Research, Society and Development, v. 11, n. 6, e26611629143, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.29143

Retail display life extension of beef by gelatin-chitosan edible coating incorporated with allspice leaves extract

Extensão do tempo de exposição refrigerada de carne bovina por revestimento comestível de gelatina-quitosana incorporado com extrato de folhas de pimenta da Jamaica

Prolongación del tiempo de exhibición refrigerada de la carne de vacuno mediante un recubrimiento comestible de gelatina-quitosana incorporada con extracto de hojas de pimienta de Jamaica

Received: 04/12/2022 | Reviewed: 04/21/2022 | Accept: 04/23/2022 | Published: 04/27/2022

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Abstract
This study was aimed to examine the potential use of ethanolic allspice extracts as antioxidant agent on the edible gelatin-chitosan coating film to preserve the beef during retail display. Beef sirloins steaks were used in control (without edible coating), GEL (coating film) and AEXT (coating film containing 300 mg/kg of allspice extract) treatments, overwrapped in oxygen-permeable film and evaluated up to 10 days, in refrigeration (2.5°C). The effects and their interactions were analyzed using the F test (ANOVA) and, when statistically significant ($P < 0.05$), the means were compared using Duncan test. The psychrotrophic bacteria multiplication was not affected by treatments, but lactic acid bacteria growth was retarded in the coated meat samples. The edible coatings decreased weight losses and lipid oxidation of the meat compared to the control during storage, but the AEXT coating was most effective as an antioxidant. The coatings did not affect the myoglobin redox forms but increased the color stability, leading to samples with lighter (higher L* values) and bright red (higher h values) colors for up to 8-days against 6-days to control. However, these changes in instrumental color were considered small. The use of allspice extract on the edible coating film as an antioxidant is feasible, but more studies are needed to reach the ideal blend to reduce meat discoloration during display.
Keywords: Meat color; Oxidation; Packing system.

Resumo
Este estudo teve como objetivo examinar o potencial uso de extratos etanólicos de pimenta da Jamaica como agente antioxidante em filmes de revestimento comestível a base de gelatina-quitosana para preservar a carne bovina durante a exibição no varejo. Bifes de contrafilé foram utilizados nos tratamentos controle (sem cobertura comestível), GEL (filme de revestimentos) e AEXT (filme de revestimento contendo 300 mg/kg de extrato de pimenta da Jamaica),

embalados em filme permeável ao oxigênio e analisadas no período de 10 dias, sob refrigeração (2,5°C). Os efeitos e suas interações foram analisados pelo teste F (ANOVA) e, quando estatisticamente significantes (p<0,05), as médias foram comparadas pelo teste de Duncan. A multiplicação de bactérias psicrotróficas não foi afetada pelos tratamentos, mas o crescimento de bactérias lácticas foi retardado nas amostras revestidas. Os revestimentos comestíveis diminuíram as perdas de peso e a oxidação lipídica da carne em relação ao controle durante o armazenamento, mas o revestimento AEXT foi mais eficaz como antioxidante. Os revestimentos não afetaram as formas redox da mioglobina, mas aumentaram a estabilidade da cor, gerando amostras com cores mais claras (maiores valores de L*) e tom vermelho brilhante (maiores valores de h) por até 8 dias contra 6 dias para o controle. No entanto, essas mudanças na cor instrumental foram consideradas pequenas. O uso de extrato de pimenta da Jamaica no filme de revestimento comestível como antioxidante é viável, porém mais estudos são necessários para chegar à mistura ideal para reduzir a descoloração da carne durante a exposição refrigerada.
Palavras-chave: Cor da carne; Oxidação; Sistema de embalagem.

Resumen
Este estudio objetivou examinar el uso potencial de extractos etanólicos de pimienta de Jamaica como agente antioxidante en la película de recubrimiento comestible de gelatina y quitosano para preservar la carne de res durante la exhibición. Solomillos de res se usaron en tratamientos de control (sin recubrimiento comestible), GEL (película comestible) y AEXT (película comestible que contenía 300mg/kg de extracto de pimienta de Jamaica), se envolvieron en una película permeable al oxígeno y se evaluaron durante un período de 10 días (2,5 °C). Los efectos y sus interacciones se analizaron mediante la prueba F (ANOVA), cuando fueron estadísticamente significativos (p<0,05), las medias se compararon mediante la prueba de Duncan. La multiplicación de bacterias psicrotróficas no se vio afectada por los tratamientos, pero el crecimiento de bacterias ácido-lácticas se retrasó en las muestras de carne rebozada. Los recubrimientos comestibles redujeron las pérdidas de peso y la oxidación de lípidos de la carne en comparación con el control, pero AEXT fue más efectivo como antioxidante. Los recubrimientos no afectaron las formas redox de mioglobina, pero aumentaron la estabilidad del color, lo que dio lugar a muestras con colores más claros (valores L* más altos) y rojo brillante (valores h más altos) durante hasta 8 días frente a 6 días para el control. Sin embargo, estos cambios en el color instrumental se consideraron pequeños.El uso de extracto de pimienta de Jamaica en la película comestible como antioxidante es factible, pero se necesitan más estudios para llegar a la mezcla ideal para reducir la decoloración de la carne durante la exhibición.
Palabras clave: Color de la carne; Oxidación; Sistema de embalaje.

