

MICROBIOLOGICAL QUALITY AND OTHER CHARACTERISTICS OF REFRIGERATED CHICKEN MEAT IN CONTACT WITH CELLULOSE ACETATE-BASED FILM INCORPORATED WITH ROSEMARY ESSENTIAL OIL

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ABSTRACT

Antimicrobial active packaging delays or inhibits microorganism growth in packed products, and it can be used in a variety of food systems. The objective of the present research was to develop packaging incorporated with natural antimicrobial agents (active film). The effects of the active film on the spoilage, pathogenic microorganism counts, pH and color of the refrigerated chicken breast cuts were analyzed. Cellulose acetate-based active films incorporating two concentrations (20% and 50%, v/w) of rosemary (*Rosmarinus officinalis* L.) essential oil were manufactured and placed in contact with the chicken breast cuts for six days. An analysis of variance and mean comparison tests (Tukey's test, $p < 0.05$) were performed on the results. The films that contained 20% essential oil and were intercalated with chicken breast samples did not demonstrate significant effects on the control of psychrotrophic or total coliform microorganisms during the storage period; however, the films incorporated with 50% essential oil demonstrated efficacy toward the control of coliforms during the storage of the samples (6 days, $2 \pm 2^\circ\text{C}$). The pH was related to the psychrotrophic microorganism count and was not influenced by the treatment. The color was not influenced by the time of storage or the treatment. The results demonstrate that active films incorporating 50% rosemary essential oil are effective at controlling certain microorganisms in chicken breast cuts.

Key words: active packaging, antimicrobial activity, cellulose acetate, *Rosmarinus officinalis* L., chicken.

INTRODUCTION

Microbiological changes are the most important modifications that occur in food because certain microorganisms are pathogenic and directly affect consumer health (49, 52), whereas others cause spoilage and diminish

shelf life. Refrigerated chicken meat is a highly perishable product in which modifications are related to the growth of spoilage-inducing or pathogenic microorganisms (34, 39, 40). Contamination can occur during the storage of the poultry or during slaughter due to human manipulation or through contact with the environment or contaminated water (13, 44, 54, 57).

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The use of heat, cold, drying or chemical preservatives have the primary function of reducing the spoilage effects on meat (17); however, when considering fresh foods, such as poultry, most of the aforementioned methods should not be applied because they cause sensory changes that modify the product's characteristics (43). Food packaging also constitutes a preservation method as it creates a physical barrier between the product and the environment, thereby preventing contamination after packing and reducing biochemical and microbiological modifications (1, 38).

Many studies have been conducted to create "active packaging" methods that are not only able to protect but also to interact with the product (31, 55). This technology includes antimicrobial active packaging methods that are able to reduce the population growth, extend the lag phase and/or inactivate the microorganisms present in the food or in the package itself through contact (4, 43). There are several types of antimicrobial packaging methods, such as sachets or tablets with volatile antimicrobials, polymers that form films with inherent antimicrobial effects, polymers that are coated or incorporated with volatile or non-volatile antimicrobials (4, 17). With the exception of systems that use volatile agents, direct contact between the active packaging and the food is necessary (18). These packaging methods should not only have antimicrobial efficacy but they should also not negatively affect the sensory characteristics of the food (31).

Packaging materials can be manufactured from synthetic polymers, although the use of polymers will be more desirable when they do not cause a negative environmental impact due to disposal (49, 53). Antimicrobials used in packaging can be synthetic as well; however, there is a great interest in using natural practices to meet consumers' demands (19). Essential oils are natural compounds, the majority of which are Generally Recognized as Safe, and they can be used in food with no harm to the health of those who consume them (4). In Brazil, the use of vegetable oils in food packaging is allowed with no restrictions (10). The incorporation of clove, thyme, oregano and cinnamon essential oils in packaging to increase

the shelf life of refrigerated meat has been previously described (24, 26, 37).

Rosemary essential oil is a product of variable composition with oxygenated monoterpenes and hydrocarbons as primary components (29). The antimicrobial activity of rosemary oil has been demonstrated (8, 14, 16, 22); however, although there are reports of the incorporation of such compounds in films based on whey protein, the action of rosemary essential oil after the incorporated within packaging has not been thoroughly investigated (48).

