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ORIGINAL ARTICLE

Pectin-based films with thyme essential oil: production, characterization, antimicrobial activity, and biodegradability

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Abstract

This work aimed to incorporate thyme essential oil into films composed of pectin to provide antimicrobial action to them. The effect of adding essential oil on the films' mechanical, physical-chemical, and barrier properties and their degradability was evaluated. Essential oil addition was possible by using Tween® 20 as an emulsifier, and it was possible to observe antimicrobial activity in the films containing 1.0 wt.% and 2.0 wt.% essential oil. The films containing thyme essential oil were more elastic and thicker but less resistant, with high permeability to water vapor and more hydrophilic relative to other formulations. Scanning electron microscopy analysis showed the presence of heterogeneities in the formulations with essential oil. The films produced using the optimized formulation (30 wt.% glycerol, 1.0 wt.% thyme essential oil, and 0.5 wt.% Tween® 20 relative to pectin mass) degraded entirely after 24 days of exposure to standard soil.

Keywords: *active packaging, biological activity, biopolymer, terpenes.*

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1. Introduction

The use of polymers made transporting and storing various products more convenient, securing their protection, and increasing shelf-life. However, the high turnover of plastic products and their large-scale production causes the accumulation of waste in the environment. These degradation-resistant materials remain for years in the soil. Researchers, governments, and the productive chain are searching for ways to reduce plastic waste generation. Research is also being conducted to develop materials capable of decomposing faster using biopolymers[1].

Among the materials from renewable sources that can be used to produce polymer films, polysaccharides, lipids, and proteins stand out. These raw materials give a renewable characteristic to the polymer and, generally, its degradability. However, more in-depth studies are essential for improving these materials' mechanical and barrier properties and production processes so they can compete with synthetic plastics[2-3].

Another property that can be conferred to biodegradable polymers is antioxidant and/or antimicrobial activity by inserting additives into the polymer matrix. The addition of essential oils to biofilms is being researched since some essential oils show antimicrobial activity, and their use complies with the need for safer food[2,4]. Using films with antimicrobial activity expands the field of applications to products prone to quick deterioration due to microbial action. The use of pectin, a byproduct of apple juice extraction, in the production of biofilms can be a potential destination for this waste[5-6].

Recently, materials were developed to be used in fruit coatings (apples and oranges), such as shellac and carnauba wax, being used alone or in combination[7]. The advantages of this application for biodegradable polymers are the reduction of synthetic plastics as packaging materials and the possibility of adding preservatives and other ingredients to the polymer matrix. The latter option is a possible solution to the growing demand for safe and environmentally friendly food[3].

Among the biopolymers reportedly used in the production of biofilms are alginate[8-9], chitosan[10-11], soy protein[12], whey[13-15], and pectin[2,16], among others. Research also focuses on adding antimicrobial and antioxidant agents to film formulations. However, studies using pectin as the main biopolymer in producing this type of film are scarce[17].

Traditionally, pectin is mainly used in the food industry as a jellifying, thickening, or stabilizing agent. Its application is diverse, being able to be present in products based on fruits, dairy, confectionery, bakery, and even in products from the pharmaceutical industry[2,18]. Commercially used pectin may be extracted from citrus fruits or apple residues[19]. Historically, apple was considered the primary pectin source. However, in the last few years, the use of citrus wastes as a feedstock

for pectin obtainment increased substantially. More recently, beet pulp is also being used as a source of pectin. Europe and citrus-producing countries such as Brazil and Mexico compose the main pectin production centers worldwide[18].

Natural antimicrobial agents can be added to pectin-based films, satisfying consumer demand for food free from chemical additives. Usually, compounds with these characteristics that are added to pectin films are antimicrobial peptides, essential oils, and polyphenols[6]. In this context, antimicrobial agents are added to film formulations and food coatings to avoid or delay food deterioration and reduce the risk of pathogen contamination. The most commonly used substances are organic acids, plant extracts, and essential oils[17].

The antimicrobial activity of essential oils is generally attributed to their terpene content. These substances, due to their lipophilicity, interact with the cell membrane. The action of these compounds causes the disorganization of the cell membrane of the microorganisms, increasing their permeability to ions and eventually leading to the rupture of the membrane[17,20-21].

