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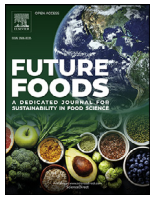

# Antimicrobial properties of chitosan and galactomannan composite coatings and physical properties of films made thereof

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ABSTRACT

The aim of this study was to produce antimicrobial coatings and films based on the mixture of chitosan and galactomannan from *Adenanthera pavonina* L., with the incorporation of sodium acetate. The antimicrobial activity of the coatings was evaluated against *L. monocytogenes, S. aureus, P. aeruginosa* and *S. enteritidis*. Then the films produced, based on the coating formulations, were characterized in terms of water vapour permeability (WVP), oxygen ($O_2$) and carbon dioxide ($CO_2$) permeability, moisture content (MC), water solubility (S), tensile strength (TS), elongation at break (EB), elastic modulus (EM), opacity and color. The composite coatings with Chi were effective against Gram-negative and Gram-positive bacteria. Films Chi presented the low water solubility and high values of component b*, which indicates the predominance of yellowish coloration and the highest TS values. Films of Chi-Gal-NaA present lower values of S, MC, WVP and EB and is the film presenting the higher value of EM. While for the films of Gal-NaA there was a reduction of the $O_2$ permeability and an increase of $CO_2$ permeability. Chi in combination with NaA can be used in the development of antimicrobial coatings and films for food applications, therefore contributing to food preservation and shelf-life extension.

## 1. Introduction

The primary function of the packaging is the protection of the external environment from physical and microbiological factors, among which are: the water vapor present in the atmosphere, water, odor, dust and micro-organism (Robertson, 2012), this external protection in the food helps maintaining quality and food security. The use of food packaging in the preservation of quality is already well established in the industry, however the raw material is usually of petroleum origin, which ends up generating a concern with the accumulation in the environment (Mathew and Radhakrishnan, 2019). A new alternative for the replacement of petroleum-based packaging has been growing in recent years, with emphasis for edible packaging due to the material from renewable and biodegradable sources, which has aroused the interest of the global market (Cerqueira et al., 2016).

The edible films and coatings still cannot completely replace conventional packaging (petroleum origin), but they can be used to extend the stability of foods in the exchange of moisture, lipids, volatiles and gases between food and the surrounding environment (Mohamed et al., 2020). Due to these reasons, they have been investigated for their ability to prevent dehydration or absorption of water by food, loss of flavor and limiting the penetration of oxygen into food, reducing oxidation reactions. Therefore, edible films and coatings can be used to extend the shelf life of different products (Costa et al., 2018; Montero-Calderón et al., 2016; Nunes et al., 2016).

The production of films combining different polysaccharides, such as chitosan (Chi) and galactomannan (Gal), is one of the useful methods to obtain new materials with the desired functional properties, thus combining the intrinsic properties of the different biopolymers (Rao et al., 2010; Martins et al., 2012b). However, the combination of Chi and Gal, and to the best of the authors' knowledge has never been tested, despite the existing examples between Chi and tara gum (Antoniou et al., 2015) and Gal and carrageenan (Martins et al., 2012b). Chi is a polysaccharide obtained from the alkaline hydrolysis of N-acetyl group of chitin, the main component of the crustacean shells and is known to be biodegrad-

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able, biofunctional, biocompatible and non-toxic, qualifying it to be used in the food industry (Cao et al., 2012; Abdollahi et al., 2012; Arkoun et al., 2017; Santos et al., 2017). Several studies have shown that Chi has antimicrobial activity against a wide range of microorganisms and that can be used to increase shelf life of foods (Aider 2010; Cao et al., 2012; Elsabee and Abdou 2013; Jayathilakan et al., 2012; Li et al., 2013). Gal are polysaccharides consisting of a linear chain of mannose units linked by $\alpha$- (1 → 4) bonds and branched by D-galactose units linked by $\beta$-(1→6) bonds. They are present in the endosperm of various vegetable species and have different functions such as carbohydrate reserves. These hydrocolloids are food grade, present thickening capacity and used in on different food applications (Jiang et al., 2011; Cerqueira et al., 2011).

The active properties of edible films and coatings can be improved by the addition of some organic acids, such as lactic, acetic, malic, citric acids and sodium acetate (NaA), that when added to the edible films allow it to inhibit microorganisms (Campos et al., 2011; Sallam, 2007). Several works explored the incorporation of active and bioactive compounds in edible and films and use it to extend the food shelf-life, through the decrease of microbiological growth (Guimarães et al., 2018). The use of NaA was tested as antimicrobial in hydroxypropyl methylcellulose coatings (Karaca et al., 2014) but never evaluated in combination with chitosan and galactomannan.

The objective of this work was to determine the antimicrobial and physical properties of composite coatings and films, respectively, based on Chi from crustacean, Gal from *Adenanthera pavonina* L. and NaA. In a first stage the antimicrobial activity of the composite coatings formulations was studied. Afterwards, the films produced based on the coating formulation were evaluated in terms of their physical properties, namely mechanical and barrier properties, moisture content, solubility in water, color and opacity.

