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Production of antimicrobial biobased packaging and application in sliced cooked ham

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ABSTRACT. In this work, active sheets composed by thermoplastic starch and poly (lactic acid) (PLA) coated with silver nanoparticles (AgNPs) were obtained. The mechanical properties and water vapor permeability of the sheets were not affected by the presence of the silver nanoparticles. As a proof of concept, the sheets were applied to pack sliced cooked ham for 7 days at 10°C. Migration of metallic silver from the sheets to the sliced ham was detected in a considered safe concentration, according to literature data, by ICP-MS. The sheets coated with AgNPs were able to significantly hinder psychrotrophic and mesophilic bacteria growth during 7 days of storage when compared to the control sample (sheets without AgNPs). Furthermore, lipid oxidation occurred in a higher proportion in the ham packaged with AgNPs, probably due to the catalyst effect of silver. It may be concluded that the sheets composed by starch and PLA acted as an effective support for the AgNPs, as well as an active packaging for sliced cooked ham.

Keywords: thermoplastic starch; poly (lactic acid); silver nanoparticle; extrusion; food conservation.

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Introduction

Packages produced from biopolymers allow the incorporation of additives, such as antioxidants, antimicrobials, colorants, and other nutrients. Biobased polymers such as thermoplastic starch (TPS), poly (lactic acid) (PLA) and their respective blends are preferred due to environmental concerns and also because they can operate as support for active compounds, in addition to protect foodstuff from external agents. In the case of antimicrobial agents, active packages may act increasing products shelf life, as well as maintaining their sensory and nutritional properties (Pizzoli et al., 2016; Singh, Jairath, & Ahlawat, 2016). Antimicrobial films and coatings act inhibiting microbial growth on food surface, since there is a direct contact with the package (Appendini & Hotchkiss, 2002; Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Nano-food packaging based on metallic nanocomposites with antimicrobial properties represent a new generation of active packages (Panea, Ripoll, González, Fernández-Cuello, & Albertí, 2014). Among the metallic antimicrobial agents of interest to active packaging, silver nanoparticles (AgNPs) have been highlighted due to the ease of production and the broad antimicrobial activity spectrum including gram-negative and gram-positive bacteria, fungi, protozoa and certain viruses (Kumar & Münstedt, 2005; Onitsuka, Hamada, & Okamura, 2019). The AgNPs application in different products have been extensively studied, such as to edible films and food products (An, Zhang, Wang, & Tang, 2008; Marchiore et al., 2017), to cotton cloths (Onitsuka et al., 2019), and as antimicrobial films and packages (Braga, Pérez, Soazo, & Machado, 2019; Hannon et al., 2018; Pizzoli et al., 2016), showing that there is a potential for AgNPs application in foodstuff preservation.

Sliced meat products are usually produced aiming at consumer´s easy manipulation, however, slicing increases the exposed surface area that could lead to improved discoloration, rancidity and microbial deterioration (Fernandes et al., 2015). Antimicrobial coated packaging would be of interest for sliced

cooked ham and other meat because in such cases microbiological contamination occurs primarily in the food surface (Muppalla, Kanatt, Chawla, & Sharma, 2014; Pizzoli et al., 2016). Different materials containing antioxidant and antimicrobial compounds were already applied to meat products as active packagings, such as low density polyethylene (LDPE) containing antioxidants plant extracts (rosemary extract, oregano essential oil and green tea extract) applied to beef and foal meat (Barbosa-Pereira, Aurrekoetxea, Angulo, Paseiro-Losada, & Cruz, 2014; Lorenzo, Batlle, & Gómez, 2014); carboxymethyl cellulose and poly (vinyl alcohol) films containing clove oil applied to chicken ground meat (Muppalla et al., 2014); and LDPE nanocomposites made with zinc oxide and silver nanoparticles (AgNPs) to preserve chicken breast fillets (Azlin-Hasim, Cruz-Romero, Morris, Cummins, & Kerry, 2015; Panea et al., 2014). However, the use of TPS/PLA blends as a support to AgNPs deserves investigation due to its potential in packaging sliced meat products.

Migration studies are also on demand to determine if silver would be found in the food itself after long storage times (Hannon et al., 2018). Investigation on the use of silver nanoparticles is important due to the concerns about safety and the effects of silver ions on the body (Dąbrowska-Bouta et al., 2016; Liu, Guan, Ren, & Yang, 2012). In this work, TPS/PLA sheets coated with AgNPs were obtained and applied to sliced cooked ham. The silver nanoparticles and the polymer films were characterized in relation to their technological properties, as well as the physicochemical and color properties of the packaged sliced ham during 7 days. The migration of silver from the polymer sheets to the sliced ham and microbiological growth (psychrotrophic and mesophilic bacteria) were also evaluated.