Several essential oils are reported as antimicrobial additives for biofilms, such as oregano[9,13-14], cinnamon[22], rosemary[13], lavender[23], basil[24], garlic[13,25], sage[26], clove[9,23], anise[23], and thyme[22-23], among others. Thyme essential oil has antimicrobial activity, but few works used this essential oil as an antimicrobial additive in biopolymer-based films[23]. So, this study also aimed to evaluate the characteristics of pectin films with thyme essential oil.

Igarashi[27] evaluated the antimicrobial activity of an edible alginate film, testing the addition of thirteen essential oils. The results showed that adding some essential oils made the film capable of inhibiting microorganisms of the genera *Salmonella* and *Pseudomonas* and the bacterium *Listeria monocytogenes*. Among the essential oils investigated, only clove essential oil inhibited all strains of microorganisms tested. This essential oil also increased the permeability to water vapor, tensile strength, and elongation at break of the film.

Souza[28] reported that cassava-based films with 2.0 wt.% clove essential oil inhibited *P. commune* and *E. amstelodami*, fungi commonly found in bakery products.

Ojagh et al.[29] developed a biodegradable film composed of chitosan and cinnamon essential oil, evaluating the produced film's mechanical, physical, and antibacterial properties. Essential oil concentrations between 0.4 v.% and 2.0 v.% were tested. It was found that after the addition of essential oil, there was an increase in the antimicrobial activity of the film, as well as a decrease in moisture content, water solubility, and water vapor permeability.

Thus, this work aimed to produce pectin-based films containing thyme essential oil (*Thymus vulgaris* L.) as an additive to verify the impact of the presence of the essential oil on the mechanical, physicochemical, barrier, degradability, and antimicrobial capacity of the films.

2. Materials and Methods

2.1 Determination of the formulation and film preparation

Preliminary tests were conducted to determine the optimal formulation of the films containing pectin, glycerol, and thyme essential oil. Pectin concentration in the filmogenic solution was varied in the proportions of 0.5 wt.%, 1.0 wt.%, 2.0 wt.%, and 3.0 wt.%[30-31], using commercial pectin (CAS number 9000-69-5, > 75 wt.% galacturonic acid, Sigma Aldrich, USA). After determining the adequate pectin concentration, glycerol (CAS number 51-86-5, 99% purity, Sigma Aldrich, USA) addition was tested at 10 wt.%, 20 wt.%, and 30 wt.% relative to pectin mass[32].

Aiming to evaluate the emulsifier that promoted the best interaction between the essential oil and the polymer matrix, soy lecithin (CAS number 8002-43-5, Sigma Aldrich, USA), xanthan gum (CAS number 11138-66-2, Sigma Aldrich, USA), Tween® 20 (VWR Life Science, USA), and Emustab® (Mix Ingredientes, Brazil) were tested, at the concentration of 0.5 wt.%. Thyme essential oil was kept at 1.0 wt.% in all tests. The essential oil was obtained by steam distillation, purchased from Tekton company (Viamão, Brazil); the essential oil was of the thymol chemotype, with 33 wt.% thymol and 25 wt.% p-cymene as the major compounds.

For the previous selection of the optimal concentrations of each component, a visual analysis of the films was carried out, considering aspects such as uniformity, presence of cracks, homogeneity, and flexibility. When choosing the emulsifier, visual and tactile aspects of the filmogenic solution and the formed films were evaluated. The homogeneity of the film-forming solution and the uniformity, roughness, and opacity of the films produced were considered.

To evaluate the most adequate essential oil concentration aiming for an antimicrobial effect, films containing thyme essential oil concentrations of 0.5 wt.%, 1.0 wt.%, and 2.0 wt.% (relative to pectin mass) were produced[33-34].

All films were produced by casting, following the procedures described, regardless of the formulation. The ingredients were weighed and dissolved in heated distilled water (75 °C) under constant stirring with a magnetic stirrer (120 rpm). After the complete dissolution of the ingredients, the solution was left to rest for 20 min to remove bubbles, and the solution was poured into glass Petri dishes with a diameter of 11 cm. The cast solutions were dried for 48 h at 26 °C to form the films.