## 2. Materials and methods

### 2.1. Materials

The materials used to prepare the coatings and film-forming solutions were: high molecular weight chitosan, obtained in the POLYMAR Indústria e Comércio LTDA (Brazil) with a degree of deacetylation of 90%; lactic acid, magnesium nitrate hexahydrate, NaA and glycerol 99% (plasticizer) were obtained in VETEC Quimica Fina (Sigma-Aldrich – USA) and distilled water. Gal was extracted from Adenanthera pavonina L. seeds endosperm (Cerqueira et al., 2009a), collected in the Federal University of Ceará, Campus of Pici, Fortaleza, CE, Brazil. Adenanthera pavonine L. was registered in the Brazilian National System of Management of GeneticHeritage and Associated Traditional Knowledge (SisGen) by registration number AE7BAB9. Lyophilized strains of *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 1026) and *Salmonella enteritidis* (ATCC 1132) were obtained from a supplier Microbiologics (USA).

### 2.2. Experimental design

The effect of NaA addition to the coatings and films of Chi and Gal was assessed in their antimicrobial performance and physical properties. The experiment were prepared using a $3^3$ factorial design that included Chi (0.0 and 1.0 g/100 mL), Gal of *A. pavonina* L. (0.0 and 1.0 g/100 mL) and NaA (0.0 and 0.05 g/100 mL).

### 2.3. Production of film-forming solution

#### 2.3.1. Production of Chi coatings and film-forming solutions

Coating and film-forming solutions of Chi were prepared according to the methodology presented by Vásconez et al. (2009) and Souza et al. (2010), with some modifications. Chi 1% (w/v) was dispersed in 1% (v/v) lactic acid solution under gentle stirring during 1 hour at room temperature. Glycerol was then added at a concentration of 0.6% (v/v). The formulation with NaA was prepared by adding 0.05% (w/v) of NaA to the previously prepared solution and left to stir during 1 h. These solutions were named Chi solution and Chi–NaA solution, respectively.

#### 2.3.2. Preparation of Gal coatings and film-forming solutions of A. pavonina L

Coatings and film-forming solutions were prepared according to the methodology described by Cerqueira et al. (2009b) and Vásconez et al. (2009), with adaptations. Briefly, lyophilized Gal 1% (w/v) was dissolved in distilled water and glycerol added at a concentration of 0.6% (v/v). This mixture was then magnetically stirred with heating (60°C) for 5 h. The formulation with NaA was prepared by adding 0.05% (w/v) of NaA to the previously prepared solution and left to stir during 1 h. These solutions were named Gal solution and Gal-NaA solution, respectively.

#### 2.3.3. Preparation of composites coatings and film-forming solutions

The composites were obtained by adding the Gal film-forming solution to the Chi film-forming solution. The composites were magnetically stirred with heating (60°C) for 1 h. The final concentrations of the components of the film-forming solution were: Chi 1% (w/w), Gal 1% (w/v) and glycerol 0.6% (v/v). For the formulation containing NaA, the final concentrations were: Chi 1% (w/w), Gal 1% (w/v), glycerol 0.6% (v/v) and NaA 0.05% (w/v). These formulations were named composite of Chi-Gal and composite of Chi-Gal-NaA, respectively.

### 2.4. Evaluation of the antimicrobial activity of the coatings

#### 2.4.1. Preparation of the inoculants

The different strains have undergone the protocol indicated by the supplier. A well-isolated colony with a standardized size was obtained of each microorganism (*L. monocytogenes* and *S. enteritidis*: 1 mm; *S. aureus*: 2 mm; *P. aeruginosa*: 4 mm). *S. aureus, P. aeruginosa* and *S. enteritidis* were incubated in tryptic soy agar (TSA/Difco) and *L. monocytogenes* in tryptic soy-extract of yeast agar (TSA-YE/Difco) at 35°C for 24 hours. *S. aureus, P. aeruginosa* and *S. enteritidis* were transferred to tubes containing 5 mL of broth infusion brain heart (BHI) and *L. monocytogenes* was inoculated into tube containing 5 mL of tryptic soy broth (TSB/Difco). The strains were incubated at 35°C for 24 h to obtain a final concentration around $10^8$ CFU/mL for *L. monocytogenes* and $10^9$ CFU/mL for other microorganisms. The concentrations of the inoculants were adjusted with peptonized water 0.1% (Difco) making dilutions to $10^5$ CFU/mL. The concentrations were confirmed, after incubation, by the count of viable colonies in selective/differential means for each microorganism: *L. monocytogenes*: PALCAM agar base (at 35°C/48 hours); *S. aureus*: Baird-Parker Agar (at 35°C /48 hours); *S. enteritidis*: Agar enteric de Hecktoen (at 35°C/24 hours); *P. aeruginosa*: Pseudomonas agar base (at 25°C/24 hours).