Material and methods

Materials

Soluble starch (Merk, Germany), D-glucose (Isofar, Brazil) and silver nitrate (Proquímios, Brazil) were used for silver nanoparticles synthesis. The sheets were produced with cassava starch (Indemil, Brazil), glycerol (Dinâmica, Brazil) and PLA Ingeo 4043D (Natureworks LLC, Cargill, USA). Sliced ham was acquired in the local market of Campo Mourão, PR, Brazil. Malonaldehyde bis (dimethyl acetal) (1,1,3,3–tetramethoxypropane, TMP), 2-thiobarbituric acid (TBA), propyl gallate (Sigma Aldrich, Germany), trichloroacetic acid (TCA) and ethylene diamine tetra acetic acid (EDTA) (Vetec, Brazil) were used in thiobarbituric acid-reactive substance (TBARS) assay. Plate count agar (Biomark, Brazil) was used for microbiological analysis.

Silver nanoparticles synthesis and characterization

AgNPs were synthesized according to the procedure described in a previous study (Pizzoli et al., 2016). Briefly, a silver nitrate aqueous solution (2 mL, 25 mM) was mixed with a starch solution (50 mL, 1 wt%) and an aqueous D-glucose solution (4 mL, 25 mM). After that the mixture was autoclaved (121 ºC, 15 psi, 15 min) resulting in a silver colloidal nanoparticles dispersion. The plasmon absorption analysis was performed using an UV–Vis spectrophotometer (Ocean Optics, USB650UV, USA) and the full-width at half maximum (FWHM) was determined to estimate nanoparticles size.

TPS/PLA sheets production and AGNPs coating procedure

The procedure adopted to obtain the extruded sheets was described in details by (Pizzoli et al., 2016). First, a mixture of 50 wt% PLA, 37.5 wt% starch and 12.5 wt% glycerol was extruded as cylindrical profiles in a single-screw extruder (BGM, EL-25 model, Brazil, screw diameter of 25 mm, screw length of 28 D, screw speed of 30 rpm, and temperature profile of 90/180/180/180 ºC at the four heating zones). Then, the cylindrical profiles were pelletized and extruded again in a pilot co-rotating twin-screw extruder (BGM, D-20 model) coupled with a calender (AX-Plasticos, Brazil, screws diameter (D) of 20 mm, screws length of 35 D, temperature profile of 100/170/170/170/175ºC, screw speed of 100 rpm, and feed speed of 30 rpm) for sheet production. In the calender, the distance between the rolls was equal to 0.8 mm.

To coat the TPS/PLA sheets surface with the synthesized AgNPs, the obtained AgNPs solution was diluted with sterile distilled water to 37.5 μg mL^{-1}. This concentration was chosen since it corresponds to Minimum Inhibitory Concentration (MIC) determined previously (Pizzoli et al., 2016) against *Staphylococcus aureus* (ATCC 6538). The sheets (10 cm x 20 cm) were dipped into AgNPs solution for 30

seconds and dried in an air convection oven (Cienlab, Brazil) at 40°C for 12h. Control sheets were dipped in distilled water following the procedure described above.

Sliced cooked ham packaging

The slices of cooked ham (~3 mm thickness) were placed on the surface of the TPS/PLA sheets (coated with AgNPs and control) immediately after purchasing. For each sheet two slices (20 g each) were placed side by side, as showed in Figure 1. After that, a poly(vinyl chloride) film was used to wrap the samples, which were stored in a refrigerator at 10°C for 7 days to simulate a commercial condition. The experiment was carried out in triplicate.

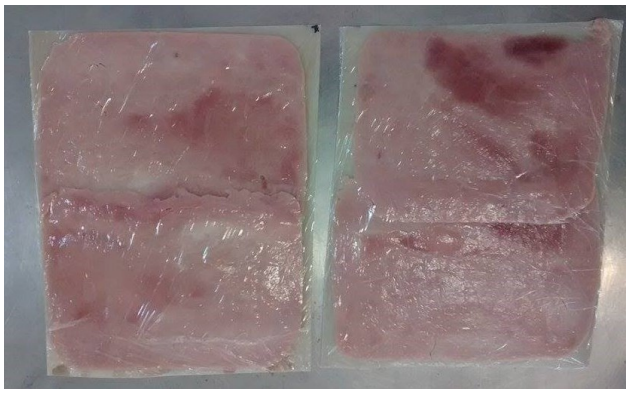

Figure 1. Sliced ham packaged in TPS/PLA biodegradables sheets containing AgNPs.

Migration of silver from TPS/PLA sheets to the sliced cooked ham

Metallic silver migration from the sheets to the sliced cooked ham was evaluated during the storage period (0, 3 and 7 days) in triplicate. For the initial time (0 day) the sliced cooked ham was kept in contact with the package for 15 min. The sliced cooked ham samples (40 g) were crushed in a domestic blender with distilled water (1 L), centrifuged (Mini Spin Plus Eppendorf centrifuge, Germany, 30 min at 14,500 rpm), then the supernatant was collected and analyzed by Inductive Coupled Plasma-Mass Spectroscopy (ICP-MS, Perkin Elmer, NexIon 300 D, Shelton, USA) (Marchiore et al., 2017). The procedure was carried out in triplicate and the results were expressed as $ng_{Ag}\ g_{cooked\ ham}^{-1}$.