1. Introduction

Due to the consumer's demand for foods that have better quality indicators, such as color, texture, taste, and nutritional value, the industries have been stimulated to develop new technologies, such as the use of packaging systems capable of delaying the major changes that occur in beefs during retail display (Cardoso, et al., 2019). Two of the biggest challenges for the meat industry are to increase food safety and the shelf life of fresh products and, in this context, new packaging technologies are developed to optimize the conservation and maintenance of the quality of these products.

Packaging protects products against deteriorative effects, which may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity, and other measurable factors (Zhou, et al., 2010). Fresh meat is a highly perishable product due to its biological composition and several factors influence its shelf life but in terms of a quality maintenance, the development of a packaging system for fresh beef should, as a matter of priority, delay the main indicators of quality depreciation, such as loss of red color, development of unpleasant aromas and odors (due to lipid oxidation and microbial degradation), and superficial dehydration (Antoniewski, et al., 2007; Cardoso, et al., 2019).

In Brazil, the use of a modified atmosphere in meat conservation during retail display is still very uncommon, being the vacuum packaging generally used to package and market primal and subprimal cuts of fresh beefs. However, when beef is stored under a vacuum, it retains a purple-red color that makes the product less attractive to consumers, and, therefore, the Brazilian supermarkets remove the original vacuum packaging and re-packaging in an air-permeable overwrap package, which ensures the formation of the attractive bright red color at the time of consumer purchase (Cardoso, et al., 2016). However, the continuous exposure to oxygen in this system favors the development of aerobic microorganisms and the lipid and myoglobin oxidations, which reduces not only the meat shelf-life but also its display life since accelerates discoloration from a red color to a brown color (Jeremiah, 2001; Robertson, 2005).

An alternative to the problem of fresh meats packaged in an air-permeable overwrap system is the use of edible coating films, which allow controlling microbial growth, lipid oxidation, and discoloration on the surface of the product, as well as excessive loss of meat exudate (Cardoso, et al., 2016). Over the past few years, some researchers (Cardoso, et al., 2019; Cardoso, et al., 2016; Lashkari, et al., 2020; Majdinasab, et al., 2020; Samani, et al., 2022; Vital, et al., 2016) have attempted to develop edible films and coatings prepared from organic substances such as carbohydrates, lipids, and proteins to extend the shelf life of meat during retail display. Cardoso and his collaborators (Cardoso, et al., 2019; Cardoso, et al., 2016) have successfully developed chitosan-gelatin coating films to extend the shelf life and display life of meats during retail storage. Coating films effectively decreased weight loss, lipid oxidation, microbial growth, and discoloration of beef steaks during retail display. These authors chose gelatin and chitosan due to their good gel-forming properties, which make them suitable for the preparation of flexible water- and oxygen-barrier films, and also due to the antibacterial and antioxidant properties of chitosan.

Another advantage of coating beef steaks is the possibility of incorporating other compounds with functional properties in addition to the blend of biopolymers used. Natural compounds extracted from plants that exhibit antimicrobial and antioxidant properties had attract interest as additives in the food industry since consumers are highly interested in the incorporation of these compounds into food products, especially as a replacement for synthetic antioxidants (Atarés & Chiralt, 2016; Lashkari, et al., 2020). A plant with great potential use in the meat industry as a natural antioxidant and/or antimicrobial is the allspice (Pimenta dioica Lindl), also known as Jamaican pepper. A great antioxidant and antimicrobial effectiveness of allspice have been reported both in ethanolic extracts (Murali, et al., 2021) and in essential oils (Andrade, et al., 2022) obtained from its leaves.