#### 2.4.2. Antimicrobial activity of the coatings

The determination of the antimicrobial activity of the coatings was performed using the dilution method described by Kim et al. (1995), with modifications. In test tubes containing 3.95 mL of tryptic soy broth (TSB/Difco) sterile, an aliquot of 50 μL of the bacterial suspension in a concentration of $10^5$ CFU/mL and 1.0 mL of the coating were added. After the incubation period, 100 μL of the culture was inoculated with agar plates containing selective/differential means for each microorganism in the spread plate. The plates were incubated according to the time and temperature described above, and subsequently analyzed visually to verify that there was bacterial growth, was expressed as follows: (+): presence or (-) absence of microorganisms). The following controls were conducted: culture medium + NaA; culture medium + NaA + inoculum; positive control (culture medium + inoculum). The pH of the coatings and the pH of the coatings + culture medium were determined by direct

reading in a pre-calibrated potentiometer with buffer solutions of pH 4.0 and 7.0.

### 2.5. Production of films

The films were obtained by the casting technique (or solvent evaporation), according to Souza et al. (2010), with adaptations: 28 mL of film-forming solution were dispensed on the surface of Petri dishes (9 cm of diameter) and dried in a controlled temperature chamber at 35°C for 16 h. After the film formation, it was separated from the dish to obtain standalone films. All the samples were conditioned inside desiccators containing silica gel, which provided a relative humidity (RH) of 10% at 25°C, until further characterization.

### 2.6. Physical properties of films

#### 2.6.1. Film thickness

The film thickness was measured with a digital micrometer (Digimess, São Paulo, Brazil), having a sensitivity of 0.001 mm. Three thickness measurements were taken on each testing sample in different, randomly chosen points. The mean value of film thickness was used to calculate water vapor permeability (WVP), oxygen permeability ($O_2P$), carbon dioxide permeability ($CO_2P$) and tensile strength (TS).

#### 2.6.2. Mechanical properties

Traction tests were conducted for the mechanical characterization of the films tensile strength, elongation at break and elastic modulus, in the Biomass Technology Laboratory of Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil. Before conducting the tests, the films were packed in a desiccator containing magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$) to keep them in an environment with relative humidity and temperature surrounding, respectively, of 50 ± 5% and 24 ± 2°C for at least 40 h. In traction trials, the test bodies with a length of 63.24 mm and width of 12.86 mm were fixed by means of claws engaged to a movable platter of the universal test machine, following the ASTM-D-882-91 guidelines. The equipment used for analysis was the EMIC (Universal testing machine, model DL-3000, China), with a load cell of 100 N. The grip separation and crosshead speed were defined at 30 mm and 50 mm $min^{-1}$, respectively. TS was expressed in MPa and EB was expressed in percentage (%). The TS was calculated by dividing the maximum load (N) by the initial film cross-sectional area ($m^2$) of the film. EB was expressed as a percentage of the change in the initial length of the specimen at the point of break. TS and EB tests were replicated at least five times for each film.

#### 2.6.3. Water vapor permeability (WVP) measurement

The water vapor permeability (WVP) of films was gravimetrically assessed, according to the protocol B of ASTM (1995) with the adaptations proposed by Debeaufort et al. (1993) specifically for edible films. Circular aluminum cups, with a diameter of 8 cm and a depth of 5 cm, were accordingly used. Deionized water (30 mL) was placed in each test cup, to expose the lower film face to a high RH. The films samples were mounted with the upper surface facing the RH (50 ± 2%) of the environment-controlled room. Theweight loss of the cups was monitored over a 72 h-period, with weights recorded at 4 h-intervals. WVP of the film was calculated as follows:

$$WVP = (\Delta W x FT)/(S x \Delta P) \quad (1)$$

Where $\Delta W$ is the weight loss of the cup per day (g $d^{-1}$) (i.e. the slope of the linear behaviour), FT is the film thickness (mm), S is the area of ex posed film ($m^2$) and $\Delta P$ is the vapor pressure differential across the test film (Pa). At least 3 replicates were produced from each film type.

#### 2.6.4. Oxygen permeability ($O_2P$) and carbon dioxide permeability ($CO_2P$)

Oxygen permeability ($O_2P$) and carbon dioxide permeability ($CO_2P$) analyses were conducted in the Laboratory of Industry and Processes, at the University of Minho, Portugal. The analyses were determined based on the ASTM-D-3985-02 method. The films were sealed between two chambers, having each one two channels. In the lower chamber $O_2$ (or $CO_2$) was supplied at a controlled (J & W Scientific, ADM 2000, USA) flow rate to maintain its pressure constant in that compartment. The other chamber was purged by a stream of nitrogen, also at controlled flow. Nitrogen acted as a carrier for the $O_2$ (or the $CO_2$). In the case of $O_2P$ measurement, the flow leaving this chamber was connected to an $O_2$ sensor (Mettler Toledo, Suisse) which measured the $O_2$ concentration in that flow on-line. In the case of $CO_2P$ measurement the flow leaving this chamber was collected in a syringe for $CO_2$ quantification. To determine $CO_2$ concentration, 1 mL of sample was injected in a gas chromatograph (Chrompack 9001, Middelburg, Netherlands) at 110°C with a column Porapak Q 80/ 100 mesh 2 m x 1/8" x 2 mm SS, using a flame ionization detector (FID) at 110°C. Helium at 23 mL $min^{-1}$ was used as carrier gas. A standard mixture containing 10% $CO_2$, 20% $O_2$ and 70% $N_2$ was used for calibration. The flows of the two chambers were connected to a manometer to ensure the equality of pressures (both at 1 atm) between both compartments. As the $O_2$ (and the $CO_2$) was carried continuously by the nitrogen flow, it was considered that partial pressure of $O_2$ (and the $CO_2$) in the upper compartment is null, therefore ΔP is equal to 1 atm. Three replicates were obtained for each film for permeability ($O_2P$ and $CO_2P$).

