Use of allyl isothiocyanate and carbon nanotubes in an antimicrobial film to package shredded, cooked chicken meat

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A B S T R A C T

We developed antimicrobial packaging incorporated with allyl isothiocyanate (AIT) and carbon nanotube (CNT), and this packaging was used for shredded cooked chicken meat inoculated with Salmonella Chole-
raesuis. The following parameters were analysed during the 40 days of storage: microbial counts, colour characteristics and changes in the oxidation of the meat as well as changes in the mechanical properties of the film, the structure of the antimicrobial film and the diffusion of the antimicrobial agent into the food. The incorporation of AIT into the films increased the elongation at the break (E) value of the films and decreased the tensile strength (TS) value of the films. The CNT was important to retain the AIT which is a volatile substance in the film. The diffusion of the AIT from the film into the chicken reduced the microbial contamination, controlled oxidation and reduced the colour changes. Thus, these packages were effective for the 40 days of storage.

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1. Introduction

Poultry belong to a class of highly perishable foods, and the main concern in poultry industries is extension shelf life of products. In addition to microbiological safety, the colour and lipid sta-

tability of the meat are important quality characteristics that influence product acceptance by consumers (Calatayud et al., 2013). Minced muscle undergoes changes and develops oxidative rancidity faster than intact muscle because grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto, O’Grady, Kerry, & Buckley, 2005).

Studies have shown that allyl isothiocyanate (AIT), a volatile sulphur compound, has antioxidant and antimicrobial properties that inhibit a variety of pathogens at low concentrations, especially in frozen or chilled meat products (Nadarajah et al., 2005). In Japan, the use of AIT from natural sources is allowed, and it is classified as safe by the Food and Drug Administration (FDA) of the United States (Lemay et al., 2002; Wang, Chen, & Ho, 1998).

Chacon, Buffo, and Holley (2006) assessed AIT against Esche-
richia coli O157:H7 in refrigerated, nitrogen packed, finely chopped beef and they also reported than beef had a spicy smell just after opening the packages. This odour might be pleasing for some foods such as meat.

Some authors have pointed out that the antimicrobial activity of AIT may be decreased when it reacts with constituents of meat, such as thiols, amino acids and the sulphhydryl groups of proteins (Nadarajah et al., 2005). Another important factor that may affect the antimicrobial activity of AIT is its solubility in the fatty acids of the meat. Spontaneous degradation reactions involving hydrox-
ide ions and nucleophilic attack by water (Chen & Ho, 1998) and the adsorption of the antimicrobial in packaging materials (Nadarajah et al., 2005) have been reported as being responsible for reducing the stability of AIT. In addition, the fat in foods can provide a protective layer around the contaminating bacteria or absorb the lipophilic part of AIT, reducing its effectiveness in the product (Chacon et al., 2006).

Technology based on the encapsulation of active substances into nanoparticles in biodegradable films may offer a potential alternative to protect and control the release of antimicrobial agents, improving their effectiveness and stability for food applications. (Bouwmeester et al., 2009; Guarda, Rubilar, Miltz, & Galotto 2011; Lemay et al., 2002; Malheiro, Daroi, Silveira, & Brandelli, 2010; Mitsumoto, O’Grady, Kerry, & Buckley, 2005; Persico et al., 2009; Sanchez-Garcia, Ocio, Gimenez, & Lagaron 2008). The carbon nanotube (CNT), which is characterised by one or several concentrically wound graphene sheets, is a nanoparticle that can possibly hold active substances in its hollow inner cavity (Zarbin, 2007).
More research is needed to achieve a conclusive understanding regarding the migration of these nanoparticles. There are no available data on the possible migration of nanoparticles embedded in food packaging materials (Azeredo, 2009), which is mainly due to the lack of methods for the detection of nanoparticles in food matrices (Bouwmeester et al., 2009). Using physical and chemical concepts, Šimon, Chaudhry, and Bakos (2008) made predictions about the migration of nanoparticles, concluding that the migration of nanoparticles from packaging to food would be slow and that only a few nanoparticles would migrate. Furthermore, research thus far has indicated that CNTs are not toxic (Firme III & Bandaru, 2010; Lagaron & Lopez-Rubio, 2011).