The incorporation of allspice extracts in the gelatin-chitosan coating film can enhance the antimicrobial and/or antioxidant properties of chitosan, preserving the beef display life. Therefore, this study was performed to evaluate the effects of chitosan gelatin-based coating film application, incorporated with allspice ethanolic extract, on lipid oxidation, microbial development, and color stability in beef steaks during retail display.

2. Material and methods

2.1 Materials and edible coating solutions

The commercial bovine gelatin powder Type B (250 Bloom) was donated by Gelita do Brasil (Cotia, SP, Brazil) and glycerol was purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Allspice extract (Pimenta dioica L.) was obtained from previously dried leaves (in a ventilated oven at 35 °C/40 days) in ethanol solution (1:9 p/v) under reflux at 45 °C/24 h. The extract was concentrated under vacuum up to 10% of the initial volume, presenting an original pH of 4.20.

The biopolymer blend used to produce the edible coatings for this study was based on the report of Cardoso, et al. (2016), being composed of 3% gelatin (w/v) and 6% glycerol (w/w, based on dry gelatin). Two solutions were prepared, one containing only gelatin and glycerol (GEL) and the other also containing allspice ethanolic extract (AEXT) as an antioxidant at a concentration of 300 mg/kg. The edible coating solution was prepared by first stirring the gelatin in distilled water for 30 min to allow complete dissolution and then heating at 70 °C for 10 min. The hot gelatin solution was cooled to 40 °C, the glycerol and allspice extract were added according to each treatment and the mixture was homogenized (model TE-102; Tecnal Equipamentos, Piracicaba, SP, Brazil) at 14,000 rpm for 30 s. Then the coating was immediately applied to the beef.

2.2 Beef coating and storage

Four vacuum-packed samples of striploin (Longissimus lumborum muscle, LL) beef were obtained at 48 h postmortem directly from a slaughterhouse in the State of Minas Gerais, Brazil. From each striploin cut, 27 steak pieces of

approximately 1.5-cm thickness were obtained, individually arranged in polypropylene trays, and kept for 45 min (at ± 4º C) for blooming before being randomized into three treatments: uncoated (CONT); GEL coated; and AEXT coated. Nine steaks (three of each treatment) were divided in half for carrying out the microbiological analysis.

The coating application technique used was immersion in a film-forming solution to polymerize the films directly on the steaks as described by Cardoso, et al. (2016). The steaks were individually weighed, immersed in the coating solution (at 40 °C) for 20 s and allowed to drip for 30 min (at 4 °C) hung by hooks. The coated steaks were weighed again and overwrapped in a polypropylene tray with an oxygen-permeable film of polyvinyl chloride (PVC) to simulating the condition of storage in supermarkets. CONT steaks were immersed in distilled water, proceeding in the same manner as done for the coated samples.

The packaged samples were stored at 2.5 ± 0.5 °C in a climate chamber (EL202; EletroLab, São Paulo, SP, Brazil) and analyzed at days 0 (approximately 1 h after coating), 2, 4, 6, 8, and 10 of storage.

2.3 Microbiological evaluation

Populations of psychrotrophic aerobic (PAB) and lactic acid (LAB) bacteria's were monitored during retail display, by following the methods described by Silva, et al. (2017). A 25 g sample of each steak was aseptically obtained, added to 225 mL of 0.1% (w/v) sterile peptone water, and homogenized (490 strokes/min) in a stomacher (1204M; Marconi, Sao Paulo, Brazil) for 2 min. The homogenized slurries were decimally serially diluted with peptone water and plated under the following conditions: the PAB were determined on plate count agar (PCA; Merck, Darmstadt, Germany) after incubation at 7 °C for 10 days; and the LAB were determined on de Man Rogosa Sharpe broth (MRS; Merck, Darmstadt, Germany) incubated in microaerophilic atmosphere at 30 °C for 48 h. The results are reported as the logarithm of the colony-forming units per gram of food (log CFU/g).

2.4 Weight loss, pH, and lipid oxidation

The weight loss during storage was measured removing the steaks from their trays and weighed again on a semi-analytical scale. The results were expressed as percentages of mass lost relative to the initial mass at day 0. The pH was determined with a digital pH meter DM20 (Digimed, São Paulo, SP, Brazil) after sample homogenization in distilled water (at the ratio of 1:10 w/v). Lipid oxidation was evaluated measuring the number of thiobarbituric acid reactive substances (TBARS) according to the methodology proposed by Raharjo, et al. (1992), with modifications described by Cardoso, et al. (2016). The TBARS values were reported in milligrams of malonaldehyde (MDA) per kilogram of the sample (mg MDA/kg) by means of a standard calibration curve using 1,1,3,3-tetraethoxypropane in 20% trichloroacetic acid.