TPS/PLA extruded sheets characterization

Sheet thickness was determined with the use of a digital micrometer (Starrett, 0.001 mm resolution). Ten random points were measured from each sample. Tensile strength tests were done in a texturometer Stable Micro Systems, model TA-TX2, determining tensile strength (MPa) and elongation at break (%) according to the American Society for Testing and Material (American Society for Testing and Materials, 2001). The test was performed ten times for each treatment. Water vapor permeability (WVP) was obtained according to American Society for Testing and Material (American Society for Testing and Materials, 2000) using appropriate aluminum diffusion cells, with a relative humidity (RH) of 2% inside the cell and 75% outside the cell. All tests were carried out in triplicate. Mechanical properties and water vapor permeability (WVP) were determined on the sheets before being in contact with the sliced ham and after 7 days of contact with sliced ham.

The AgNPs-coated sheet was evaluated by Scanning Electron Microscopy (SEM, Philips, FEI Quanta 200, Japan) at 20 kV. Samples were dried (14 days in desiccator with silica) and gold coated (Sputter Coater, BAL-TEC, SCD-050, Balzers, Germany) before analysis.

Physicochemical and microbiological evaluation of packaged sliced cooked ham

Instrumental color parameters L*, a* and b* of the sliced ham was determined using a Mini Scan EZ (Hunter Lab, USA) at ten random points on the sample surface. A contact potentiometer (Testo, Brazil) was used to obtain pH values in triplicate. For lipid oxidation, thiobarbituric acid-reactive substances (TBARS) assay was carried out in triplicate according to the procedure described by Bruna, Ordóñez, Fernández, Herranz, and de la Hoz (2001) with minor modifications. Sliced ham samples (5 g) were mixed with 25 mL of TCA solution (7.5 wt/v% TCA, 0.1 wt/v% propyl gallate and 0.1 wt% EDTA) and

homogenized in a blender for 30 seconds. After filtration, 5 mL of the filtrate was added to 5 mL TBA solution (0.02 mol/L) in a test tube. The test tubes were incubated in boiling water for 40 min. Then, absorbance was measured at 538 nm using a UV–Vis spectrophotometer (Ocean Optics, USB650UV, USA). TBARs value was expressed as $mg_{malonaldehyde}$ $Kg_{product}^{-1}$.

For the microbiological evaluation, 25 g of each sample (in triplicate) were homogenized in saline solution (0.85 wt%) with 0.1 wt% peptone in a stomacher (ITR, MR1204, Brazil). Appropriate dilutions of the sample homogenates were prepared in sterile peptone water (0.1 wt%) and inoculated in triplicate into growth media plates (Plate Count Agar, PCA) to estimate microbial counts. Counting of psychrotrophic bacteria were performed incubating the PCA plates at 7°C for 10 days (Silva et al., 2007). For mesophilic bacteria, samples were plated in depth in PCA and incubated in a bacterial culture incubator (Ethik, Brazil) under aerobic conditions at 35 °C for 48 h. Counting results were expressed as log CFU/g.

Statistical analysis

The obtained results were evaluated using Student's t-test analysis, ANOVA and Tukey's test at the 5% significance level ($p < 0.05$) using the software Statistica 7.0 (Statsoft, USA).

Results and discussion

Silver nanoparticles and sheets characterization

Figure 2A presents the UV-Vis absorbance spectra of metal nanoparticles and a SEM surface image of the AgNPs-coated TPS/PLA sheet is presented in Figure 2B.

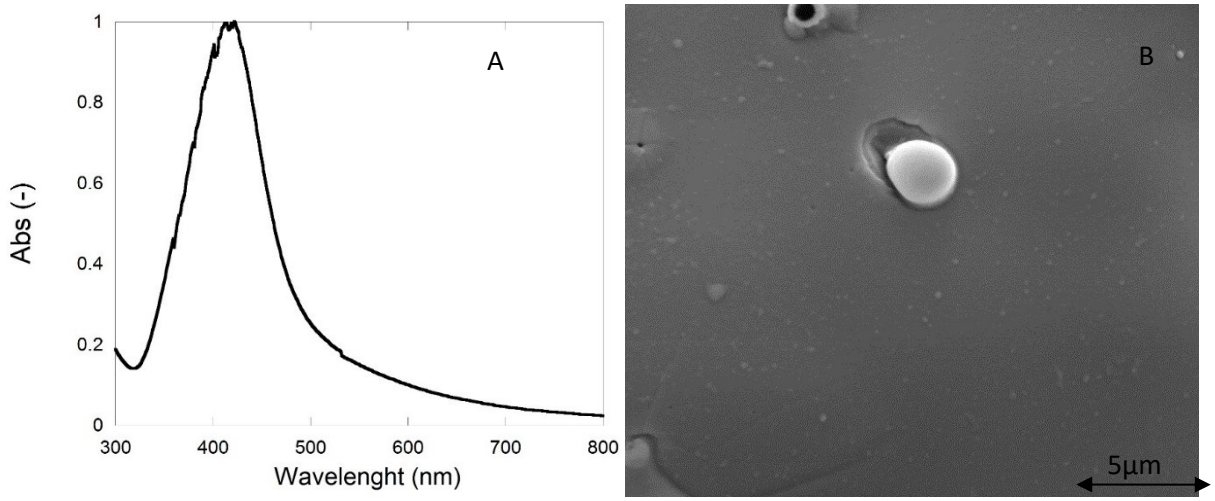

Figure 2. (A) UV–Vis spectrum of the AgNPs colloidal suspension and (B) surface Scanning Electron Microscopy image of TPS/PLA sheets with AgNPs (magnification 12,000x).