Based on the aforementioned arguments, this objective of this study was to investigate the effect of films incorporated with rosemary essential oil on microbial growth, pH and the color of chicken breast cuts stored at $2 \pm 2^\circ\text{C}$.

MATERIALS AND METHODS

Materials

Cellulose acetate was obtained from Rhodia (Freiburg, Germany), and acetone P.A. was obtained from Protec Produtos Científicos (Goiânia, GO, Brazil). Food-grade rosemary essential oil (*Rosmarinus officinalis* L.) was purchased from Petite Marie Química Nova (Itaquaquecetuba, SP, Brazil).

Antimicrobial film preparation

The films were prepared using the casting method described by Soares (51) with some modifications. The cast film-forming solution was prepared by adding cellulose acetate flakes to acetone (10% w/v; cellulose acetate/acetone), and the mixture was left to stand until its complete solubilization (10 h, $25 \pm 2^\circ\text{C}$). Rosemary essential oil was added to the emulsion at final concentrations of 20% and 50% (v/w; rosemary essential oil/cellulose acetate). Aliquots of the mixture were spread onto a glass plate (previously sanitized with alcohol at 70%) followed by drying. Control films were prepared with no addition of the essential oil. Films of $25 \pm 5 \mu\text{m}$ thickness were used (average of 10 measurements at random parts of the films

using a micrometer Mitutoyo 0-25 mm), and all treatments were submitted to UV light for 2 min. on each side prior to being placed in contact with the chicken breast cuts.

Tray assembly

The chicken breast cuts, weighing approximately 40 g and measuring 5-8 mm in thickness, were purchased at a local market inspected by the Municipal Inspecting Service (Goiânia, GO, Brazil). The inner surfaces of polystyrene trays were completely covered by an antimicrobial film, and five chicken breast cuts were placed one over the other in each tray and interspersed with antimicrobial films so that both sides of the cuts were in contact with the film. As a control, trays with no films were assembled. Each tray contained approximately 200 g of meat. Subsequently, the trays were wrapped with polyvinyl chloride (PVC) film (15 µm) and stored under refrigeration ($2 \pm 2^\circ\text{C}$).

Microbiological analysis

Counts of psychrotrophs, total coliforms, thermotolerant coliforms and coagulase-positive *Staphylococcus* were performed at days 0, 3 and 6 during storage. The assessment of *Salmonella* levels was conducted at day 0 and, for safety reasons, at day 3. The repetition of the tests occurred at day 6 only in the event of the presence of microorganisms at day 0 or 3. The laboratory analyses followed a valid methodology for animal-origin products (9).

Chicken breast samples (25 g) from each treatment were aseptically weighed inside stomacher bags (Interlab, São Paulo, SP, Brazil), and 225 mL of 0.1% peptone water (Apijã, Goiânia, GO, Brazil) was poured into the bags and homogenized for 2 min. Serial dilutions were prepared using 0.1% peptone water, and the inoculation was performed thereafter. To count the total number of psychrotrophs, 0.1 mL aliquots of the dilutions were inoculated onto a Petri dish surface containing plate count agar (Merck, São Paulo, SP, Brazil) and incubated at $8 \pm 1^\circ\text{C}$ for 7 days. The total and thermotolerant coliform counts were performed using the agar-

overlay method of inoculation for isolation with 1 mL aliquots from dilutions within the Petri dish using violet red bile agar (Merck, São Paulo, SP, Brazil) and incubation at $36 \pm 1^\circ\text{C}$ for 48 h. Confirmation of the total coliforms was obtained using 2% brilliant green bile broth (Merck, São Paulo, SP, Brazil) at $36 \pm 1^\circ\text{C}$ for 24 to 48 h; for thermotolerant coliforms, EC broth (Merck, São Paulo, SP, Brazil) was used at $45 \pm 0.2^\circ\text{C}$ for 24 to 48 h.

