimicrobial effect impact on the meat surface. This assumption is supported by Fernández et al. (2010), who also found an effect of silver on the microbial content of the drip but not on the meat.

With regard to the meat color on all days, the L* values were significantly ($P < 0.05$) higher in the MAP than in the vacuum packages. On day 4, significantly ($P < 0.05$) higher L* values could be detected for the non-nano-silver film packages (Table 3). The effects of the “packaging variant” agree with the results of Veberg et al. (2006) in minced pig and turkey, Gómez and Lorenzo (2012) in foal and Blacha et al. (2014) and Deus et al. (2017) in turkey meat. However, Mastromatteo et al. (2009) and Blacha et al. (2013) couldn’t find any L* differences between vacuum- and high-oxygen -packaged poultry patties and pork cutlets, respectively. The effect of the “silver coating” shouldn’t be overestimated, as the significant difference didn’t persist during further storage. Azlin-Hasim et al. (2015, 2016) and Deus et al. (2017) also found no clear impact of a nanosilver film on the L* values of chicken or turkey meat. Only on day 4, significantly ($P < 0.05$) higher a* results of the MAP compared to the vacuum packed cutlets could be found, whereas no influence of the “silver coating” was obtained (Table 3). The small impact of the “packaging variant” partly agrees with the results of Veberg et al. (2006), who also found no clear influence of the high oxygen and vacuum packaging on the surface a* values of minced pig and turkey meat. However, Blacha et al. (2013) found generally higher a* results in high-oxygen compared to vacuum packaged turkey and pig meat and Martínez et al. (2006) in pork sausages. Orkus et al. (2013) and Deus et al. (2017) presented the opposite effect with higher a* values in vacuum packaged goose and turkey meat, respectively. The results of the nanosilver coated film mainly agree with Azlin-Hasim et al. (2015, 2016) who only found significantly higher a* values of the nanosilver wrapped chicken meat on day 6 respectively day 3 of storage. However, Deus et al. (2017) showed that the nanosilver coated film significantly decreased the a* values of the turkey meat on days 1, 4 and 8. The generally inconsistent color results in this study and other publications are difficult to compare in particular the

Table 2 Least square means (LSM) and standard error of the means (SEM) of total viable count (TVC), *Pseudomonas* spp. and *Enterobacteriaceae* counts of pork cutlets depending on packaging variant and storage time

Parameter	Day	V 1	MAP 1	V 2	MAP 2	SEM	P	S	P × S
TVC	4	2.46	2.63	2.71	2.20	0.11	NS	NS	NS
	8	5.55	5.52	5.15	5.21	0.12	NS	NS	NS
	12	5.91	6.34	5.60	6.15	0.10	*	NS	NS
<i>Pseudomonas</i> spp.	4	1.81	1.26	1.54	0.91	0.18	NS	NS	NS
	8	4.30	3.76	4.55	3.78	0.13	*	NS	NS
	12	4.34	4.13	4.11	4.39	0.11	NS	NS	NS
<i>Enterobacteriaceae</i>	4	0.06	0.08	0.00	0.00	0.03	NS	NS	NS
	8	1.63	0.03	1.07	0.15	0.16	*	NS	NS
	12	2.08	1.20	1.50	0.30	0.21	*	NS	NS

All data in log₁₀ colony forming units/g meat; V 1: vacuum and non-Nano-Ag-layer, MAP 1: 80% O₂, 20% CO₂ and non-Nano-Ag-layer, V 2: vacuum and Nano-Ag-layer, MAP 2: 80% O₂, 20% CO₂ and Nano-Ag-layer; effects of packaging variant (P), silver coating (S), and their interaction (P × S) were significant (* $P < 0.05$) or not significant (NS); n = 12 cutlets per packaging method and day

Table 3 Least square means (LSM) and standard error of the means (SEM) of lightness (L*) and redness (a*) results of pork cutlets, determined through the wrapped layer and the vacuum bag, depending on the packaging method and storage time

Parameter	Day	V 1	MAP 1	V 2	MAP 2	SEM	P	S	P × S
L*	4	50.00	53.94	49.04	52.70	0.24	*	*	NS
	8	50.29	53.52	49.51	53.03	0.27	*	NS	NS
	12	50.97	53.97	51.15	53.40	0.32	*	NS	NS
a*	4	6.83	7.47	7.17	7.29	0.09	*	NS	NS
	8	6.99	7.42	7.16	7.05	0.11	NS	NS	NS
	12	7.36	7.33	7.45	7.29	0.17	NS	NS	NS

V 1: vacuum and non-Nano-Ag-layer, MAP 1: 80% O₂, 20% CO₂ and non-Nano-Ag-layer, V 2: vacuum and Nano-Ag-layer, MAP 2: 80% O₂, 20% CO₂ and Nano-Ag-layer; effects of packaging variant (P), silver coating (S), and their interaction (P × S) were significant (* $P < 0.05$) or not significant (NS); numbers of cutlets per packaging method: days 1 and 4, n = 48; day 8, n = 32; day 12, n = 16 cutlets per packaging method

lack of clear a* differences, although L* values of the high oxygen and vacuum stored cutlets vary significantly. As myoglobin (Mb) is the principle protein responsible for the color of the meat (Mancini and Hunt 2005), we also analyzed the Mb redox form percentages of the meat to clarify the color effects.