Formation of the AgNPs was denoted by the solution of synthesis color change, forming a yellowish-brown color. The characteristic surface plasmon absorption band may be seen at 420 nm (Figure 2) and the FWHM diameter was calculated as being 96 nm. According to Bankura et al. (2012), AgNPs absorb radiation at visible range (380-450 nm) and a well-defined peak is an indicative of an adequate degree of dispersion, as well as of nanoparticles with spherical shape and narrow sizes distribution.

Control and AgNPs-coated sheet presented average thickness of 463 ± 23 µm and 444 ± 21 µm, respectively and no significant difference between them was detected ($p > 0.05$). It is possible to observe in the SEM image the presence of starch granules with approximately 2.4 µm diameter, as well as AgNPs agglomerates with approximately 230 nm diameter. The nanoparticles-coated sheet presented a homogeneous surface indicating that the dipping method followed by drying was adequate to incorporate the silver nanoparticles.

Table 1 presents the mechanical properties (elongation at break and tensile strength), as well as the Water Vapor Permeability (WVP) results of the TPS/PLA extruded sheets before and after 7 days of contact with sliced ham.

Table 1. Mechanical properties and water vapor permeability results of TPS/PLA control sheets (no AgNPs added) and with AgNPs-loaded sheets before (T_0) and after 7 days (T_7) of contact with sliced ham.

	Tensile Strengh (MPa)		Elongation at break (%)		WVP (x 10^6 g.(m.day.Pa)$^{-1}$)	
Sample	T_0	T_7	T_0	T_7	T_0	T_7
Control	$17.9^{aA} \pm 2.2$	$21.5^{aA} \pm 3.6$	$34.3^{aA} \pm 4.8$	$3.4^{aB} \pm 0.8$	$1.55^{aA} \pm 0.16$	$2.37^{aB} \pm 0.13$
AgNP	$19.8^{aA} \pm 1.4$	$21.8^{aA} \pm 2.0$	$28.9^{aA} \pm 6.4$	$3.6^{aB} \pm 0.5$	$1.81^{aA} \pm 0.06$	$2.69^{aB} \pm 0.59$

Average ± standard deviation, treatments conducted in triplicate; ^{a,b} Different letters in the same column indicate significant difference ($p < 0.05$) by Student's t-test between the treatments in the same time; ^{A,B} Different letters in the same line indicate significant difference ($p < 0.05$) by Student's t-test between the storage time for the same treatment.

No significant differences were found between control and AgNPs-coated sheets immediately their preparation ($p > 0.05$) in the mechanical properties, which is in accordance with previous findings (Pizzoli et al., 2016). A significant elongation at break reduction was detected after 7 days storage in contact with the sliced ham in both cases, suggesting that the materials became more rigid. On the other hand, tensile strength values were statistically the same ($p > 0.05$). The reduction on the elongation at break probably is related to the contact between the packaging material and the exudate generated by the sliced ham. The ham, as other meat products, present water loss as a product characteristic (Pereira, Soares, Monteiro, Gomes, & Pintado, 2018). Water can lead to a plasticizing effect on TPS, in this case probably water from the sliced ham exudate modified TPS microstructure, changing its compatibility with PLA. Since TPS is a highly hydrophilic polymer, and PLA has a hydrophobic character, this different polarity is responsible of the lack (or very low) affinity between these two polymers and this leads to a phase separation (Ferri, Garcia-Garcia, Sánchez-Nacher, Fenollar, & Balart, 2016). Thus, the significant reduction on the elongation at break of the sheets before and after being used to pack the sliced ham must be associated with the reduction on the compatibility between PLA and TPS.

WVP increased 44 and 53% for the AgNPs-coated and control sheets, respectively, after 7 days in contact with sliced ham. Since cooked ham usually presents high water activity ($a_w = 0.98$) (Garriga, Grèbol, Aymerich, Monfort, & Hugas, 2004), it is possible that water had migrated to the sheets due to the hydrophilic character of starch. Starch microstructure is prone to change throughout storage time (Cano et al., 2015) which may be accelerated by the swelling of the polymer chains. Polymer recrystallization and the modification in chain conformation may explain the changes in mechanical properties. Changes in free volume within the microstructure could also lead to increased water vapor permeation.

Migration of AgNPs from the sheets to the sliced ham

Migration of AgNPs from PLA/TPS sheet to sliced cooked ham was evaluated during 7 days of storage using ICP-MS as presented in Figure 3.