An additional phase was used to quantify *Staphylococcus* and assess the occurrence of *Salmonella*. To count the coagulase-positive *Staphylococcus*, aliquots of 0.1 mL from the dilutions were inoculated onto a Petri dish surface containing Baird-Parker agar (Merck, São Paulo, SP, Brazil), egg yolk and 3.5% potassium tellurite (Merck, São Paulo, SP, Brazil). The occurrence of coagulase-positive *Staphylococcus* was confirmed was obtained by culturing typical and atypical colonies within tubes containing brain heart infusion broth (Merck, São Paulo, SP, Brazil) to perform a coagulase test using rabbit serum (Quimilab, Goiânia, GO, Brazil). To assess the occurrence of *Salmonella*, chicken breast samples (25 g) from each treatment were aseptically weighed inside stomacher bags, and 225 mL of buffered peptone water at 0.1% was poured into the bags and then homogenized for 2 min. The bags were incubated for 24 h at $36 \pm 1^\circ\text{C}$, and 0.1 mL aliquots were then incubated in Rappaport-Vassiliadis and Express (SX2-T) broths (Interlab, São Paulo, SP, Brazil) for 24 h at $41 \pm 1^\circ\text{C}$. After incubation, 2 mL of Express broth was heated in boiling water for 15 min. to proceed with the analysis using the mini Vidas® IVD1202737 equipment (bioMérieux, Marcy l'Étoile, France).

The analyses were performed in two phases. In the first phase, trays with films incorporated with 20% (v/w) of rosemary essential oil were assembled and analyzed; in addition, trays with films having no essential oil and the control (no film) were assembled. In the second phase, only trays with films incorporated with 50% (v/w) of rosemary essential oil were assembled and analyzed in addition to the control.

pH measurement

The pH was measured after the homogenization of 50 g of chicken breast samples with 20 mL of distilled water. The pH measurement was performed using a pH meter combined with an electrode for pH (Logen Scientific - LS 300, 5204). The measurements were done in triplicate.

Color analysis

The analysis of the color of the chicken breast samples was performed only during the second phase of the experiment to identify whether the films would interfere with this attribute. The analysis was performed using a ColorQUEST II colorimeter (Hunterlab, 6553) with Universal Software Version 3.6 operating on a CIE L*a*b* standard. The system was operated using Standard Illuminant D65 with a 10° angle, and measurements were made directly on the samples (three measurements at random parts of each chicken breast cut). The colorimeter was calibrated prior to the evaluation with the parameters black, white and gray with white selected for use as the parameter (L* = 94.18, a* = -0.93, b* = 0.48).

Statistical analyses

A completely randomized design was selected. The experiment was conducted in three repetitions. The results were

submitted to an ANOVA with an F test, and when relevant, a Tukey's test was applied at a 5% probability. The *Statistical Analysis System* 6.0 program was used for the calculations (47).

RESULTS AND DISCUSSION

Antimicrobial activity of films in contact with chicken meat

In a previous study, the antimicrobial activity of films incorporating 0, 10%, 20%, 30%, 40% and 50% (v/w) of rosemary essential oil was evaluated *in vitro*. The procedure was based on the disk diffusion in agar method of the National Committee for Clinical Laboratory Standards, which is recommended by the National Health Surveillance Agency (3) for testing the *in vitro* susceptibility of aerobic bacteria to antimicrobial agents. In the *in vitro* system, between 10⁴ and 10⁵ CFU.g⁻¹ of mesophilic microorganisms obtained from the chicken meat were used (data submitted for publication). The results obtained from this previous work guided the use of essential oils in the present study.

The results of the counts of psychrotrophs, total coliforms, thermotolerant coliforms, coagulase-positive *Staphylococcus* and occurrence of *Salmonella* in the chicken breast samples treated with films without the incorporation of rosemary essential oil (0% film) or films incorporated with 20% (v/w) rosemary essential oil (20% film) and controls are presented in Table 1.

Table 1. Microbial activity (log CFU.g⁻¹) in chicken meat submitted to treatments during storage at 2 ± 2°C.

Microorganism	Treatment	Day ¹		
		0	3	6
Psychrotrophic	Control	5.45 ^{Aa}	6.61 ^{Ab}	7.57 ^{Ac}
	0% film	5.45 ^{Aa}	6.59 ^{Ab}	7.52 ^{Ac}
	20% film	5.45 ^{Aa}	6.77 ^{Ab}	7.71 ^{Ac}
Total coliforms	Control	4.40 ^{Aa}	3.99 ^{Aa}	3.58 ^{Aa}
	0% film	4.40 ^{Aa}	4.39 ^{Aa}	4.80 ^{Aa}
	20% film	4.40 ^{Aa}	4.42 ^{Aa}	3.36 ^{Aa}
Thermotolerant coliforms	Control	< 1	< 1	< 1
	0% film	< 1	< 1	< 1
	20% film	< 1	< 1	< 1
<i>Salmonella</i>	Control	absence	absence	-
	0% film	absence	absence	-
	20% film	absence	absence	-
Positive coagulase <i>Staphylococcus</i>	Control	< 1	< 1	< 1
	0% film	< 1	< 1	< 1
	20% film	< 1	< 1	< 1

¹Figures correspond to the average of three replicates. Capital letters represent the treatment, and small letters indicate the time. Averages followed by the same capital letter in the column and followed by a small letter in the row are not significantly different (p>0.05).