2.2 Determination of the antimicrobial activity of the films

The disk-diffusion method[35] was used to evaluate the antimicrobial activity of the films containing different concentrations of thyme essential oil. The produced films were cut into 1.0 cm x 1.0 cm squares and put into Petri dishes with a diameter of 11 cm containing agar nutrient medium, previously inoculated with *Escherichia coli* (ATCC 25922). The Petri dishes were incubated in a B.O.D. at 35 °C for 24 h. The antimicrobial activity of the films was determined by the qualitative evaluation of the inhibition halo formed in the medium, considering the samples with more antimicrobial activity and those with higher inhibition halos relative to the control (without essential oil). The lowest essential oil concentration that produced an antimicrobial effect was determined. This concentration, along with pectin and glycerol concentrations of the filmogenic solution, was used to determine the optimal formulation. For the other tests and characterization analyses, three formulations were tested, using the optimal concentrations determined

previously, being denominated: Pec – film composed only of pectin; Pec/Gly – film composed of pectin and glycerol, and Pec/Gly/EO – film composed of pectin, glycerol, and thyme essential oil.

2.3 Film characterization

Film thickness was measured using a digital micrometer (Mitutoyo, Japan) with a measurement range of 0.001 – 25 mm and a resolution of 1.0 µm at five random points of the film, and calculating the arithmetic mean of the measurements. The determination of the mechanical properties of the films followed the ASTM D882-00 standard. The tensile tests were performed using an EMIC universal testing machine, model DL 3000, with a spacer clearance speed of 50 mm·min^{-1} and initial spacing of 5 cm.

The films' permeability to water vapor (PWV) was determined following the ASTM E96-00 standard. Film solubility in water was measured following the methods described by Bierhalz[36] and Tong et al.[37]. The contact angle of the films with water was measured[38] using the Surftens software to calculate the contact angles.

The microstructure and morphology of the surface of the films were assessed by scanning electron microscopy (SEM). The presence of metals on the film surface was also assessed by energy dispersive spectroscopy (EDS). The tests were conducted using an SEM-FEG microscope Mira 3 LM (TESCAN, Czech Republic).

2.4 Degradability tests

The films' degradability was evaluated using a standard soil, following the ASTM G160-12 standard[15]. Film samples made using the optimal formulation were packed with inert net packaging to protect the samples against physical damage and help identify them in the soil. The net packages containing the samples were visually evaluated daily until the complete degradation of the film samples.

2.5 Experimental design and statistical analysis

The tests followed a completely randomized design, with three replicates for each treatment (film formulation). The data regarding the optimal formulation underwent analysis of variance (ANOVA), followed by Tukey's multiple range test at a 5% error probability ($p = 0.05$). The statistical analyses were conducted using the Statistica 12 software (StatSoft, USA).

3. Results and Discussions

3.1 Determination of the optimized film formulation

The first stage of the study consisted of evaluating the pectin concentration. The concentration of 2.0 wt.% in the filmogenic solution was chosen among the other concentrations tested because it generated films more resistant to handling and easier to release from the Petri dish. Film-forming solutions with very high pectin concentrations result in brittle films, and the increase in the viscosity makes the handling and casting process difficult[39].

In the stage evaluating the plasticizer concentration, it was noticed that in all tested concentrations, there was an improvement in the flexibility of the formed films. This could be the result of the interaction of this component with the pectin polymer chains. According to Camargo et al.[40], the plasticizer acts on the intermolecular forces between the pectin chains, decreasing their intensity and increasing the free space in the polymer matrix. Such a phenomenon facilitates movement between the polymer chains, consequently increasing film flexibility.

In this context, due to the greater flexibility, the film containing 30 wt.% glycerol relative to pectin mass (0.6 wt.% in the filmogenic solution) was chosen to compose the other formulations, together with the pectin at 2.0 wt.%. Although visually, this glycerol concentration was the most suitable (by improving film malleability), higher concentrations of plasticizer can also decrease film strength and increase the permeability to water vapor due to the increase in free space in the polymer matrix, facilitating the passage of water vapor[40].

Considering that thyme essential oil is immiscible in the filmogenic solution, it was necessary to use an emulsifier to incorporate it into the film. For the choice of emulsifier, the pre-stipulated concentrations of pectin and glycerol were maintained, and a thyme essential oil concentration of 1.0 wt.% (relative to pectin mass) was used. The emulsifiers soy lecithin, xanthan gum, Tween® 20, and Emustab® were tested at 0.5 wt.% (relative to pectin mass).