#### 2.6.5. Water solubility

The film solubility in water was determined according to the method reported by Gontard et al. (1992). It was defined by the content of dry matter solubilized after 24 h immersion in water. The initial dry matter content of each film was determined by drying to constant weight in an oven at 105°C. Two disks of the film samples (2 cm diameter) were cut, weighed and immersed in 50 mL of water. After 24 h of immersion at 20°C with agitation (60 rpm), the pieces of film were taken out and dried to constant weight in an oven at 105°C, to determine the weight of dry matter, which was not solubilized in water. Three replicates were obtained for each sample.

#### 2.6.6. Moisture content

The moisture content (MC) was determined according to the method reported by Martins et al. (2012b). It was expressed as the percentage of water removed from the initial mass sample. MC was determined gravimetrically by drying film samples at 105°C for 24 h. The experiments were performed on each film sample in triplicate.

#### 2.6.7. Opacity and color

The color of the films was determined with a Minolta colorimeter (CR 400; Minolta, Japan). A white standard color plate for the instrument calibration was used as a background for color measurements of the films, and the $L^*$, $a^*$, $b^*$ values of each film were evaluated by reflectance measurements. In this system, $L^*$ indicates the lightness (ranging from black to white), and the horizontal axes, indicated by $a^*$ and $b^*$, are the chromatic coordinates (ranging from $-a^*$: greenness, $-b^*$: blueness to $+a^*$: redness, $+b^*$: yellowness). The values of $a^*$ and $b^*$ approach zero for neutral colors and increase as the color becomes more chromatic and more saturated. The opacity of a material is an indication of how much light passes through it and is calculated from reflectance measurements. The opacity of the samples was determined according to the Hunter lab method, as the relationship between the opacity of each sample on a black standard and the opacity of each sample on a white standard. The measurements were repeated three times for each film.

### 2.7. Statistical analysis

The results were evaluated using the analysis of variance (ANOVA) and the samples were compared by the Tukey test to the significance level of 5%. Statistical analysis was performed using the SigmaStat software (version 3.5).

**Table 1**
pH values of the coatings with and without adding the culture medium.

| Coatings | pH Coating solution | Coating + BHI | Coating + TSB |
|---|---|---|---|
| Chi | 2.98 ± 0.18$^{e}$ | 4.95 ± 0.15$^{c}$ | 5.34 ± 0.09$^{c}$ |
| Chi-NaA | 3.06 ± 0.17$^{e}$ | 4.90 ± 0.18$^{c}$ | 4.99 ± 0.06$^{d}$ |
| Gal | 5.65 ± 0.20$^{b}$ | 6.60 ± 0.04$^{a}$ | 6.99 ± 0.07$^{a}$ |
| Gal-NaA | 6.47 ± 0.04$^{a}$ | 6.60 ± 0.03$^{a}$ | 6.99 ± 0.05$^{a}$ |
| Chi-Gal | 3.72 ± 0.06$^{d}$ | 6.02 ± 0.12$^{b}$ | 6.08 ± 0.03$^{b}$ |
| Chi-Gal-NaA | 3.84 ± 0.01$^{c}$ | 6.08 ± 0.13$^{b}$ | 6.12 ± 0.04$^{b}$ |

*Data represent mean values ± standard deviation. Letters indicate significant differences ($p<0.05$).

## 3. Results and discussion

### 3.1. Antimicrobial activity of the coatings

The development of active coatings for food applications is of great importance when the main objective is to delay or prevent the growth of microorganisms. A coating presenting antimicrobial activity can have a broad number of applications in the food industry (e.g. direct application in food surfaces or functionalization of packaging surfaces). In this work, coatings with different formulations were evaluated regarding their antimicrobial activity.

One of the parameters that should be studied when Chi is used for coatings production is the pH. Chi is soluble only in acidic medium, so it was necessary to determine the pH of the coatings with and without the addition of culture media (brain heart infusion broth (BHI) and tryptic soy broth (TSB)) to verify if this factor exerted any effect adverse effects on the growth of microorganisms. According to Franco and Landgraf (2008), the pH limits for the growth of *S. enteritidis* and *S. aureus* are ranged between 4.0 and 9.0, while *P. aeruginosa* and *L. monocytogenes* do not grow at pH less than or equal to 4.5. Besides that, chitosan has its bactericidal or bacteriostatic effect well studied in the literature, this effect is possible due to the relationship of the free amino group with the degree of deacetylation. Vale et al., 2020 reports higher degree of deacetylation more amino group is found in the chitosan molecule, which reduces the growth of microorganisms. Another aspect is relation pH, high-deacetylation chitosan will give a larger amino group for the reaction of the molecule in acidic pH. Goy et al., 2016 and Vale et al., 2020 emphasize that the degree of deacetylation is an important factor in the physico-chemical quality, highlighting the greater solubility of chitosan.