The percentages of the myoglobin (Mb) redox forms were influenced by the packaging variant and the silver coating (Table 4). Vacuum packaged pork cutlets had significantly ($P < 0.05$) higher OxyMb and lower MetMb percentages on day 8 and 12 compared to the MAP cutlets. The OxyMb percentages were significantly ($P < 0.05$) higher in the nano-silver stored cutlets on day 4 and 12, whereas on day 8 the results were opposite. On day 4, the MetMb and DeoxyMb percentages were significantly ($P < 0.05$) lower in nano-silver stored cutlets. During further storage, the MetMb and DeoxyMb percentages in these packages were higher ($P < 0.05$) on day 8 and again lower ($P < 0.05$) on day 12 in comparison to the cutlets wrapped in non-nano-silver films. Considering the effects of the packaging variant, Martínez et al. (2006) or Orkusz et al. (2013) also presented higher MetMb and

lower OxyMb ratios in high O₂ compared to vacuum stored fresh pork sausages or goose meat. Zakrys et al. (2008) support our results showing a negative correlation between O₂ and OxyMb content. The data indicate that O₂ in the MAP not only influences oxygenation of Mb, accompanied with an increase of OxyMb, but to a higher extent oxidation of Mb and formation of MetMb. From this assumption it could be suggested that in the present study the missing a* differences between MAP and vacuum stored cutlets on days 8 and 12 are related to the higher MetMb percentages in the MAP stored pork. This is supported by Martínez et al. (2006) who presented that after an increase of the MetMb ratio in high oxygen stored pork sausages a clear reduction of the a* values of this products could be found. From the presented effects of the silver coating, in particular the differences between day 8 and the other days, it could be suggested that silver has an influence not only on the oxidation, but also on the oxygenation of the Mb molecules. However, as the results are inconsistent during storage, they are difficult to generalize, indicating no clear impact of the silver coating on the myoglobin results.

Table 4 Least square means (LSM) and standard error of the means (SEM) of the percentages of the different myoglobin redox forms of the pork cutlets depending on the packaging variant

Parameter	Day	V 1	MAP 1	V 2	MAP 2	SEM	P	S	P × S
Oxy-myoglobin (%)	4	48.74	46.28	55.48	50.32	1.29	NS	*	NS
	8	58.80	52.94	54.98	46.54	1.25	*	*	NS
	12	47.63	34.34	55.78	42.85	1.79	*	*	NS
Met-myoglobin (%)	4	37.37	39.87	33.86	38.73	0.84	NS	*	NS
	8	30.85	36.54	33.58	40.62	0.94	*	*	NS
	12	37.52	48.71	32.74	42.87	1.26	*	*	NS
Deoxy-myoglobin (%)	4	13.34	13.36	9.89	10.30	0.49	NS	*	NS
	8	9.51	9.76	10.72	12.28	0.38	NS	*	NS
	12	14.23	16.58	10.66	13.76	0.70	NS	*	NS

V 1: vacuum and non-Nano-Ag-layer, MAP 1: 80% O₂, 20% CO₂ and non-Nano-Ag-layer, V 2: vacuum and Nano-Ag-layer, MAP 2: 80% O₂, 20% CO₂ and Nano-Ag-layer; effects of packaging variant (P), silver coating (S), and their interaction (P × S) were significant (* $P < 0.05$) or not significant (NS); n = 12 cutlets per packaging method and day

There was a significantly ($P < 0.05$) higher liquid loss of the vacuum stored pork cutlets compared to the MAP products on all days (data not shown). This effect of the vacuum storage was also presented by Huang et al. (2005) in pork and by Blacha et al. (2013) in pig and turkey meat. The higher liquid loss might be due to physical compression during packaging. No effect of the packaging variant and the silver coating on the pH and EC results could be found (data not shown). The pH results correspond to those of Huang et al. (2005), Martínez et al. (2006) and Blacha et al. (2013) who also couldn't observe pH differences during storage of pork, pork sausages and turkey meat in vacuum and high-oxygen packages. Blacha et al. (2013) also couldn't find impacts of vacuum and high oxygen packaging on pork and turkey meat on the EC results.

Conclusion

The results of our study show that storage of pork slices in MAP is more advantageous in comparison to vacuum packages due to better appearance and lower liquid loss. However, MAP results in only low benefit of microbial spoilage compared to vacuum packed meat. The silver-coating of the film which was clearly proven by different analytical methods, but the silver has no impact on microbial spoilage of pork during storage, especially after spiking the cutlets with high *S. aureus* concentrations. Only the Mb redox form percentages on all days and the L* values on day 4 were influenced by the silver-coating, but the Mb redox form effects were inconsistent. Therefore, the use of the commercial nano-silver-coated films for meat storage seems to be useless.

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