In the films produced with soy lecithin, phase separation was observed in the filmogenic solution, probably caused by partial emulsification of the oil. Although the mixing was carried out at a low temperature (50 °C), the formulation also showed the formation of white clots, possibly due to the denaturation of lecithin. Regarding the formulation containing xanthan gum, a homogeneous filmogenic solution was observed, and there was no phase separation, although the films were opaque. In addition, this polysaccharide could interfere with the evaluation of films because xanthan gum is a polymer also used to formulate biodegradable films[17].

Meydanju et al.[17] reported the preparation of lemon peel-based films with the addition of xantham gum and TiO_2-Ag nanoparticles. The added materials greatly improved the films' physical-chemical and antimicrobial properties, and that xantham gum alone influenced film color and increased the moisture content. The same authors commented that homogeneous films were formed using xantham gum, probably due to the formation of a homogeneous network between the xantham gum and the polymeric matrix.

The films produced with Tween® 20 and Emustab® as emulsifiers had the best visual appearances among the four formulations. However, films containing Tween® 20 as the emulsifier were more uniform when compared to those produced with Emustab®. The visual aspect of the films produced using each of the four tested emulsifiers is shown in Figure 1.

The Tween® 20 emulsifier is widely used in various formulations containing essential oils and also acts as a stabilizer in terpene-containing emulsions and essential oil encapsulation processes, producing microcapsules and nanocapsules. Tween® 20 acts as an emulsifier by stabilizing the dispersion of essential oil throughout the film structure by forming micelles, helping to distribute

and incorporate the essential oil components along the film profile. This mechanism also avoids the coalescence of essential oil particles, a phenomenon caused mainly due to density differences between the essential oil and the other components of the film solution[41-42].

Based on the visual aspect, it was decided to use the emulsifier Tween® 20 to compose the formulations containing essential oil since this emulsifier formed more uniform films without opacity or any other undesirable visual appearance.

3.2 Determination of the optimized film formulation

The choice of the optimum thyme essential oil concentration in the films was based on a qualitative evaluation of the antimicrobial action caused by each formulation. Film samples underwent the disk-diffusion test in agar (halo test), evaluating the antimicrobial activity of these films against the bacterium *Escherichia coli*, which can become pathogenic when ingested through food and drink, causing infections in its host[43]. The results of the disk-diffusion test of the produced films containing thyme essential oil are shown in Figure 2.

The film samples that did not contain thyme essential oil (control) and the film containing 0.5 wt.% essential oil did not show inhibiting effects on the growth of *E. coli*. However, formulations containing 1.0 wt.% and 2.0 wt.% essential oil showed inhibition halos (Figure 2), indicating the presence of antimicrobial activity. Furthermore, it is possible to observe that the inhibition of bacterial growth was proportional to the concentration of essential oil, confirming the antimicrobial activity attributed to thyme essential oil[23].

Almasi et al.[44], evaluating the antibacterial activity of pectin films containing marjoram essential oil, reported that the produced films had antimicrobial activity, although weaker than essential oil emulsions at the same concentration. Although the formulation containing 2.0 wt.% essential oil had a greater antimicrobial effect against *E. coli*, its films had an oily appearance, indicating partial incorporation of the oil into the polymer matrix and increasing the production costs of the films due to the greater amount of essential oil used. In this context, a formulation with 1.0 wt.% essential oil was chosen, considering that this was the lowest concentration to have an inhibitory action on the tested bacterium.

3.3 Film characterization

The results regarding the mechanical properties of the films produced (pectin, pectin and glycerol, and pectin, glycerol, and thyme essential oil) are shown in Table 1.