In this context, the objectives of this study were as follows: to determine the antimicrobial efficacy of cellulose-based films incorporated with AIT and CNT for shredded cooked chicken inoculated with Salmonella Choleraesuis; to investigate the influence of this film on the colour and oxidation of meat; and to analyse mechanical changes in the film, including the structure of the film and diffusion of components of the antimicrobial film into the packaged meat.

2. Materials and methods

2.1. Materials

The materials used for the development of this research were as follows: polymer derived from cellulose (cellulose acetate, Rhodia Co., Courbevoie, France), acetone (Merck, Darmstadt, Germany), AIT from oil of mustard (94%, Sigma Aldrich Chemie, Steinheim, Germany), carbon nanotubes (CNT; Ahwahnee Technology, San Jose, CA,USA), peptone water (Difco® [Becton, Dickinson and Co., Franklin Lakes, NJ, USA]), butylated hydroxytoluene (BHT; Sigma Aldrich, St. Louis, MO, USA), trichloroacetic acid (TCA; Sigma), thio-barbituric acid (TBA; Sigma), plate count agar (PCA; Difco®), and HiCrone Improved Salmonella agar (HiMed, Old Bethpage, New York, USA).

2.2. Experimental design

A central composite design with three central points was used in order to evaluate the influence of the following factors: the amount of antimicrobial AIT in the film (20–60% (v/w)), the amount of CNT in the film (0–0.1%; w/w) and the analysis period (2–40 days) (Rodrigues & Lemma, 2012). The AIT and CNT concentrations were based on previous in vitro studies. Table 1 shows the assays that were delineated using Minitab software.

To evaluate the antimicrobial effect of the film, the contaminated shredded cooked chicken meat was packaged in control films without AIT that were either cellulose-based (CB) or cellulose-based with CNT (CB + CNT), and the meat was assessed at the time points defined by the experimental delineation (2, 10, 21, 32, and 40 days). The films with AIT are described in Table 1 (films 1–17). To evaluate the changes in the films and in the chicken over the experimental time period, the films and the chicken were characterised at time zero.

2.3. Development of the films

The films (PI 1004748-4, Dias, Soares, Borges, & Medeiros 2011) were prepared by dissolving the cellulose polymer in acetone. AIT was used as the antimicrobial agent, and it was embedded in the film solution in a concentration range of 20–60% (v/w). CNT were also added to the film in the range 0–0.1% (w/w) in relation to the weight of the polymer. The film solutions were placed in a support/mould with the aid of machine casting (K-Paint applicator, port/mould with the aid of machine casting (K-Paint applicator, the weight of the polymer. The film solutions were placed in a sup-

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(C–) not detected.
Model 202 Littlington, Royston, UK), and the solvent was allowed to evaporate. The films were dried in an environment of 50 ± 2% RH and 23 ± 2 °C.

After being dried, films measured 30 × 15 cm, had a thickness of 40 μm and were opaque (white).

2.4. Preparation of cooked shredded chicken

Fresh chicken breast meat was cooked for 10 min in a jacketed open evaporator and then shredded by hand with sterilised utensils (200 ppm chlorinated water for 20 min). To confirm that the shredded cooked chicken was sterile, detection tests for Salmonella sp. and counts of aerobic mesophilic (AM) and psychrotrophic (PSI) microorganisms were performed according to rules of the American Public Health Association (APHA, 2001).

2.4.1. Activation of the S. Choleraesuis and inoculation of the shredded cooked chicken

S. Choleraesuis (ATCC 6539) bacteria was activated twice in succession in tryptic soy broth (TSB; Difco) and incubated at 35 ± 2 °C for 24 h. After incubation, the broth was centrifuged at 3783g for 5 min at 5 °C (Centrifuge 4 K-15, Sigma). The cell pellets were washed with 0.1% peptone water and centrifuged again under the same conditions. The cultures were resuspended in peptone water and diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to an absorbance of 0.3 at a wavelength of 560 nm as determined by spectrophotometry (GC UV/VIS 918, Shimadzu, Kyoto, Japan), which is equivalent to a count of 8 log CFU/mL (Nadarajah et al., 2005). The inoculum was diluted to 7 log CFU/mL, and 160 mL of this S. Choleraesuis solution was added to 8.0 kg of meat to yield a final concentration of 5.7 log CFU/mL (Nadarajah et al., 2005).