2.5 Beef surface color evaluation

The steak surface color was measured using a CM-700 spectrophotometer (Konica Minolta) with an 8-mm aperture size, illuminant A, and 10° angle of the observer. The spectral reflectance data (from 400 to 730 nm, in 10-nm intervals) were recorded, in a specular component excluded (SCE) and included (SCI) modes, by five measurements representing the entire surface of each sample. The lightness (L*), redness (a*), yellowness (b*), chroma (C*), and hue angle (hº) were recorded, and the myoglobin redox forms (oxymyoglobin, OMb; deoxymyoglobin, DMb; and metmyoglobin, MMb) estimated by the mathematical method described by Krzywicki (1979).

2.6 Statistical analysis

The experiment was carried out in a randomized block design, with the blocks consisted of different animals (n = 4),

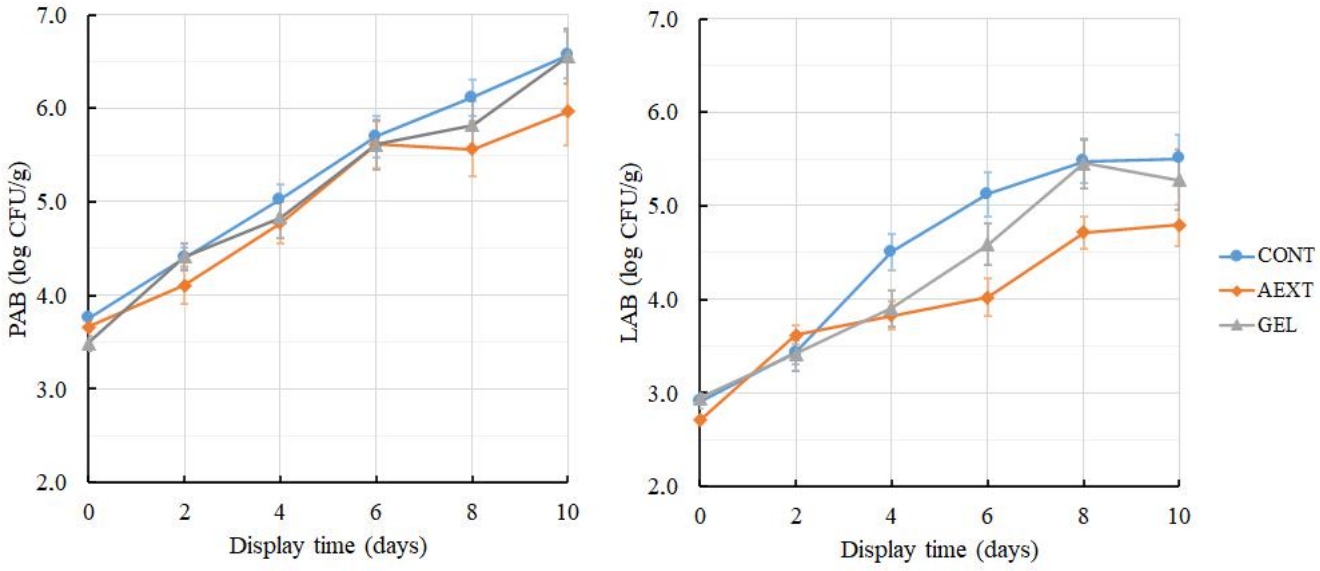
in a factorial split-plot scheme, with the three treatments (different coatings) in the whole plot and the six display times in the split-plot. The main effects and their interactions were analyzed using the F test (ANOVA) and, when statistically significant ($P < 0.05$), the means were compared using Duncan test. All statistical analyzes were performed using the statistical program STATISTICA version 8.0 (StatSoft. Inc., 2007).

3. Results and Discussion

3.1 Microbial stability

As the storage period proceeded, the psychrotrophic aerobic bacteria (PAB) and lactic acid bacteria (LAB) populations significantly ($P < 0.05$) increased in all the samples (Figure 1).

Figure 1. Microbial changes (log CFU/g) in uncoated (CONT) and coated beef steaks with gelatin-chitosan film (GEL) and gelatin-chitosan + allspice ethanolic extract film (AEXT) during display storage (at 2.5 °C): PAB = psychrotrophic aerobic bacteria; and LAB = lactic acid bacteria. Bars represent the standard error of the means (n = 5).



Source: Authors.