Film thickness varied significantly with the alteration of the formulation, in which the addition of components to the films caused an increase in thickness. This same effect was observed by Lorevice[45] when adding the surfactant Tween® 80 to pectin films, which caused an increase in film thickness. The same author also pointed out that this variation could be associated with an increase in the amount of solids in the

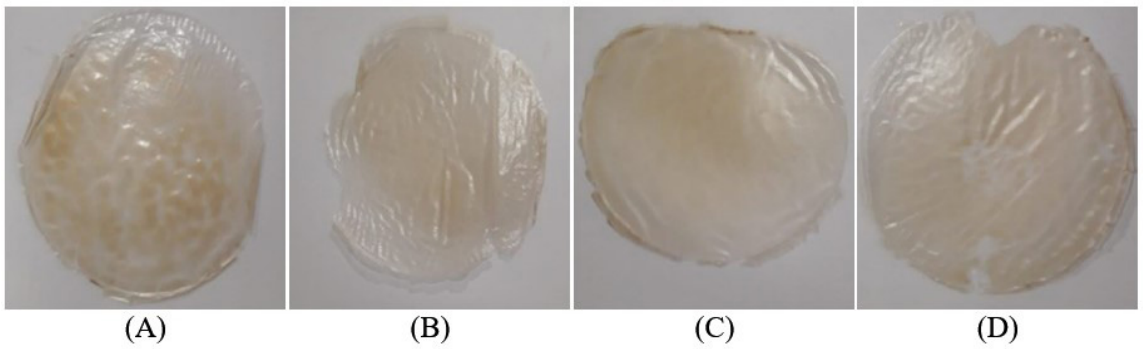

Figure 1. Visual aspect of the films produced with soy lecithin (A); xanthan gum (B); Tween® 20 (C); and Emustab® (D).

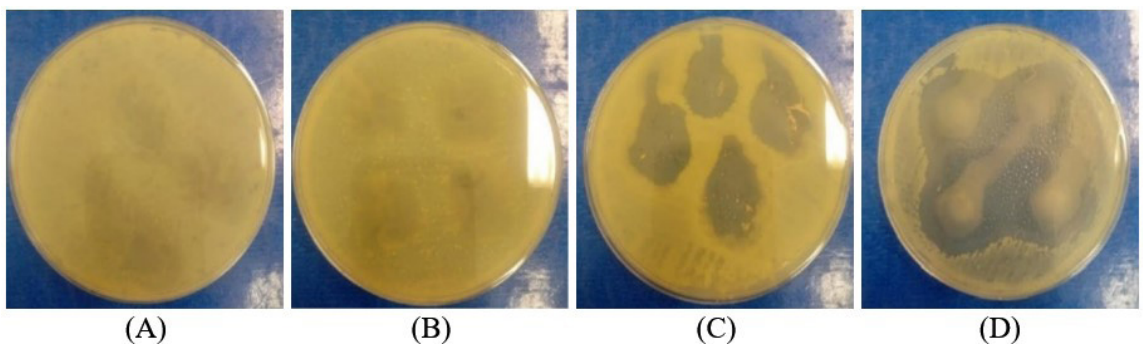

Figure 2. Disk-diffusion test in agar nutrient medium of the films with zero (A); 0.5 wt.% (B); 1.0 wt.% (C); and 2.0 wt.% (D) thyme essential oil (relative to pectin mass) against *Escherichia coli*.

matrix. However, films produced with the same formulation showed a uniform thickness between them. Almasi et al.[44] also observed an increase in the thickness of pectin films with the addition of marjoram essential oil, either through nanoemulsion or stabilized essential oil emulsion.

Caetano[46] commented on the influence of adding oregano essential oil in the polymer matrix on film thickness, causing a thickening of the film. This increase in thickness was also observed in the present work by adding thyme essential oil to the films produced, probably caused by the increase in the spacing between the polymer chains.

The tensile test showed information about the mechanical properties of the different formulations. According to Table 1, adding glycerol caused the formation of more flexible films due to the reduction of attractive intermolecular forces between the pectin chains. However, this effect also caused a reduction in mechanical strength, as evidenced by the decrease in the tensile strength and Young modulus of the films relative to the pectin-only film.

This same characteristic associated with the addition of plasticizer is reported by Camargo et al.[40], in which the addition of glycerol, even at concentrations below 15 wt.%, caused a decrease in the mechanical strength of the films. The same effects observed with the addition of glycerol also occurred with the addition of thyme essential oil, but more markedly. Essential oils also have plasticizing properties when incorporated into biopolymers such as pectin. Thus, glycerol and thyme essential oil may act together as plasticizers[47-48].