2.4.2. Storage of shredded cooked chicken in antimicrobial packaging

Shredded cooked chicken meat inoculated with S. Choleraesuis was packaged (approximately 100 g per package) in antimicrobial films and control films (CB and CB + CNT) which were placed in plastic bags (polyethylene/nylon) with dimension of 15 × 12 cm. The antimicrobial film lined the inner surface on both sides of the plastic bags. The packages were heat sealed with no vacuum applied and stored at 4 ± 1 °C, the standard temperature for meat refrigeration.

2.5. Analysis of the packaging

The mechanical properties, AIT content and structural properties (infrared spectroscopy) of the antimicrobial films and control films used in the packaging of cooked shredded chicken meat were analysed throughout the storage period.

2.5.1. Evaluation of the mechanical properties

The mechanical properties of the films were determined by the tensile test using a Universal Testing Machine (Instron model 3367 [Instron, Norwood, MA., USA]) with the following parameters: load cell of 1 kN, speed of 12.5 mm min⁻¹ and initial grip separation of 125 mm. Five samples of each film with dimensions of 25 × 175 mm were analysed. The tensile strength (TS, MPA) and elongation at break (E, %) values were measured. TS was calculated by dividing the maximum load by the cross-sectional area of the specimen and by multiplying the result by 100 (ASTM, 2002).

2.5.2. Determination of AIT in films

To quantify the AIT present in the films, three samples with an area of 90 cm² were taken from each film and placed in screw-cap test tubes. Hexane (10 mL, Sigma) was added to each film sample and mixed at 2500 rpm for 1 min. Samples were left overnight at 4 °C. After this period, the tube was shaken again for one minute and the hexane was filtered through a 0.22 micron Millipore membrane and stored in vials of approximately 2 mL (Chacon et al., 2006). With the aid of a gastight syringe (Hamilton), an aliquot (10 μL) was taken from the vial through the septum and injected into a gas chromatograph coupled to a mass spectrometer (GC/MS; HP 5050, column DB5, Shimadzu Co., Kyoto, Japan) and an autosampler (Shimadzu Co.). The GC/MS operating conditions used in the present study were previously described by Nadarajah et al. (2005). The result was expressed as mg AIT/100 mL.

To quantify the AIT compound, a standard curve was obtained from a range of known concentrations of AIT. The retention time was 3.45 min for the AIT compound.

2.5.3. Spectroscopy in the infrared region

To evaluate structural changes in the films throughout the chicken storage period, infrared spectra were obtained using a Nicolet IR200 spectrometer (Thermo Nicolet Corporation, West Palm Beach, FL, USA) in transmission mode. The range of analysis was 4000–400 cm⁻¹, and 32 scans were performed per sample.

2.6. Analysis of cooked shredded chicken meat

2.6.1. Measurement of lipid oxidation

Lipid oxidation was measured by analysing thiobarbituric acid reactive substances (TBARS) in triplicate. To perform this analysis, 0.5 mL of 0.15% BHT was added to each sample (5 g) to prevent oxidation during the preparation and then 20 mL of 5% TCA was added. The mixture was incubated for 20 min and then homogenised with a vortex homogeniser. The homogenised mixture was filtered through filter paper into 25 mL volumetric flasks, and 5% TCA was added to reach a final volume of 25 mL. Then, 2 mL of this solution was removed and added to 2 mL of 0.08 M TBA; the tubes were then sealed and placed in a boiling water bath at 95 °C for 15 min. After cooling, an absorbance reading was taken with a spectrophotometer (GC UV/VIS 918, Shimadzu, Japan) at 532 nm against a blank containing all of the reagents except the sample (Ganhão, Estévez, & Morcuende, 2011).