The initial count of psychrotrophic microorganisms in the samples was 2.8×10^5 CFU.g⁻¹. The microbial agents assessed here are the primary causes of refrigerated meat spoilage (2, 11, 34), and although Brazilian legislation has not established limits for psychrotrophic counts in chicken meat, the value presented in this manuscript is considered high according to the literature; the first signs of spoilage, such as inappropriate odors, are noticeable at counts greater than 10^7 CFU.g⁻¹ (20, 23, 30). Therefore, after the sixth day of storage, the chicken meat would be inadequate for consumption.

There was no significant difference in the growth of microorganisms between the control samples and the 0% film, thereby indicating that the cellulose acetate-based films without essential oil had no influence upon the growth of the analyzed microorganisms. Films incorporated with 20% of the essential oil were not effective at diminishing or avoiding psychrotrophic growth, thus indicating that the film at this oil concentration does not significantly contribute to an extended shelf life. The total coliform count did not significantly vary based on the days of storage ($p > 0.05$). The absence of *Salmonella* as well as thermotolerant coliform and coagulase-positive *Staphylococcus* counts of less than 10 CFU.g⁻¹ on the chicken breast samples indicated that the raw material used in the tests was of high quality; thus, it was not possible to assess the efficacy of the films upon these organisms.

The non-efficacy of the active film with 20% of the essential oil could be related to the short contact time with the meat. We can infer that to obtain a positive response, a longer contact time between the film and the system may be necessary (35). The pH of a product can also have an influence on the activities of active agents because the pH can modify the extent of ionization (dissociation/ionization) of certain compounds and consequently affect their activity (28). Gutierrez *et al.* (27) described studies using essential oils of oregano and thyme in

which a pH of 5 was favorable for the activities of the oils. According to the authors, the low pH increased the oil hydrophobicity, thereby facilitating their passage through the membranes of bacterial cells. It is worth considering that the average pH in refrigerated chicken breast meat was greater than 6 on days 0, 3 and 6, and therefore, the pH was not in the optimal range described by Gutierrez *et al.*

Perhaps the most important consideration is the food system itself, which is deeply connected to the concentration of the essential oil in the films. Devlieghere *et al.* (19) reported the possibility of interactions between antimicrobial compounds and food components, such as proteins and fat; such interactions may influence the efficacy of the antimicrobial compounds. Due to the possible interactions between compounds and ingredients, according to Moreira *et al.* (35), there is no direct contact between the agents and microorganisms in food systems. Fat and/or protein molecules act as physical barriers to the antimicrobial agents, which protects the microorganisms from the antimicrobial activity (12). In a study of hotdogs, Singh *et al.* (50) demonstrated a significant reduction in the antimicrobial activity of thyme and clove essential oils following changes in the fat level of the hotdogs from zero to 9%.

Studies have demonstrated that the control of microorganisms is improved when a greater concentration of antimicrobial agents is incorporated into the packaging (42, 58), and the quantity of such microorganisms in the product can determine the activity of certain active components (15). Ponce *et al.* (41) reported that active components have a specific concentration of maximum inhibition, and when the microorganism count is greater than this ability, growth control is not effective. The results of microbial growth during the second phase of the experiment are presented in Table 2.

Table 2. Microbial activity (log CFU.g⁻¹) in chicken meat submitted to treatments during storage at 2 ± 2°C.