The pH measurements of culture media BHI and TSB were 6.61 and 6.95, respectively. Results presented in Table 1 show that the pH of Chi coating increases when this is added to the culture medium, and thus a pH range in which the studied microorganisms can growth. No et al. (2002) showed that the antibacterial activity of Chi was inversely affected by pH, presenting higher activity at lower pH values. The antimicrobial activity of Chi has been related to the presence of positively charged amino acid groups that interacts with the negatively charged membrane of microbial cells. This interaction may slightly alter the permeability of the membrane, as well as neutralize or reverse the surface charge of the bacteria. The increase in membrane permeability leads to destabilization of the cell membrane and leakage of intracellular substances, which leads to the cell death (Mohamed et al., 2013; Pereda et al., 2011; Kong et al., 2010; Bonilla and Sobral 2016).

Fernandez-Saiz et al. (2009) added Chi films to Mueller-Hinton broth (MHB) with different pH values (6.2 and 7.4) and subsequently inoculated with *S. aureus*. They confirmed that the antimicrobial capacity of Chi is affected by the pH of the medium. In the tests performed at a lower pH (6.2) a lower bacterial count was observed, indicating a strong biocide effect. This effect is related to the pKa 6.4 of the Chi used, which is close to the value of pH 6.2. At this pH, the amount of positively charged amino groups (active groups) is close to 75%, whereas at a pH 7.4 that amount drops to approximately 10%.

According to Table 2, none of the prepared coatings were effective against *S. aureus* growth. According to Zheng and Zhu (2003), a higher antimicrobial activity against *S. aureus* is observed for Chi with high molecular weight. However, in this case the coating produced using 1% of high molecular weight chitosan was not effective against *S. aureus* growth. Chi and Chi-NaA coatings showed to be effective against Gram-negative bacteria (*P. aeruginosa* and *S. enteritidis*) and Gram-positive (*L. monocytogenes*). However, this effect can be attributed only to the action of Chi, since the controls containing the culture media, inoculum and NaA (0.05%) did not present antibacterial action.

Gal coating does not present any antimicrobial activity and the addition of sodium acetate (Gal-NaA) does not affect this behavior and the resulting coatings do not present any antimicrobial activity. However, when Chi is added to the Gal solution, it was observed that the coating showed to be effective against *P. aeruginosa* and *L. monocytogenes*. There is no bactericidal effect of the blend against *S. enteritidis*, suggesting that the antimicrobial activity of Chi was decreased. According to Vásconez et al. (2009), this action is explained by the reduction of $NH_3$ group availability of Chi in the interaction with the cell membrane of the microorganism.

### 3.2. Mechanical properties of films

The study of the mechanical properties of films can give valuable information regarding the interaction of materials, and in this case can show how composite films, with and without sodium acetate will behave in comparison with the other studied films. TS indicates the maximum tensile stress that the film can sustain, EB point is the maximum change in length of a test specimen before breaking, and the Young's modulus (EM) is a measure of the stiffness of the film (Kimura et al., 2016).

The results of the TS, EB and EM are presented in Table 3. The films with Gal in their composition exhibited reduced values of EB when compared to the Chi and Chi-NaA films, whereas the composite with the incorporation of NaA had a lower EB value (Chi-Gal-NaA: 67.11 ± 0.89%), differing significantly from the other films ($p<0.05$).

According to Sobral (2000), the most resistant films are the least flexible, presenting lower EB values. Chi films exhibited the highest values of EB and the lowest TS values, being this property also observed during the analyses, as they were relatively fragile and needed to be handled very carefully. The incorporation of NaA reduced the value of TS of Chi films ($p>0.05$). This result suggests that the incorporation of NaA in the Chi films led to a decrease in the intermolecular interactions and thus the cohesive force of the films. Mechanical behaviour of polymeric matrices is also dependent on crystallinity. An increase in the crystallinity of films can be associated with more breakable (lower TS) and elastic (higher EB) films (Bourbon et al., 2011).

In practical terms, the EM is an indicator of the stiffness of the material, and the larger the modulus, the more rigid the material (Sarantópoulos et al., 2002). It can be observed that the EM of the films increases with the presence of Gal of *A. pavonina* L. in the composition of these, being verified that higher values were obtained in the composite (Chi-Gal, Chi-Gal-NaA). The EM of the blend Chi-Gal-NaA increased by 50% when NaA was added. In other words, the energy needed to break the film increase with the addition of NaA within the film. The results also shown that the presence of Gal of *A. pavonina* L. in the composition of the films exerts influence in the property of TS, being that the composite presented the highest values in this property (Table 3).

Other work using Gal blends of *A. pavonina* L., in that case with collagen, the obtained films presented higher values of TS (8.34 Mpa) and low values of EB (47.17%) (Lima et al., 2010) when compared to blends Chi-Gal prepared in this study (7.18 Mpa and 82.21%), respectively.