The initial PAB count was in the range 3.49-3.76 log CFU/g which reached 5.96-6.57 log CFU/g after 10 days of display, still below the maximum permitted microbial load of fresh meat (7 log CFU/g) described by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) for total viable count. The highest and lowest PAB counts after 8 days of display were, respectively, related to the control (CONT) and the sample coated with chitosan-gelatin film with allspice extract (AEXT). This is partially in agreement with Cardoso, et al. (2019), who observed lower PAB counts in beef with gelatin-chitosan coatings than those of the control samples during all eight days of display time at 4°C. In addition to the chitosan effect, allspice extracts can also contribute to an antimicrobial action due to the different groups of chemical compounds present, especially the phenolic compound eugenol (Andrade, et al., 2022). In fact, these authors observed that the allspice essential oil have more effective in inhibiting Pseudomonas than its major component eugenol. Gram-negative PAB like Pseudomonas species are known as the major causes of the spoilage of fresh meat stored at low temperatures (Majdinasab, et al., 2020).

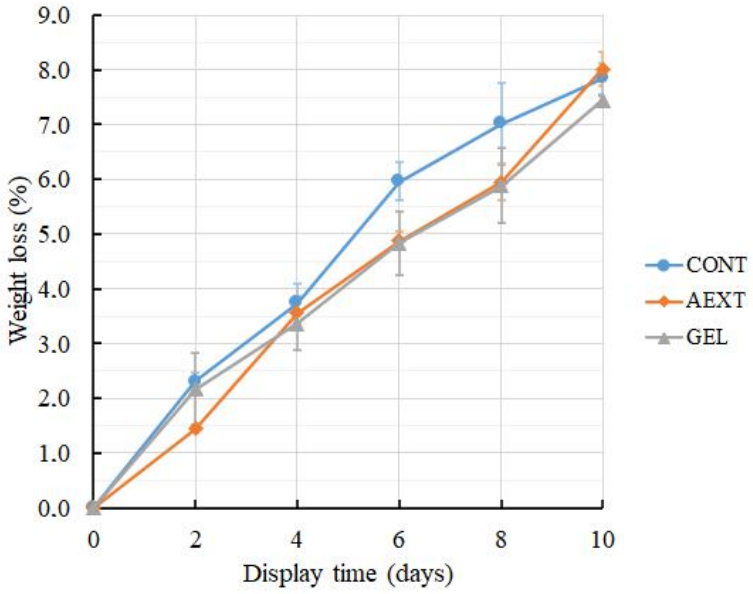
The antimicrobial effects of the edible films can be seen in the inhibition conferred on the LAB counts. Fermentative degradation rates were higher in the control samples, followed by those coated with gelatin-chitosan film, and finally in those

coated with a gelatin-chitosan film containing allspice extract. LAB constitute a heterogeneous group that has been largely associated with fresh and cooked meat products as it serves as an indicator of the occurrence of groups of spoilage microorganisms (Pothakos, et al., 2015). A reduction in LAB counts was also observed by Lashkari, et al. (2020) in veal meat coated with sodium caseinate films incorporated with Zataria multiflora essential oil.

3.2 Weight losses

The application of the edible coating significantly affected the beef weight loss during display (Figure 2), but lower losses were observed ($P < 0.05$) only on days 6 and 8 of storage. Little or no exudate from the coated samples was visible, with the greatest weight losses arising from surface evaporation. This is attributed to the low water solubility of the chitosan-gelatin polymeric blend which acts as a water barrier, trapping the exudate from the meat product, and to the fact that this coating can act as moisture-sacrificing agents rather than moisture barriers, by losing their water content first than beef (Antoniewski, et al., 2007; Cardoso, et al., 2019). This protection effect against moisture loss was also observed in coated meats by other authors (Cardoso, et al., 2019; Lashkari, et al., 2020; Vital, et al., 2016).

Figure 2. Weight losses (%) of uncoated (CONT) and coated beef steaks with gelatin-chitosan film (GEL) and gelatin-chitosan + allspice ethanolic extract film (AEXT) during display storage (at 2.5 °C). Bars represent the standard error of the means (n = 5).