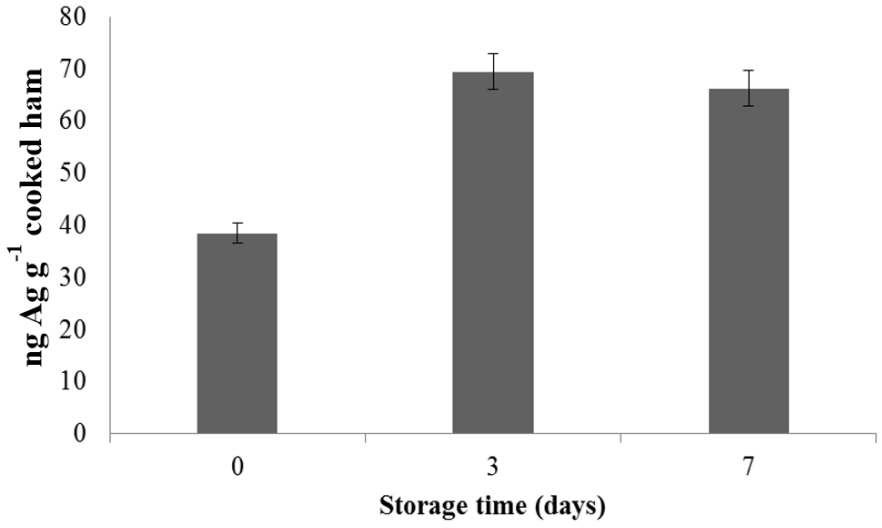

Figure 3. Metallic silver concentration determined in the sliced cooked ham during the storage time (0 days, represents 15 min of contact).

The concentration of AgNPs in the cooked ham increased in the first 3 days of storage time and remained constant until complete 7 days. Migration of silver from the sheets to the cooked ham may be favored by the high water content of the cooked ham. This is an important finding because the amount of silver in foodstuff must be controlled due to toxicological reasons. In fact, legislation regarding silver content only permits a concentration of 0.05 mg_{Ag} Kg_{food}^{-1} (Fernández, Soriano, Hernández-Muñoz, & Gavara, 2010) meaning that the amount of AgNPs that migrated from sheet to cooked ham may be considered safe. Acute oral toxicity of AgNPs in rats has been investigated (Siqueira et al., 2013) and liver cells degeneration was observed for 1 mg L^{-1} silver concentration. Authors claimed that this concentration must be restricted to packaging films and not to edible coatings.

Sliced cooked ham microbiologic analysis

Table 2 presents the total count of psychrotrophic and mesophilic microorganisms of the sliced ham.

Table 2. Microbiologic evaluation of sliced ham packaged with TPS/PLA control sheets (no AgNPs added) and with AgNPs-loaded sheets for 7 days storage time.

Storage time	Psychrotrophic (log CFU g^{-1})		Mesophilic (log CFU g^{-1})	
(days)	AgNP	Control	AgNP	Control
0	$6.00^{aA} \pm 0.01$	$6.11^{bA} \pm 0.04$	$4.15^{aA} \pm 0.24$	$4.16^{aA} \pm 0.24$
3	$6.90^{aB} \pm 0.01$	$6.95^{aB} \pm 0.03$	$5.61^{aB} \pm 0.01$	$5.63^{aB} \pm 0.03$
7	$7.70^{aB} \pm 0.01$	$8.16^{bC} \pm 0.06$	$7.87^{aC} \pm 0.06$	$8.62^{bC} \pm 0.02$

Average ± standard deviation, treatments conducted in triplicate; ^{a,b} Different letters in the line indicate significant difference ($p < 0.05$) by Student's t test. ^{A,B} Different letters in column indicate significant differences ($p < 0.05$) by Tukey test.

Both psychrotrophic and mesophilic counts did not differ until the 3rd day storage. High values for an initial time of evaluation were found when compared to literature data (Bressan et al., 2007) for vacuum packed ham. Also, proliferation of microorganisms even at low temperatures (10 °C) occurs in ham due to favorable conditions like pH (above 0) and a_w (above 0.94) (Lloret, Picouet, Trbojevich, & Fernández, 2016). For the control sheets, psychrotrophic and mesophilic counts increased by approximately 1 log cycle at the 7th day storage when compared to the 3rd day. On the other hand, the microbial count remained constant in packages containing AgNPs, confirming that AgNPs remained active after its binding in the sheets surface (An et al., 2008; Azlin-Hasim et al., 2015; Panea et al., 2014). Also, the high water content of sliced ham could have favored nanoparticles mobility and action. It is known that low amounts of silver are required to achieve biocidal effects in aqueous media (Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012) and these results are important because psychrotrophic bacteria, especially *Leuconostoc* and *Pediococcus*, are capable of producing multiple modifications on foodstuff such as slime (mainly exopolysaccharides), gases, lactic acid, ethanol and small amounts of short chain fatty acids (Abreu et al., 2015).

Sliced cooked ham physicochemical characterization

Results obtained for pH, color (L*, a* and b*) and lipid oxidation (TBARS) from packaged sliced hams in biodegradable antimicrobial sheet for 7 days are shown in Table 3.

Table 3. pH, color and lipid oxidation results of sliced ham sliced ham packaged with TPS/PLA control sheets (no AgNPs added) and with AgNPs-loaded sheets for 7 days storage time.