Considering the plasticizing effect of the essential oil, obtaining a more malleable film may be interesting for packaging applications. On the contrary, the decrease in Young modulus may render the film too compliant, making it prone to rupture even when strained by smaller forces. Thus, it is important to consider that, depending on the application envisaged, a reinforcing agent may be necessary to avoid excessive film maleability[48]. Another possible option is reducing plasticizer (glycerol) content, aiming to balance the plasticizer effects of it and the essential oil[45,48].

Table 1. Mechanical properties of the films composed of pectin (Pec), pectin and glycerol (Pec/Gly), and containing pectin, glycerol, and thyme essential oil (Pec/Gly/EO).

Parameter	Pec	Pec/Gly	Pec/Gly/EO
Thickness (μm)	85.2±4.5 c	101.9±6.3 b	146.0±5.8 a
Elongation at break (%)	3.1±1.0 c	6.0±2.9 b	12.1±3.1 a
Tensile strength (MPa)	50.7±7.1 a	26.9±6.0 b	16.9±2.2 c
Young modulus (MPa)	42.7±13.6 a	22.6±7.2 a	13.3±2.2 b

Means in row followed by the same lowercase letter do not differ statistically by Tukey's multiple range test at a 5% error probability (p = 0.05).

Lorevice[45] mentioned that Tween® 20, added to the filmogenic solution to emulsify the essential oil, may have a plasticizing effect. Thus, the presence of all these components can justify the increase in elongation at break and the decrease in tensile strength and Young modulus, as observed in the Pec/Gly/EO formulation. However, it is important to note that the addition of essential oil may not have a plasticizing effect in all cases, and it is possible to reduce the elongation at break, especially if the interaction between the polymer and the essential oil is impaired by the presence of components with a destabilizing effect[46].

Considering that commercial films, such as poly(vinyl chloride) films, present elongation at break in the range of 120 – 250% and tensile strength in the range of 15 – 21 MPa[49], it can be observed that the films produced were much less flexible, but their strength was comparable to that of commercial films. For the formulation containing only pectin (Pec), the tensile strength was superior to commercial films (42.7±13.6 MPa).

Table 2 shows the physicochemical properties of solubility, permeability to water vapor, and contact angle with water of the films produced. The images of contact angle measurement are shown in Figure 3.

It was not possible to determine the degree of solubility of the films, as they were completely solubilized after remaining in contact with distilled water for approximately 10 min, preventing the test from being carried out. Ngo et al.[50] also reported complete solubilization of films composed of pectin as a base polymer and about 45% solubility in films composed of 75 wt.% pectin and 25 wt.% chitosan nanoparticles. Since pectin is highly hydrophilic, like most polysaccharides, the films were expected to be poorly water-resistant[51].

The permeability to water vapor (PWV) values observed show that the addition of glycerol and thyme essential oil caused an increase in the permeability of the films. Despite many studies involving biodegradable polymers, the difficulty in producing films with barrier properties similar or superior to those of plastic films of fossil origin is recurrent[36].

The PWV values determined for each film produced aligned with the values reported in the literature. This parameter can be influenced by the type of process used to prepare the films or the composition of the filmogenic solution. Batista[5] determined the PWV values of pectin films, observing average values of 6.80 $g \cdot day^{-1} \cdot mm^{-1} \cdot kPa^{-1}$, close to the PWV values determined in this study (6.81 $g \cdot day^{-1} \cdot mm^{-1} \cdot kPa^{-1}$). On the other hand, Ngo et al.[50], observed a decrease in PWV with the addition of chitosan nanoparticles to pectin films (from 1.33 $g \cdot mm^{-1} \cdot day^{-1} \cdot kPa^{-1}$ in films composed only of pectin to 0.27 $g \cdot mm^{-1} \cdot day^{-1} \cdot kPa^{-1}$ in the films containing 75 wt.% pectin and 25 wt.% chitosan nanoparticles). Nisar et al.[52]

Table 2. Physical-chemical properties of the films composed of pectin (Pec), pectin and glycerol (Pec/Gly), and containing pectin, glycerol, and thyme essential oil (Pec/Gly/EO).

Parameter	Pec	Pec/Gly	Pec/Gly/EO
Permeability to water vapor ($g \cdot day^{-1} \cdot mm^{-1} \cdot kPa^{-1}$)	6.8±1.0 c	8.6±0.2 b	11.4±1.0 a
Contact angle with water (°)	84±1 a	73±1 b	39±1 c

Means in row followed by the same letter do not differ statistically by Tukey's multiple range test at a 5% error probability (p = 0.05).