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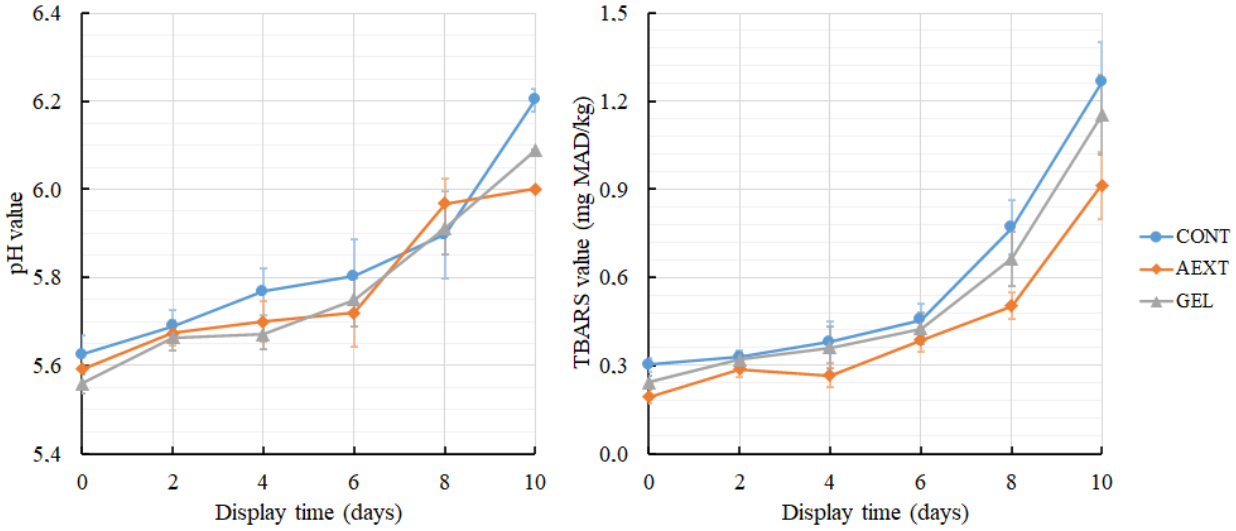
3.3 pH values and lipid oxidation

There were no differences ($P > 0.05$) in pH values among the coated samples but significant differences in pH and TBARS values were observed during display storage (Figure 3).

Figure 3. Changes in the pH and TBARS (mg MAD/kg) values of uncoated (CONT) and coated beef steaks with gelatin-chitosan film (GEL) and gelatin-chitosan + allspice ethanolic extract film (AEXT) during display storage (at 2.5 °C). MAD = malonaldehyde. Bars represent the standard error of the means (n = 5).



Source: Authors.

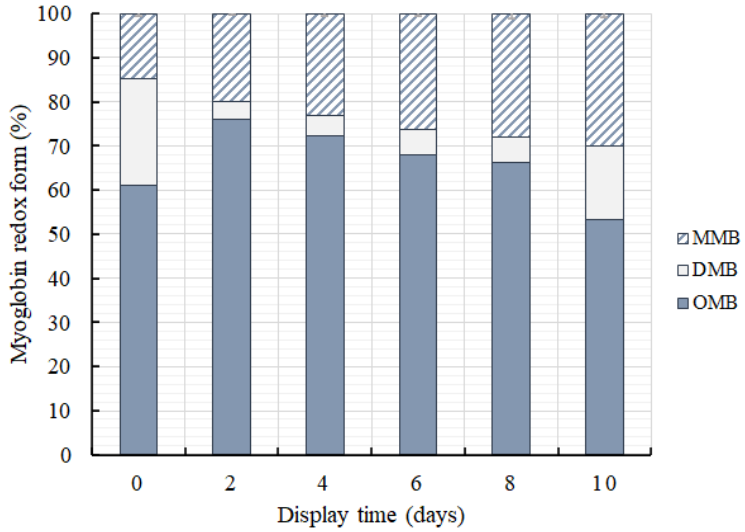
All beef samples had considerable rises in pH values during display. The initial pH of the samples ranged from 5.56 to 5.63 which reached the range of 6.09–6.20 after the 10 days of storage. An increasing pH value during storage may be an indication of microbial growth, which is reinforced by the observed changes in the microbial counts of the samples during storage (Figure 1). With the storage, the meat texture is degraded by the enzymatic activity of endogenous proteases or microorganism action, which leads to an increased concentration of nitrogenous compounds such as ammonia and finally to an increase in the meat pH (Ramos & Gomide, 2017). Vital, et al. (2016) also found no effect on pH values in beef coated with alginate film containing rosemary and oregano essential oils. However, Cardoso, et al. (2019) reported lower pH values in beef steaks coated with gelatin-chitosan film than control samples during display storage.