Microorganism	Treatment	Day ¹		
		0	3	6
Psychrotrophic	Control	< 1 ^{Aa}	5.21 ^{Ab}	6.57 ^{Ab}
	50% film	< 1 ^{Aa}	4.49 ^{Ab}	6.86 ^{Ab}
Total coliforms	Control	< 1 ^{Aa}	3.84 ^{Ab}	4.56 ^{Ab}
	50% film	< 1 ^{Aa}	2.62 ^{Aa}	2.39 ^{Ba}
Thermotolerant coliforms	Control	< 1	< 1	< 1
	50% film	< 1	< 1	< 1
<i>Salmonella</i>	Control	absence	absence	-
	50% film	absence	absence	-
Positive coagulase <i>Staphylococcus</i>	Control	< 1	< 1	< 1
	50% film	< 1	< 1	< 1

¹Figures correspond to the average of three replicates. Capital letters represent the treatment, and small letters indicate the time. Averages followed by the same capital letter in the column and followed by a small letter in the row are not significantly different (p>0.05).

Although the initial psychrotrophic count was less than 10 CFU.g⁻¹, the count was approximately 10⁷ CFU.g⁻¹ on day 6. The observed microbial growth is related to the favorable temperature for these microorganisms (2), high level of nutrients, high water activity and the pH, which make poultry meat susceptible to spoiling (21).

Even considering the low initial microbial count, there was no significant difference (p>0.05) between the control and treatments on different days with respect to the psychrotrophic growth; however, the total coliform growth was greatly reduced on day 6 (p≤0.05) for the 50% films relative to the control, which represents an average difference of 2 logarithmic growth cycles. The results indicate that there was no reduction in the total coliform count, although the growth of these microorganisms was controlled during storage. The control of microorganism growth by antimicrobial agents can occur through a reduction in the growth rate, an increase in the lag phase or through direct inactivation by contact between the active agents and microorganisms (43). Therefore, the treatment may have presented at least one of these roles. According to the results for the films with 50% rosemary essential oil, this packaging method is effective against certain total coliforms and is likely effective against other microorganisms.

Studies conducted by Santiago-Silva *et al.* (46) indicate that films made with cellulose acetate incorporating 25% or 50% of pediocin (w/w) were effective in the control of *Listeria innocua* within sliced ham samples stored at 12 ± 1°C, and the reduction was proportional to the concentration of pediocin within the films (2 logarithmic cycles in samples treated with 50% films and less than 1 cycle in samples treated with 25% films). However, the treatments were not as effective in the control of *Salmonella* sp. Zinoviadou *et al.* (58) also reported a significant reduction in the growth of the assessed microorganisms as the concentration of oregano essential oil was increased from 0.5% to 1.5% in whey protein isolate films. In addition, it was demonstrated that lactic acid bacteria were more sensitive to the active components of oils than *Pseudomonas*. Both studies demonstrate that the efficacy of antimicrobial films is influenced by the susceptibility of each microorganism to the active agents in addition to other factors.

pH value

In the first phase of the experiment, no statistical variance (p>0.05) in the pH of the samples was detected. The initial average pH was 6.08, and the maximum value of 6.13 was reached on day 6. Foods that are stored under aerobiosis and rich in proteins and free amino acids, such as chicken meat,

commonly present a pH increase as the numbers of microorganisms that cause spoilage rises (5, 32, 36); i.e., their proteolytic activity results in the production of basic compounds (56), thereby causing a slight pH increase. In the second phase, there was no significant pH variance between the days and treatments; the values were 6.09 on day 0 and 6.04 on day 6.

Color analysis

The color analysis of the control samples and the 50% film demonstrated no significant variance ($p>0.05$) related to the $L^*a^*b^*$ parameters between the days and treatments. Probably, neither lipid oxidation nor relevant metmyoglobin formation were evident during this period. It has been reported that rosemary compounds possess antioxidant action (6, 25, 33, 45) and are able to contribute to the maintenance of meat color if the formation of metmyoglobin and lipid oxidation are avoided. However, such results were not observed in the present study.

The aforementioned results have been taken into account, as the primary objective of antimicrobial packaging is to control microbial growth; the fact they have not caused negative variations on meat color is highly positive (7).

CONCLUSION

The use of cellulose acetate-based active films incorporating 20% rosemary essential oil did not significantly reduced the growth of psychrotrophic microorganisms and total coliforms within the refrigerated chicken meat; however, films incorporating 50% rosemary essential oil were effective in controlling total coliforms. The slight variation in the pH value of the chicken meat was related to the growth of psychrotrophs, and there was no significant variance between the treatments. The color of the meat was not influenced by the use of films incorporating 50% rosemary essential oil, and the color did not significantly vary during storage.

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