Parameter	Treatment	Storage times (days)		
		0	3	7
pH	Control	$6.24^{C} \pm 0.01$	$5.35^{aB} \pm 0.23$	$5.20^{aA} \pm 0.05$
	AgNP		$5.51^{bB} \pm 0.12$	$5.37^{bA} \pm 0.09$
L*	Control	$60.49^{A} \pm 6.75$	$62.93^{bB} \pm 1.48$	$62.47^{bB} \pm 1.87$
	AgNP		$60.75^{aA} \pm 3.03$	$60.25^{aA} \pm 2.58$
a*	Control	$13.61^{A} \pm 3.28$	$12.96^{aA} \pm 1.57$	$16.86^{aB} \pm 2.35$
	AgNP		$14.19^{bA} \pm 1.24$	$17.81^{aB} \pm 1.32$
b*	Control	$12.31^{A} \pm 0.96$	$12.88^{aA} \pm 1.22$	$21.00^{aB} \pm 2.32$
	AgNP		$12.43^{aA} \pm 0.93$	$20.16^{aB} \pm 1.92$
Lipid oxidation (mg MDA kg^{-1} ham)	Control	$0.6934^{A} \pm 0.003$	$2.1910^{aB} \pm 0.204$	$3.5302^{aC} \pm 0.102$
	AgNP		$2.6590^{bB} \pm 0.081$	$5.4022^{bC} \pm 0.122$

Average ± standard deviation, treatments conducted in triplicate; ^{A,B} Different letters on the line indicate significant difference ($p < 0.05$) by Student's t test. ^{a,b} Different letters in column indicate significant differences ($p < 0.05$) by Tukey test.

The initial pH found for both samples was equal to 6.2 which is close to that reported by Han et al. (2011) (pH = 6.40) for sliced ham treated by high hydrostatic pressure. The pH values decreased during storage and this effect was more pronounced in control samples possibly due to the growth of psychrotrophic bacteria (Lorenzo et al., 2014). This result is consistent with those obtained for psychrotrophic and mesophilic count (Table 2).

Luminosity (L*) presented statistical difference between samples packaged with the control sheet and with AgNPs treated sheet ($p < 0.05$). Control sample presented an increase of luminosity during storage time but this effect was not detected in the case of the AgNPs-coated sheets, suggesting that AgNPs avoid the ham browning. A similar lightening behavior was verified by Azlin-Hasim et al. (2015) in chicken breast fillets packaged with conventional and AgNPs-coated films. Increase in luminosity can be related to the conversion of myoglobin into metamyoglobin due to the low oxygen pressure induced by the package that could modify the incident light reflection and to protein conformational changes (Veeck, Boligon, Athayde, & Emanuelli, 2013). This is in accordance with the findings that the presence of AgNPs influenced water vapor permeation (Table 1) since an increase in the free volume of material structure could also be associated with an increase in the oxygen permeability. In the same manner, parameters a* and b* presented significant increase ($p < 0.05$) after 7 days of storage which could also be related to meat discoloration and the accumulation of metamyoglobin at the meat surface (Wambura & Verghese, 2011).

Lipid oxidation of the sliced ham significantly increased ($p < 0.05$) for control and AgNPs-coated sheets, however oxidation was higher in the case of the sheets containing the nanoparticles. This behavior has already been described for chicken breasts in low density polyethylene containing AgNPs (Panea et al., 2014), and also AgNPs applied directly on chicken sausages (Marchiore et al., 2017).

Conclusion

TPS/PLA sheets characterization demonstrated that mechanical properties and WVP were not affected by the presence of the AgNP meaning that the surface deposition technique was appropriated to create bioactive films without impacting mechanical performance. Silver was found to migrate to the ham in small amounts, and remained below maximum values suggested by the literature. The lipid oxidation increased due to the presence of the AgNPs as expected, while ham luminosity remained unchanged when AgNPs were added to the sheets. The nanoparticles-coated sheets were more effective than control sheets to control psychrotrophic and mesophilic bacteria growth.

Acknowledgements

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References

Abreu, A. S., Oliveira, M., Sá, A., Rodrigues, R. M., Cerqueira, M. A., Vicente, A. A., & Machado, A. V. (2015). Antimicrobial nanostructured starch based films for packaging. *Carbohydrate Polymers*, *129*, 127-134. doi: 10.1016/j.carbpol.2015.04.021

American Society for Testing and Materials. (2000). Standard test methods for water vapor transmission of materials ASTM E96-00. In American Society for Testing and Materials, *Annual book of ASTM*. Philadelphia, PA: Author.

American Society for Testing and Materials. (2001). Standard test methods for tensile properties of thin plastic sheeting D882-00. In American Society for Testing and Materials, *Annual book of ASTM*. Philadelphia, PA: Author.