Regarding lipid oxidation, the TBARS values were increased during storage for all treatments. In general, the TBARS values increased slightly until the six days of storage, after which they increased dramatically until the end of the display (Figure 3). However, during storage, the highest and lowest TBARS values were, respectively, related to the control (CONT) and the sample coated with the gelatin-chitosan film containing allspice extract (AEXT). This change in TBARS behavior during display was very similar to that observed by Sasaki, et al. (2001) in beef overwrapped with oxygen-permeable PVC film and stored (at 4 °C) for up to 10 days in the dark. The TBARS assay measures the secondary oxidation products, such as malonaldehydes, responsible for oxidative rancidity (Dutra, et al., 2017). Therefore, the beef coating, especially with film solutions added of allspice extracts, significantly decreased the lipid oxidation during storage. This could be due to the antioxidative property of chitosan, which is related to the metal ion chelation or lipid complexation abilities of its residual amino groups, making those components unavailable for oxidation reactions (Alishahi & Aïder, 2012). Moreover, both gelatin and chitosan are considered compounds capable of forming polymers with a good oxygen barrier capacity, being able to reduce the access and diffusion of oxygen to the meat surface, delaying the lipid oxidation process (Cardoso, et al., 2016). In addition to chitosan, the presence of the allspice extract potentiated the antioxidant action by the coating film. The allspice antioxidant activity is due to the presence of phenylpropanoids, especially eugenol as its main active component, and other aromatic monoterpenes such as p-cymene (Andrade, et al., 2022; Kikuzaki, et al., 1999).

3.4 Color stability

There were no differences ($P > 0.05$) in all relative pigment contents among the coated samples, but significant differences were observed during display storage (Figure 4). The redox form of the myoglobin determines the meat color; depending on its access to oxygen, this pigment is in the form of deoxymyoglobin (DMb; purple red color) or oxymyoglobin (OMb; desirable bright red color), and its oxidation form the metmyoglobin (MMb; undesirable brown color), affecting the beef surface color and, therefore, the consumer acceptance (Mancini & Hunt, 2005).

Figure 4. Changes in the myoglobin redox forms proportion (%) on surface of beef steaks (uncoated and coated) during display storage (at 2.5 °C). MMb = metamyoglobin; DMb = deoxymyoglobin; and OMb = oxymyoglobin.