TBARS were expressed as mg malonaldehyde (MDA equivalent)/1000 g sample.

2.6.2. Colour analysis

Colour measurements were determined using the Color Quest XE colorimeter (Huber Lab, Tampa, FL, USA) and a CIELab system with a D65 illuminant and observation angle of 10°. The following parameters were obtained: L*, lightness; a*, intensity of red/green; b*, intensity of yellow/blue; saturation index difference (DC*); and grey colour difference (DE*). The ΔL*, Δa* and Δb* were calculated from the difference between the sample value and the value of the standard. The cooked chicken sample at time zero was used as a standard. The parameters c*,

\[ c^* = \sqrt{a'^2 + b'^2} \]  
\[ DC^* = C_{\text{sample}} - C_{\text{standard}} \]  
\[ DE^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \]

2.6.3. Determination of AIT in shredded cooked chicken

To quantify the AIT diffused from the film into the chicken, 5.0 g of shredded cooked chicken packaged with antimicrobial film was weighed in a screw-cap test tube. The AIT concentrations in these samples were determined as described above (Section 2.5.2.).
2.6.4. Microbiological analysis

Plate count agar (PCA) was used for counting the aerobic mesophilic (AM) and psychrotrophic (PSI) microorganisms, and HiCrome Improved Salmonella agar was used for counting S. Choleraesuis (SAL). A sample of shredded cooked chicken meat (25 g) was aseptically collected and added to 225 mL of peptone water. The mixture was homogenised for 30 s in a stomacher (model 1240, ITR Ltd., Pillar, Rio Grande do Sul, Brazil), and serial dilutions were made. Aliquots (0.1 mL) were plated on the culture media. Plates incubated at 35°C for 24 h were used for counts of AM and SAL microorganisms, and plates incubated at 7 ± 2°C for 10 days were used for counts of PSI microorganisms. Plates containing 25–250 colonies were selected for counting, and the results were expressed as logCFU/g (APHA, 2001).

2.7. Principal component analysis

To provide an overview of the behaviour of the films and meat, principal component analysis (PCA) was applied to the results of the assays of the central composite design elements (films and meat) and the control assays. These data were autoscaled. PCA was also applied to the spectroscopic data that had been processed with multiplicative scatter correction (MSC). After pre-processing the data, the data matrices were analysed by PCA using Matlab (version 7.5).

The samples (films and chicken packaged in respective films) were identified in the PCA plot as follows: CB (cellulose-based film) and CNT (cellulose-based film + CNT) followed by numbers indicating the analysis time: 0, 2, 10, 21, 32, and 40 days. The samples coded with numbers from 1 to 17 represent the assays of the central composite design (Table 1) and numbers from 1 to 17 followed by a 0 represent the film and the shredded cooked chicken of the design at time zero.

The films were characterised by the results of the mechanical analysis and the AIT content, identified in plot PCA by TS (tensile strength), E (elongation at break) and CG film (determination of AIT in films), respectively.

The shredded cooked chicken meat was characterised by lipid oxidation (TBA), colour (L*, a*, b*, DC* and DE*), determination of AIT in chicken (GC chicken) and microbiological content (AM, PSI, and SAL). The influence of independent variables (AIT, time, and CNT) on the analysis was also evaluated.

3. Results and discussion

3.1. Results of the physical and chemical analyses

Table 1 shows the assay results for the central composite design. Analysis of assay 1 and 3; 6 and 8; 11 and 15 revealed that increasing the amount of CNT increases the amount of AIT in the films (film GC).

The values of tensile strength and elongation at break for the antimicrobial films ranged from 26.62 to 50.34 MPa and 1.63% to 4.91%. Gemili, Yemenicioglu, and Altinkaya (2009) found intermediate values for cellulose acetate films.

After forty days of storage, the shredded cooked chicken still had AIT (GC chicken), indicating the efficiency of the films. In relation to colour analysis, shredded cooked chicken presented DC* < 0 indicating that the colour of the sample is less saturated than the colour of the standard (shredded cooked chicken at time 0) (Ramos & Gomide, 2007) probably due to loss of pigment.