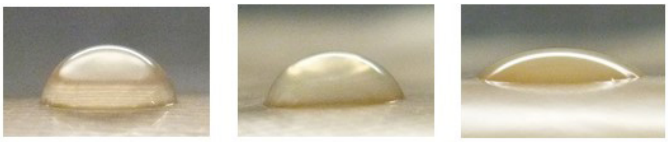

Figure 3. Images of contact angle measurements of the films composed of pectin (Pec), pectin and glycerol (Pec/Gly), and containing pectin, glycerol, and thyme essential oil (Pec/Gly/EO).

reported a decrease in PWV of chitosan films containing increasing proportions of clove essential oil.

Caetano[46], studying the effects caused by the variation of the concentration of glycerol and essential oil of oregano on the PWV of cassava starch films, reported an inverse relationship between the PWV values and the glycerol concentration used in the production of the films. The plasticizing effect potentiated by the combined action of glycerol and essential oil can weaken interactions between polymer chains and would cause an increase in film permeability.

This fact may explain the increase in PWV in films containing only glycerol and even higher PWV values in films with thyme essential oil relative to films containing only pectin, which had the lowest PWV. However, Ezati and Rhim[53] did not observe a statistical difference in the PWV of pectin films with the addition of curcumin and sulfur nanoparticles, indicating that not all additives used would cause an increase in film PWV.

Regarding the contact angles of the films with water, it can be observed that the addition of glycerol and thyme essential oil caused a reduction in the contact angle in relation to the film composed only of pectin (Pec) and the formulation containing both glycerol and essential oil (Pec/Gly/EO) showed the smallest angle (39°) between the three formulations. Sriamornsak et al.[54] observed water contact angles in the range of 60 – 90° for films composed only of pectin with different specifications. With the addition of mucin, the contact angles of the films were reduced to less than 40°, except for only one type of pectin, which showed a reduction in the contact angle from 85° to 80°. On the other hand, Ezati and Rhim[53] observed an increase in the contact angle of pectin films by adding curcumin and sulfur nanoparticles. Ngo et al.[50] reported a water contact angle of 62° for pectin films. The presence of chitosan nanoparticles influenced the contact angle; the contact angles increased with an increasing nanoparticle content.

Considering that all formulations had contact angles below 90°, the films had a hydrophilic character[55], which was accentuated with the addition of glycerol and essential oil. It is also important to note that the PWV of the produced films showed similar behavior to that of the contact angles of the films with distilled water since these two parameters are related to the type of interaction between the film and water, which was attractive (hydrophilic).

Although thyme essential oil and other essential oils have a hydrophobic character, adding this material increased the hydrophilicity of the films. It is important to observe that the dispersion of essential oil (and other hydrophobic materials) throughout a polar polymeric matrix must be stabilized, and this occurs by micelle formation[50,53-54]. Thus, considering that the polar part of the micelles is pointed outward, the presence of these polar moieties may enhance the polarity of the film, easing interaction with water and its transport, which reflects in a higher permeability to water vapor and decreased contact angle, even with the presence of dispersed hydrophobic particles in the polymeric matrix.

In addition, this increase in water permeability and reduction in contact angle may be a hindrance when considering using this material as a packaging material for materials or foods with considerable amounts of water. On the other hand, this behavior can make this material feasible to be used as a water absorber in some packages and for agricultural applications, such as seed coating.

3.4 Microstructure and morphology of the produced films

SEM analysis was used to evaluate the morphological aspects of the developed films. The Pec and Pec/Gly formulations were regular and without substantial defects or heterogeneities. However, the films containing thyme essential oil (Pec/Gly/EO) showed irregularities on their surface. The SEM images of the surface of the three formulations studied are shown in Figure 4.

The occurrence of irregularities can be associated with the formation of lipid droplets dispersed in the matrix that were not fully incorporated[56]. Siracusa et al.[57] also observed morphological changes in the films containing the oil, which the authors attributed this behavior to an uneven dispersion of the essential oil into the polymer matrix.

According to energy-dispersive X-ray spectroscopy (EDS) analysis, alkaline and alkaline earth metals were observed in all formulations. Most likely, these elements come from the pectin obtainment processes or even contaminants from the raw material from which the pectin was extracted[58].