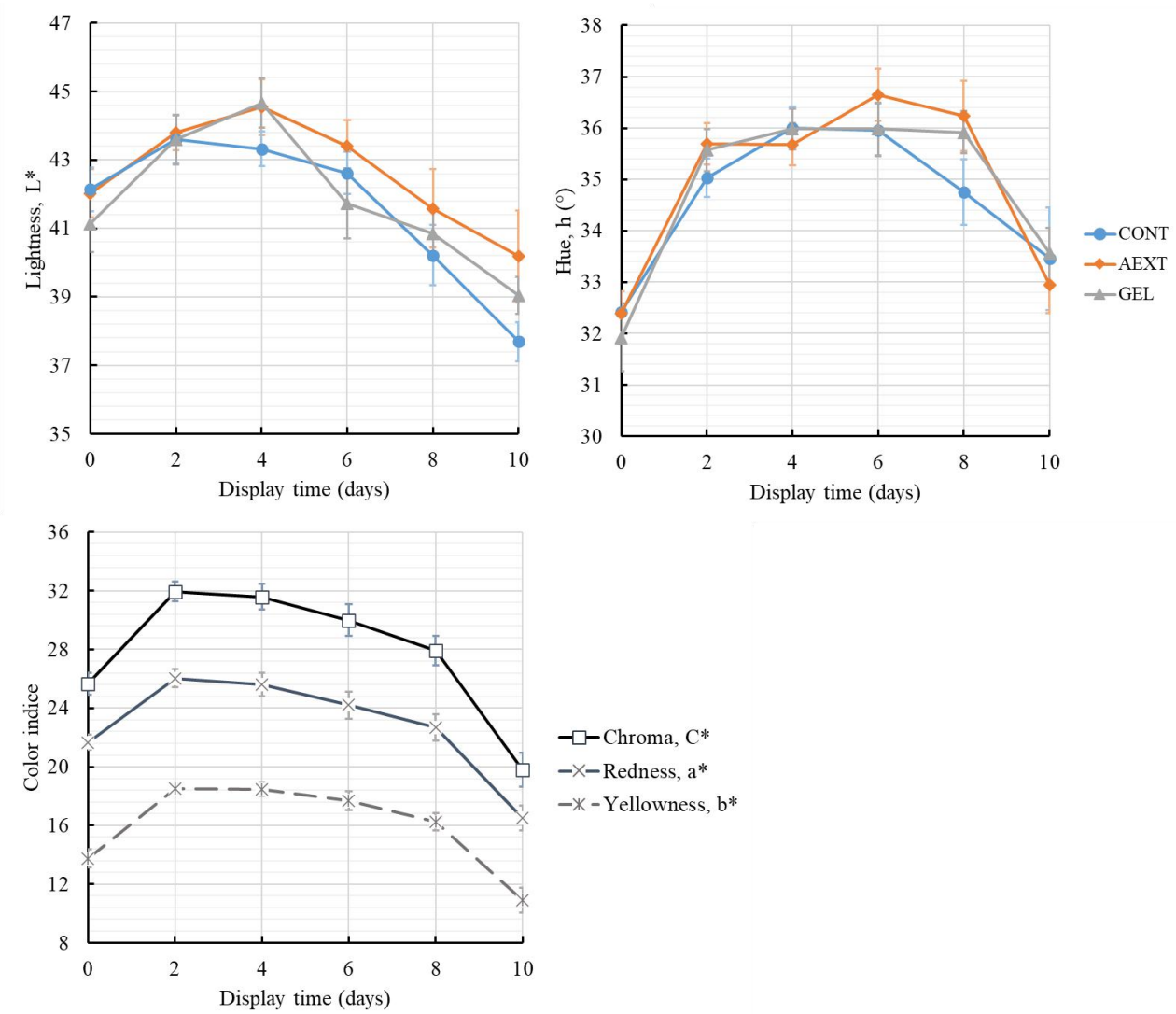


Source: Authors.

The OMb content on the beef surface increased in the first two days and then decreased until the end of storage, being gradually oxidized to MMb form. These initial increase in OMb could be explained by the higher diffusion of oxygen into the meat due to the reduction in the residual mitochondrial respiration (reduction in the oxygen consumption rate - OCR) leading to the formation of a larger layer of OMb. The subsequent drop in OMb levels is due to a reduction in the MMb reductase activity (MRA) of the meat during display, thereby contributing to a MMb accumulation on the meat surface (Cardoso, et al., 2019; Ramos & Gomide, 2017).

Although the proportion of myoglobin chemical forms on the surface can determine the meat's color, it is also affected by meat structure and, in this case, also by the coating polymer layer (Cardoso, et al., 2016). The CIE color indices of the beef samples during display storage are presented in Figure 5. Only lightness (L*) and hue angle (h) values were affected ($P < 0.05$) by the treatments.

Figure 5. Changes in the CIE color indices of uncoated (CONT) and coated beef steaks with gelatin-chitosan film (GEL) and gelatin-chitosan + allspice ethanolic extract film (AEXT) during display storage (at 2.5 °C). Bars represent the standard error of the means (n = 5).



Source: Authors.

Overall, the changes that occurred in the color indices followed the changes in the myoglobin redox forms, especially OMb, with an increase in values being observed in the first days, followed by a reduction. The decrease in the CIE color indices is an indication of color deterioration during retail display, due to the oxidation of OMb to MMb occurred by oxidizing reactions and microbial growth (Cardoso, et al., 2019). Generally, the meat's red color is described by the redness indice (a*). However, although the chromaticity indices (a* and b*) can reflect important color changes, color is a three-dimensional attribute, being better described by its lightness (L*) and by the angular coordinates chroma (C*) and hue (h), which are determined by the chromaticity indices (Ramos & Gomide, 2017). During storage, the beef color (uncoated and coated) became less intense (determined by C* values). The reduction in L* was smaller in the coated samples than in the control, making the meat color lighter in those samples. Moreover, in the coated samples the bright red hue color (higher h values) was maintained until day 8, while in the control samples the color has changed to a more purple hue (lower h values) from day 6.

4. Conclusion

The gelatin-chitosan edible coatings effectively decreased the weight loss and retarded the lactic acid bacteria growth of beef during retail display. The addition of allspice extract in the polymeric blend of the film contributed positively to the reduction of lipid oxidation of the steaks during storage and extended the instrumental color stability by 2 days. These results suggest that the use of chitosan-gelatin coating added to allspice leaves extract as a natural antioxidant is an interesting technological solution to extending the display life of beef during retail storage. However, the changes in color due to coatings were small, barely perceptible to the naked eye, requiring an adjustment regarding the amount of extract added to improve color stability and conducting a sensory analysis to determine if the changes are noticeable; including the detection of possible changes associated with aroma and flavor after preparation of beef.

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 430206/2016-0) and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; CVZ APQ-02904-17) for their financial support. The authors also grateful to the Government of Mozambique and to the Brazilian agency Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship granted to the first (Doctoral/CNPq) author.

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