The TBA value was 0.131 and 0.221 mg malondialdehyde (MDA) equivalents/1000 g sample for shredded cooked chicken and stored in antimicrobial films for 2 and 40 days respectively.

3.2. Microbiological counts

Salmonella sp. were undetectable and less than $1 \times 10^1$ CFU/g AM and PSI microorganisms were found in 25 g of the shredded cooked chicken before the chicken was inoculation with S. Choleraeuis.

Fig. 1 shows the counts of S. Choleraeuis (SAL), aerobic mesophilic (AM) and psychrotrophic (PSI) microorganisms in the shredded cooked chicken meat inoculated with 5.7 logCFU/g of S. Choleraeuis and stored for 40 days packaged with either antimicrobial films (defined by central composite design with three central points; see Table 1) and control film lacking AIT and CNT.

The film with 28% AIT and 0.02% CNT (assay 1) and the film with the same amount of AIT but 0.08% CNT (assay 3) had SAL counts of 5.7 and 3.9 logCFU/g, respectively, by 10 days of storage. Only the second film with a higher concentration of CNT reduced the counts in shredded cooked chicken meat, indicating the importance of CNT for retaining AIT.

The film from assay 1 (28% AIT and 0.02% CNT) with 32 days of storage had counts of 5.7, 6.3 and 6.5 logCFU/g for the SAL, AM and PSI microorganisms, respectively. This was the only film that allowed the growth of PSI microorganisms.

Fig. 1. The counts and error bars of S. Choleraeuis, psychrotrophic and aerobic mesophilic microorganisms in shredded cooked chicken meat packaged in antimicrobial and control films.
After 10 days of storage, the film prepared with 52% AIT and 0.08% CNT (assay 4) had reduced the counts of SAL and AM microorganisms to 1.9 and 3.8 log CFU/g, respectively. The SAL and AM counts were reduced by 4 and 2 logarithmic cycles of the initial inoculation, respectively.

With up to 10 days of storage, it was clear that the reduction of SAL microorganisms was greater than the reduction of AM microorganisms, which may have been due to the presence of gram-positive microflora that are more resistant to AIT. Nadarajah et al. (2005) and Chacon et al. (2006) observed this same behaviour for mesophiles in beef treated with AIT.

The other treatments analysed after 21, 32 and 40 days had counts of less than 1.0 log CFU/g for the SAL, AM and PSI microorganisms.

The shredded cooked chicken meat packaged with films without antimicrobial and without CNT had counts of 8.4, 8.0 and 7.9 log CFU/g for SAL, AM and PSI microorganisms, respectively, after 40 days of storage. These values are higher than those found for the chicken packaged in antimicrobial films, which indicated the effectiveness of these films in inhibiting microbial growth in this product.

3.3. Global characterisation by PCA analysis

Fig. 2 shows the plot of scores and loadings for the first and second principal components (PCs) of the analysis performed for the samples of shredded cooked chicken meat and the films used to package it. The first and second principal components (PC1 and PC2) together explained 64.98% of the data variability with 49.54% and 15.44% of the variation explained by the first and second PCs, respectively.

Fig. 2 shows that three groups were formed. The first group, located on the left of the plot, consisted of the assays from time zero, i.e., films without contact with chicken and films 4 and 8 (52% AIT and 0.08% CNT at 10 and 32 days, respectively). These films had high levels of AIT (GC film) and elongation at break (E) values. The shredded cooked chicken in this group was associated with high values of $a^*$, $b^*$, $L^*$, and DC.