**Table 2**
Antimicrobial effect of the coatings against Gram positive and Gram negative bacteria. +: Presence of microorganisms. -: Absence of microorganisms.

| Coatings | *S. aureus* | *P.Aeruginosa* | *S. enteritidis* | *L. monocytogenes* |
|---|---|---|---|---|
| Chi | + | - | - | - |
| Chi-NaA | + | - | - | - |
| Gal | + | + | + | + |
| Gal-NaA | + | + | + | + |
| Chi-Gal | + | - | + | - |
| Chi-Gal-NaA | + | - | + | - |
| Control (Acetate (0.05 %) + BHI + Inoculum) | + | + | + | + |
| Control (Acetate (0.05 %) + TSB + Inoculum) | + | + | + | + |
| Control (BHI + Inoculum) | + | + | + | + |
| Control (TSB + Inoculum) | + | + | + | + |
| Control (BHI + Acetate (0.05 %)) | - | - | - | - |
| Control (TSB + Acetate (0.05 %)) | - | - | - | - |

**Table 3**
Tensile strength (TS), elongation at break (EB) and elastic modulus (EM) of the films.

| Film | TS (MPa) | EB (%) | EM (MPa) |
|---|---|---|---|
| Chi | 1.29 ± 0.03[c] | 145.47 ± 9.49[a] | 1.21 ± 0.01[d] |
| Chi-NaA | 0.82 ± 0.30[d] | 105.64 ± 6.68[b] | 1.02 ± 0.28[d] |
| Gal | 4.24 ± 0.31[b] | 81.85 ± 1.04[c] | 10.27 ± 0.18[c] |
| Gal-NaA | 3.71 ± 0.40[b] | 85.31 ± 1.81[c] | 10.12 ± 0.34[c] |
| Chi-Gal | 7.18 ± 0.22[a] | 82.21 ± 3.12[c] | 23.79 ± 1.38[b] |
| Chi-Gal-NaA | 6.91 ± 0.53[a] | 67.11 ± 0.89[d] | 35.68 ± 0.64[a] |

*Data represent mean values ± standard deviation.
[a] xxx
[b] xxx
[c] xxx
[d] Different letters in the same column indicate statistically significant differences ($p$<0.05).

### 3.3. Moisture content and water solubility

The amount of water present in films provides an indication of the films' hydrophilicity, being the more hydrophilic films those that present the highest values of MC (Bourbon et al., 2011). Table 4 presents the MC values of the films. The Chi film with the incorporation of NaA there was an increase in water absorption that did not differ significantly (p<0,05) of films Chi-NaA, the same was observed film Gal with film Gal-NaA. In the Chi-NaA and Gal-NaA films, the little increase in moisture content may be linked to the hydrophilic nature of NaA, which favors hydrogen formation with water in film. Liu et al., 2015 report interaction between sodium acetate and water in the gelatine film matrix, which leads to an increase in water absorption.

Incorporating NaA in films led to an increase of MC, with the exception of the composite film. This increase of moisture content in the films may also be related to the increase of charged molecules, which present a higher ability to adsorb water (Anker 1999).

In the Chi-Gal film compared to Chi-Gal there was a reduction in water absorption with the addition of NaA, the interaction between the chitosan polymer and galactomannan decreased the hydrophilic action of sodium acetate, however it did not reduce the water vapor permeability (WVP), however NaA was important in reducing WVP to chitosan and galactomannan Table 4.

The solubility of the film provides information about its integrity in aqueous systems and indicates its resistance to water. Furthermore, it can provide an indication of the film's biodegradability, which is an important feature if it is used as packaging (Vicente et al., 2010; Martins et al., 2012a).

When the objective is to preserve foods with large amounts of water and release of antimicrobials, low-solubility films are needed, as an antimicrobial film with low water resistance will dissolve quickly, resulting in low concentrations of the antimicrobial agent at the food surface, where it should act. In some cases, moderate solubility is desirable, for example, when cooking coated foods, since the film will be ingested together with the product. The polysaccharide structure is one of the factors that most affect its solubility in water (Elsabee and Abdou 2013; Pinheiro et al., 2010; Vicente et al., 2010). Table 4 presents the solubility values of the films where it is shown that films with Chi in their composition have lower water solubility. This fact can be attributed to poor solubility of Chi at neutral pH, such as water (Rabea et al., 2003; Fernández-Saiz et al., 2010; Feng et al., 2012). The amino group of Chi is not protonated in alkaline or neutral medium and therefore it is insoluble in water (Ahmed and Ikram 2015).

According to Vásconez et al. (2009), the hydrophobicity of Chi may be responsible for the existence of less interaction between the matrix of the film and water. Lima et al. (2017) report that Chi films have low solubility in water due to natural crystalline and rigid chitosan structure. According to Souza et al. (2009), the solubility of the Chi films is related to the crystallinity of the sample, where the weak solubility of Chi is attributed to its partially crystalline structure. Films that exhibit low solubility in water are needed to protect foods with high water activity.

The water solubility obtained for the Chi film (32.68 ± 1.59%) is in agreement with results reported by Darbasi et al. (2017) presenting 30.99 ± 1.95% and Ren at al. (2017) presenting 32.73 ± 0.17%, but is lower than the value found by Bourbon et al. (2011) (42.05 ± 3.46%). The water solubility values of Gal films (Gal and Gal-NaA) are significantly different from the other films produced ($p$<0.05). This behaviour can be explained by galactomannan solubility in water (Sousa et al., 2017). The presence of sodium acetate (Gal-NaA) does not change the galactomannan films behavior and the water solubility values are maintained.