An, J., Zhang, M., Wang, S., & Tang, J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *LWT – Food Science and Technology*, *41*(6), 1100-1107. doi: 10.1016/j.lwt.2007.06.019

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies*, *3*(2), 113-126. doi: 10.1016/S1466-8564(02)00012-7

Azlin-Hasim, S., Cruz-Romero, M. C., Morris, M. A., Cummins, E., & Kerry, J. P. (2015). Effects of a combination of antimicrobial silver low density polyethylene nanocomposite films and modified atmosphere packaging on the shelf life of chicken breast fillets. *Food Packaging and Shelf Life*, *4*, 26-35. doi: 10.1016/j.fpsl.2015.03.003

Bankura, K. P., Maity, D., Mollick, M. M. R., Mondal, D., Bhowmick, B., Bain, M. K., Chackraborty, A., Sarkar, J., Acharya, K., & Chattopadhyay, D. (2012). Synthesis, characterization and antimicrobial activity of dextran stabilized silver nanoparticles in aqueous medium. *Carbohydrate Polymers*, *89*(4), 1159-1165. doi: 10.1016/j.carbpol.2012.03.089

Barbosa-Pereira, L., Aurrekoetxea, G. P., Angulo, I., Paseiro-Losada, P., & Cruz, J. M. (2014). Development of new active packaging films coated with natural phenolic compounds to improve the oxidative stability of beef. *Meat Science*, *97*(2), 249-254. doi: 10.1016/j.meatsci.2014.02.006

Braga, L. R., Pérez, L. M., Soazo, M. V., & Machado, F. (2019). Evaluation of the antimicrobial, antioxidant and physicochemical properties of poly (vinyl chloride) films containing quercetin and silver nanoparticles. *LWT – Food Science and Technology*, *101*, 491-498. doi: 10.1016/j.lwt.2018.11.082

Bressan, M. C., Lodi, F., Ferreira, M. W., Andrade, P. L., Boari, C. A., & Piccoli, R. H. (2007). Influência da embalagem na vida útil de presuntos fatiados. *Ciência e Agrotecnologia*, *31*(2), 433-438. doi: 10.1590/S1413-70542007000200025

Bruna, J. M., Ordóñez, J. A., Fernández, M., Herranz, B., & de la Hoz, L. (2001). Microbial and physico-chemical changes during the ripening of dry fermented sausages superficially inoculated with or having added an intracellular cell-free extract of Penicillium aurantiogriseum. *Meat Science*, *59*(1), 87-96. doi: 10.1016/S0309-1740(01)00057-2

Cano, A., Fortunati, E., Cháfer, M., Kenny, J. M., Chiralt, A., & González-Martínez, C. (2015). Properties and ageing behaviour of pea starch films as affected by blend with poly (vinyl alcohol). *Food Hydrocolloids*, *48*, 84-93. doi: 10.1016/j.foodhyd.2015.01.008

Dąbrowska-Bouta, B., Zięba, M., Orzelska-Górka, J., Skalska, J., Sulkowski, G., Frontczak-Baniewicz, M., ... Strużyńska, L. (2016). Influence of a low dose of silver nanoparticles on cerebral myelin and behavior of adult rats. *Toxicology*, *363-364*, 29-36. doi: 10.1016/j.tox.2016.07.007

Fernandes, P. A. R., Moreira, S. A., Duarte, R., Santos, D. I., Queirós, R. P., Fidalgo, L. G., ... Saraiva, J. A. (2015). Preservation of sliced cooked ham at 25, 30 and 37°C under moderated pressure (hyperbaric storage) and comparison with refrigerated storage. *Food and Bioproducts Processing*, *95*, 200-207. doi: 10.1016/j.fbp.2015.05.004

Fernández, A., Soriano, E., Hernández-Muñoz, P., & Gavara, R. (2010). Migration of antimicrobial silver from composites of polylactide with silver zeolites. *Journal of Food Science*, *75*(3), 186-193. doi: 10.1111/j.1750-3841.2010.01549.x

Ferri, J. M., Garcia-Garcia, D., Sánchez-Nacher, L., Fenollar, O., & Balart, R. (2016). The effect of maleinized linseed oil (MLO) on mechanical performance of poly (lactic acid) – thermoplastic starch (PLA-TPS) blends. *Carbohydrate Polymers*, *147*, 60-68. doi: 10.1016/j.carbpol.2016.03.082

Garriga, M., Grèbol, N., Aymerich, M. T., Monfort, J. M., & Hugas, M. (2004). Microbial inactivation after high-pressure processing at 600 MPa in commercial meat products over its shelf life. *Innovative Food Science & Emerging Technologies*, *5*(4), 451-457. doi: 10.1016/j.ifset.2004.07.001

Han, Y., Jiang, Y., Xu, X., Sun, X., Xu, B., & Zhou, G. (2011). Effect of high pressure treatment on microbial populations of sliced vacuum-packed cooked ham. *Meat Science*, *88*(4), 682-688. doi: 10.1016/j.meatsci.2011.02.029

Hannon, J. C., Kerry, J. P., Cruz-Romero, M., Azlin-Hasim, S., Morris, M., & Cummins, E. (2018). Migration assessment of silver from nanosilver spray coated low density polyethylene or polyester films into milk. *Food Packaging and Shelf Life*, *15*, 144-150. doi: 10.1016/j.fpsl.2018.01.002