The antimicrobial agent incorporated into the film affected the polymer structure by increasing the elongation at break (E) and reducing the tensile strength (TS). The addition of AIT created a heterogeneous film structure featuring discontinuities that may have affected the stretching ability of the film. AIT is a liquid at room temperature and at 4 °C so it is present in the film in the form of easily deformed droplets, enhancing the extensibility and weakening the structure of the film by lowering its tensile strength (Chen & Lai, 2008; Zinoviadou, Koutoumanis, & Biliaderis, 2009). AIT acted as a plasticiser by reducing the intermolecular forces, decreasing the rigidity of the film structure and increasing the mobility of biopolymer chains, and thereby causing an increased capacity for elongation (Chen & Lai, 2008). Alterations in the mechanical properties of polymer matrices related to the use of active agents were reported in other studies. Sánchez-González, Chiralt, González-Martínez, and Cháter (2011) developed films incorporating different concentrations of bergamot, lemon and tea tree essential oils into hydroxypropylmethyl cellulose and observed that the use of these essential oils leads to a significant decrease in tensile strength and elastic modulus of the composite films. Espitia, Soares, Botti, and Silva (2011) developed films of the cellulose acetate with oregano, cinnamon, or lemongrass essential oils and the highest value of maximum load-at-break observed was for the film without essential oil.

Despite contact with the product, the films from assays 4 and 8 were also characterised by high levels of AIT and high elongation at break (E) values, which may be due to the high amounts of AIT and CNT (52% and 0.08%, respectively) that allow these films to have a similar behaviour to the films at time zero. Due to the entanglement of hundreds of individual tubes that are adhered (linked) to each other as a result of Van der Waals forces, CNTs form pores that provide large external surfaces that can immobilise substances (Upadhyayula, Deng, Mitchell, & Smith, 2009), and these pores could act as encapsulating agents to control the release of substances such as AIT, which is a highly volatile compound. The use of nanoparticles for the controlled release of active agents was reported by Sanchez-Garcia et al. (2008), who developed nanocomposites of polycaprolactone and natural mica modified for controlled-release of thymol, the biocidal natural. Tunc and Duman (2011) reported that the use of montmorillonite in methylcellulose film decreased the release of carvacrol.

The chicken samples in this first group had higher colour parameter values ($L^*$, $a^*$, $b^*$, and DC) when compared with other groups, indicating that the meat at time zero was lighter and had more intense red and yellow colours resulting in more saturated pigments than in the standard and consequently $ΔC^* < 0$.

The second group (Fig. 2) was formed by the film and chicken samples from assays 1, 2, 3, 5, 9 and 13 in addition to the controls without AIT (CB films and CB + CNT films) at 2, 10, 21, 32 and 40 days. The films in this group were associated with high levels of TS and the meat was associated with high microbial counts (AM, PSI and SAL microorganisms) and high values for total colour difference (DE') and lipid oxidation (TBA).

The films in contact with food had high TS values. The diffusion of AIT from the films (or the absence of AIT in the film controls) and the contact with the chicken constituents (water and fat) increased the force required to disrupt the film (TS), indicating the importance of water as a plasticiser of the film matrix. In addition, better reorganisation may occur upon hydration due to increased molecular mobility that may result in polymer chain being more cohesive (Zinoviadou, Koutoumanis, & Biliaderis, 2009). Moreover, differences among the samples with and without AIT were less apparent with higher moisture contents resulting from the film being in contact with the food, indicating the role of water as a film plasticiser. Therefore, TS values were influenced by the time of contact with the chicken and the content of AIT, and were less influenced by the presence of CNT.

The meat packaged in control films had higher microbial counts over the storage period of 40 days compared with the meat stored...
in the packaging with AIT. For the other treatments in this group, there was microbial growth due to the low concentration of AIT, the low concentration of CNT incorporated in the film to retain AIT and/or the short time of contact between the film and the chicken. The greatest variation in colour (DE’) was observed for the second group, which included the control assays. The DE’ parameter allows the determination of how much the overall colour of the sample differs from the standard and if that difference is noticeable to the consumer. According to Ramos and Gomide (2007), DE’ values greater than 5 represent differences that are detectable by the human eye. The treatments that exceeded this value included the controls and assays 1, 2, 3, 9, and 13 (group 2), which had a low concentration of AIT and/or CNT and only a few days of storage in which AIT could act as an antioxidant.