### 3.4. Water vapor permeability (WVP)

The hydrophilic properties of polysaccharide-based films are responsible for their high water vapor permeability. This hydrophilicity is one of the drawbacks, since it is often necessary to control the transfer of water vapor. In fact, one of the purposes of edible films is to block the transfer of moisture between food and the environment. Therefore, the permeability to water vapor should be as low as possible (Bourbon et al., 2011; Martins et al., 2012b). However, the low water vapor permeability can be beneficial in some cases, preventing condensation of water vapor, which may be a possible origin of microbial contamination (Pinheiro et al., 2010).

Table 4 shows the values obtained for the water vapor permeability of the films. The composite Chi-Gal presented higher WVP values, differing significantly from the composite Chi-Gal-NaA ($p$<0.05). The incorporation of NaA in the films produced with a composite of Chi and Gal leads to a reduction of 35% of the WVP values, but did not differ statistically from the other produced films ($p$>0.05). Among

**Table 4**
Thickness, Water vapour permeability (WVP), Water solubility (S), Moisture content (MC), $O_2$ and $CO_2$ permeability ($O_2P$ and $CO_2P$, respectively) of the films.

| Film | Thickness (mm) | WVP x $10^{-8}$ (g (m s Pa)$^{-1}$) | S (%) | MC (%) | $O_2P$ x $10^{-12}$ (g m (Pa s m$^2$)$^{-1}$) | $CO_2P$ x $10^{-12}$ (g m (Pa s m$^2$)$^{-1}$) |
|---|---|---|---|---|---|---|
| Chi | 0.132 ± 0.031[b] | 2.15 ± 0.47[a b] | 32.68 ± 1.59[c] | 51.52 ± 3.42[a] | 0.27 ± 0.09[a b] | 0.10 ± 0.04[c] |
| Chi-NaA | 0.131 ± 0.015[b] | 1.66 ± 0.26[b] | 35.64 ± 4.36[b c] | 55.13 ± 4.41[a] | 0.21 ± 0.04[b] | 0.12 ± 0.05[c] |
| Gal | 0.115 ± 0,039[d] | 2.32 ± 0.81[a b] | 53.07 ± 2.87[a] | 57.13 ± 4.51[a] | 0.20 ± 0.06[b] | 0.19 ± 0.06[b c] |
| Gal-NaA | 0.092 ± 0.048[e] | 1.70 ± 0.82[a b] | 56.79 ± 1.79[a] | 59.04 ± 5.58[a] | 0.18 ± 0.06[b] | 0.54 ± 0.07[a] |
| Chi-Gal | 0.176 ± 0.013[a] | 2.46 ± 0.19[a] | 32.35 ± 0.02[b c] | 46.81 ± 1.55[b] | 0.29 ± 0.07[a b] | 0.17 ± 0.02[b c] |
| Chi-Gal-NaA | 0.124 ± 0.003[c] | 1.60 ± 0.02[b] | 36.56 ± 0.75[b] | 31.76 ± 3.93[c] | 0.40 ± 0.07[a] | 0.20 ± 0.03[b] |

*Data represent mean values ± standard deviation.
[a] xxx
[b] xxx
[c] xxx
[d] xxx
[e] Different letters in the same column indicate statistically significant differences ($p$<0.05).

**Table 5**
Opacity and color of the films.

| Film | $L^*$ | $a^*$ | $b^*$ | Opacity (%) |
|---|---|---|---|---|
| Chi | 81.23 ± 1.43[d] | 3.90 ± 0.97[a] | 33.90 ± 1.14[a b] | 8.15 ± 1.46[e] |
| Chi-NaA | 83.70 ± 1.39[c d] | 2.95 ± 0.57[a] | 33.14 ± 0.07[a b] | 4.60 ± 0.23[f] |
| Gal | 95.27 ± 0.20[a] | 0.73 ± 0.05[c] | 5.89 ± 0.19[d] | 10.28 ± 0.89[d] |
| Gal-NaA | 93.86 ± 0.24[b] | 1.01 ± 0.17[c] | 6.89 ± 0.17[c] | 15.70 ± 0.52[a] |
| Chi-Gal | 82.81 ± 1.15[d] | 3.01 ± 0.55[a] | 35.85 ± 1.27[a] | 12.86 ± 0.13[c] |
| Chi-Gal-NaA | 85.71 ± 0.97[c] | 1.71 ± 0.37[b] | 32.45 ± 1.47[b] | 13.61 ± 0.08[b] |

*Data represent mean values ± standard deviation.
[a] xxx
[b] xxx
[c] xxx
[d] xxx
[e] Different letters in the same column indicate statistically significant differences ($p$<0.05).

the films with NaA, the Gal-NaA film showed the higher WVP values ($p$>0.05). WVP values of the Chi film without the incorporation of NaA (Chi) were higher ($2.15 \times 10^{-8}$ g (m s Pa)$^{-1}$) than the values found by Bourbon et al. (2010) ($1.64 \times 10^{-10}$ g (m s Pa)$^{-1}$) and by Peng et al. (2013) ($13.39 \times 10^{-11}$ g (m s Pa)$^{-1}$). These differences can be explained by the different sources of Chi, the degree of deacetylation, molecular weight, the presence of plasticizer, methods of preparation and storage of the films before the test. The NaA was important in reducing WVP, since NaA led to increased solubility in the Chi film.