Kumar, R., & Münstedt, H. (2005). Silver ion release from antimicrobial polyamide/silver composites. *Biomaterials*, *26*(14), 2081-2088. doi: 10.1016/j.biomaterials.2004.05.030

Liu, Y., Guan, W., Ren, G., & Yang, Z. (2012). The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. *Toxicology Letters*, *209*(3), 227-231. doi: 10.1016/j.toxlet.2012.01.001

Llorens, A., Lloret, E., Picouet, P. A., Trbojevich, R., & Fernandez, A. (2012). Metallic-based micro and nanocomposites in food contact materials and active food packaging. *Trends in Food Science & Technology*, *24*(1), 19-29. doi: 10.1016/j.tifs.2011.10.001

Lloret, E., Picouet, P. A., Trbojevich, R., & Fernández, A. (2016). Colour stability of cooked ham packed under modified atmospheres in polyamide nanocomposite blends. *LWT – Food Science and Technology*, *66*, 582-589. doi: 10.1016/j.lwt.2015.11.012

Lorenzo, J. M., Batlle, R., & Gómez, M. (2014). Extension of the shelf-life of foal meat with two antioxidant active packaging systems. *LWT – Food Science and Technology*, *59*(1), 181-188. doi: 10.1016/j.lwt.2014.04.061

Marchiore, N. G., Manso, I. J., Kaufmann, K. C., Lemes, G. F., Pizolli, A. P. O., Droval, A. A., ... Leimann, F. V. (2017). Migration evaluation of silver nanoparticles from antimicrobial edible coating to sausages. *LWT – Food Science and Technology*, *76*, 203-208. doi: 10.1016/j.lwt.2016.06.013

Muppalla, S. R., Kanatt, S. R., Chawla, S. P., & Sharma, A. (2014). Carboxymethyl cellulose-polyvinyl alcohol films with clove oil for active packaging of ground chicken meat. *Food Packaging and Shelf Life*, *2*(2), 51-58. doi: 10.1016/j.fpsl.2014.07.002

Onitsuka, S., Hamada, T., & Okamura, H. (2019). Preparation of antimicrobial gold and silver nanoparticles from tea leaf extracts. *Colloids and Surfaces B: Biointerfaces*, *173*, 242-248. doi: 10.1016/j.colsurfb.2018.09.055

Panea, B., Ripoll, G., González, J., Fernández-Cuello, Á., & Albertí, P. (2014). Effect of nanocomposite packaging containing different proportions of ZnO and Ag on chicken breast meat quality. *Journal of Food Engineering*, *123*, 104-112. doi: 10.1016/j.jfoodeng.2013.09.029

Pereira, J. O., Soares, J., Monteiro, M. J. P., Gomes, A., & Pintado, M. (2018). Impact of whey protein coating incorporated with Bifidobacterium and Lactobacillus on sliced ham properties. *Meat Science*, *139*, 125-133. doi: 10.1016/j.meatsci.2018.01.016

Pizzoli, A. P. O., Marchiore, N. G., Souza, S. J., Santos, P. D. F., Gonçalves, O. H., Yamashita, F., ... Leimann, F. V. (2016). Antimicrobial PLA/TPS/gelatin sheets with enzymatically crosslinked surface containing silver nanoparticles. *Journal of Applied Polymer Science*, *133*(8), article 43039. doi: 10.1002/app.43039

Silva, N., Junqueira, V. C. A., Silveira, N. F. A., Taniwaki, M. H., Santos, R. F. S., Gomes, R. A. R., & Okazaki, M. M. (2007). *Manual de métodos de análise microbiológica de alimentos* (3rd ed.). São Paulo, SP: Varela.

Singh, P. K., Jairath, G., & Ahlawat, S. S. (2016). Nanotechnology: a future tool to improve quality and safety in meat industry. *Journal of Food Science and Technology*, *53*(4), 1739-1749. doi: 10.1007/s13197-015-2090-y

Siqueira, M. C., Aouada, M. R. M., Castro, V. L. S., Brandão, H. M., Rech, R. R., Marconcini, J. M., & Mattoso, L. H. C. (2013, June 10-13). *Caracterização e avaliação da toxicidade de nanopartículas de prata para incorporação em matriz polimérica para uso em embalagens de alimentos*. VII Workshop de nanotecnologia aplicada ao agronegócio, São Carlos, SP, Brazil. Retrieved from https://bit.ly/2IDMtPd

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*, *68*(2), 408-420. doi: 10.1111/j.1365-2621.2003.tb05687.x

Veeck, A. P. L., Boligon, A. A., Athayde, M. L., & Emanuelli, T. (2013). Mate extract on lipid and color changes of dourado fillets during frozen storage. *Ciência Rural*, *43*(7), 1317-1322. doi: 10.1590/S0103-84782013005000079

Wambura, P., & Verghese, M. (2011). Effect of pulsed ultraviolet light on quality of sliced ham. *LWT – Food Science and Technology*, *44*(10), 2173-2179. doi: 10.1016/j.lwt.2011.05.016