The chicken samples from the second group had the highest level of oxidation (TBA). TBA was positively influenced by time, and negatively influenced by AIT. The highest concentration of AIT resulted in the lowest value of TBA, indicating that AIT may be a natural antioxidant. The antioxidant properties of mustard essential oil, which contains AIT as the active ingredient, have been reported by Chanjirakul, Wang, Wang, and Siriphanich (2006).

The third group was formed by the chicken samples from assays 6, 7, 10, 11, 12, 14, 15, 16, and 17; these samples had high values for AIT content (vector GC chicken) due to the diffusion of the antimicrobial agent from the film to the meat. These samples lacked microorganisms and had lower values of DE’ and TBA. According to Lemay et al. (2002), AIT increases the lag phase of several bacteria, including E. coli, Staphylococcus aureus, Proteus vulgaris, Pseudomonas fragi and Pseudomonas aeruginosa.

3.4. Characterisation by spectroscopy in the infrared region

FT-IR spectroscopy was performed to determine the characteristics of the film matrix before and after contact with chicken as well as the changes in the intermolecular interaction in the composite films (Martins, Cerqueira, & Vicente, 2012; Zhong, Song, & Li, 2011). The biplot graph in Fig. 3 shows that the first and second principal components (PC1 and PC2) explained 67.52% and 15.02%, respectively, of the variation in the infrared spectroscopic data, which was sufficient to discriminate the samples.

According to Lieber, Rao, and Ramachandran (1959), thiocyanates have characteristic vibration frequencies at approximately 2140 cm⁻¹, and thiocyanates have characteristic vibration frequencies between 2060 and 2105 cm⁻¹. The intensity of the peak in the spectrum in the wavelength region of AIT is related to the amount of this component.

The spatial separation of the films indicated the existence of two distinct chemical structural groups (Fig. 3). The lower group in Fig. 3 was formed by all films that had contact with food for different time periods. The film controls without AIT at time zero (CB0 and CNT0) and the film with 28% AIT and 0.02% CNT at time zero (1-0) belong to the same group. The contact between the product and packaging causes physical, chemical and structural changes in the polymeric materials due to constituents of the product, temperature changes, pH changes, mechanical stress, the presence of oxygen and UV radiation. These effects initiate the processes of polymer degradation, migration of chemical compounds with low molecular weights and reduction of functionality (Ozen & Floros, 2001; Shimamura & Nakamura 2009; Steinka, Morawska, Rutkowska, & Kukulowicz, 2006). Over time and with contact between the packaging and the food, the amount of AIT in the films decreased as a function of AIT diffusion into the packaged product. The diffusion of AIT from the film to the chicken can be explained by the plasticisation effect on the film produced by the water present in the chicken, which may have increased the molecular motion in the film matrix, which would trigger the release of AIT. The film 1-0 although that did not contact the food had a low amount of AIT due to low concentration of CNT used.

This result corroborated the diffusion of AIT because the chicken meat (assay 1) packaged in this film was not characterised by the GC chicken vector (Fig. 2) and yielded high counts of microorganisms (Fig 1).

The other group in Fig 3 was formed with the others films from time zero that had no contact with food and that had a high amount of AIT (2-0; 3-0; 4-0; 7-0; 9-0; 10-0; 11-0; 13-0). Therefore, a group was formed with the films that had low amount of AIT or no AIT, and another group was formed with the films that had a high amount of AIT. This leads to the conclusion that the greatest difference observed between the films by spectroscopy in the infrared region was due to the amount of AIT because there was no separation of the films with and without CNT.

4. Conclusion

AIT acting as a plasticiser that reduced the rigidity of the films, which consequently increased the elongation at break (E) values and decreased the tensile strength (TS) values. The structure of the films was changed by contact with the chicken meat through the diffusion of AIT and contact with the constituents of the chicken (water and fat) that promoted an increase in TS.

The factors studied influenced food preservation. Therefore, we recommend the use of AIT at concentrations higher than 28% combined with CNT at concentrations higher than 0.02% to achieve microbial reduction, control oxidation and reduce colour changes. These packages were effective for 40 days of storage, which represents their great potential to increase shelf life and food safety. Future sensory analysis studies may also support the application of these films for foods.

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