### 3.5. $O_2$ and $CO_2$ permeability

Table 4 presents the $O_2$ and $CO_2$ permeability of the films. The Gal-NaA films had their permeability reduced by 10% with the incorporation of 0.05% NaA. The oxygen permeability value of the composite Chi-Gal-NaA increased by 37% with the incorporation of NaA ($p$>0.05) and differed significantly from the Gal, Gal-NaA and Chi-NaA ($p$<0.05).

Butler et al. (1996) reported that Chi films present low permeability to oxygen. In studies that evaluated the permeability to $O_2$ of Chi films, the following results were obtained: $10.60 \pm 0.42 \times 10^{-16}$ (g m (Pa.s.m$^2$)$^{-1}$) and $5.17 \times 10^{-14} \pm 4.06 \times 10^{-15}$ (g m (Pa.s.m$^2$)$^{-1}$) by Souza et al. (2009) and Bourbon et al. (2011), respectively.

The permeability to $CO_2$ increased 184% when NaA was incorporated into the Gal film (Gal-NaA), differing significantly from the other films ($p$<0.05) (Table 4). The Chi films presented the lowest values of permeability to $CO_2$, and no changes were observed when NaA was added. In studies that evaluated the $CO_2$ permeability of Chi films, the following results were obtained: $6.98 \pm 0.030 \times 10^{-14}$ (g m (Pa.s.m$^2$)$^{-1}$) and $5.86 \pm 1.09 \times 10^{-13}$ (g m (Pa.s.m$^2$)$^{-1}$) by Souza et al. (2009) and Bourbon et al. (2011), respectively.

### 3.6. Opacity and color

In foods, the sensory aspects of products are essential to ensure that the application of emerging technologies, such as edible films and coatings, becomes successful. Thus, color is one of the most important parameters that have to be controlled, as it directly influences the acceptability of the consumer. The color of the coating and film is an important factor for consumer acceptance of the coating product as well as the film itself. (Pinheiro et al., 2010) A low brightness value can help prevent oxidative deterioration in packaged foods caused by exposure to visible light and ultraviolet light, causing nutrient loss, discoloration and off-flavors (Rubilar et al., 2013).

The results presented in Table 5 show that the values of $L^*$ (81.23 ± 1.43) and $a^*$ (3.90 ± 0.97) of the Chi films are lower than those found by Souza et al. (2009) (93.80 ± 0.38 and 4.04 ± 0.11, respectively). The Gal films showed the highest values of luminosity, but the incorporation of NaA reduced the values of 95.27 ± 0.20 to 93.86 ± 0.24 ($p$<0.05).

The films containing Chi presented high values of $b^*$, indicating the predominance of yellowish coloration. A similar trend was also reported by Ren et al. (2017) working on Chi and corn starch films for food packaging. The composite Chi-Gal significantly differed from the composite Chi-Gal-NaA ($p$<0.05). The Gal films of *A. pavonina* L. presented low values of $b^*$.

The opacity of a film is an indicator of the amount of light that passes through it. The higher the opacity value, the smaller the amount of light that crosses the film and this barrier can be important to control the incidence of light in food products (Pinheiro et al., 2010). Table 5 shows that opacity values varied significantly with treatments. Chi-based films were visibly transparent ($p$<0.05), but the Chi films presented higher opacity values when compared to the films containing NaA, providing better barrier against light incidence. These results suggest that the incorpora-

tion of NaA increases the transparency of the Chi film. Lima et al. (2017), studying films of Chi obtained opacity of 15.6 ± 0.18%.

The films containing Gal of *A. pavonina* L. in their composition showed high values of opacity, indicating that this hydrocolloid gives the films a lower transparency. The incorporation of NaA makes these films more opaque, in contrary to the behavior observed in Chi films. The same behavior was observed in the composite films, where the incorporation of NaA increases the opacity values.

## 4. Conclusion

The present work shows that the coatings with chitosan in their formulation were effective against Gram-negative and Gram-positive bacteria. The film of Chi-Gal-NaA presents less water solubility and lower values of MC, WVP, EB and is the film presenting higher values of EM. The composites presented the highest values of TS. The films containing Chi presented low water solubility and high values of component $b^*$, which indicates the predominance of yellowish coloration. The use of Chi in combination with NaA can used in the development of antimicrobial coatings and films for food and packaging applications, contributing to food preservation and shelf-life extension. The choice of the edible film or coating for the realization of its applicability will depend on the purpose. Future work should include shelf-life studies in order to demonstrate the expected positive effects of the application of these edible films and coatings in real food systems.

## Compliance with ethical Standards

*Compliance with ethics requirements* This article does not contain any studies with human or animal subjects.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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