The structures with cylindrical shapes were noticed in the films containing thyme essential oil. The SEM image at 5,000 x magnification, showing the presence of these structures on the surface of the films produced containing thyme essential oil, is shown in Figure 5.

These particles can be agglomerates of essential oil due to its incomplete incorporation in the polymeric matrix and its crystallization after drying the filmogenic solution and film formation[57]. Almasi et al.[44] did not observe similar particulates but reported the formation of heterogeneous films containing marjoram essential oil, attributing these heterogeneities to low incorporation of the essential oil into the pectin matrix.

The heterogeneities observed may result from poor essential oil incorporation by the polymer matrix or a

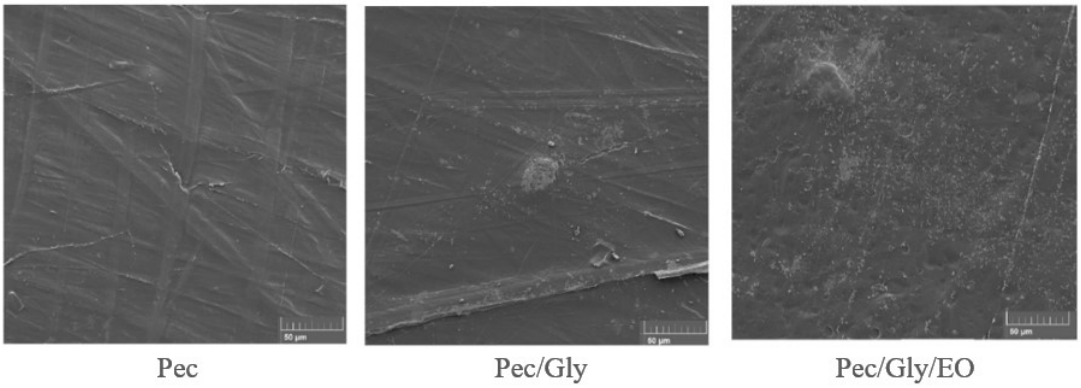

Figure 4. SEM images of the surface of the films composed of pectin (Pec), pectin and glycerol (Pec/Gly), and containing pectin, glycerol, and thyme essential oil (Pec/Gly/EO). Magnification of 1,000 x. The scale bar in the images corresponds to 50 μm.

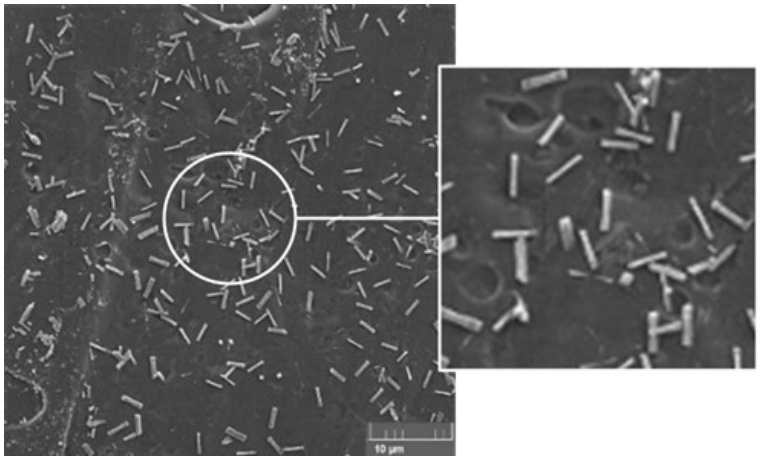

Figure 5. SEM image of the film's surface containing pectin, glycerol, and thyme essential oil (Pec/Gly/EO formulation), with a magnification of 5,000 x. The scale bar corresponds to 10 μm.

crystalization process of the essential oil, the emulsifier (Tween® 20), and the plasticizer (glycerol). As the cast film dries, the stabilization of the polymeric chains may cause part of the additives to be expelled from the matrix, building up and crystallizing at the film surface[44,57].

These surface heterogeneities may hinder food applications since the interaction between them and the food can cause these crystals to dissolve and interact with the packaged material. However, a post-treatment may remove these crystals, eliminating this issue. In addition, these crystals can also act as a barrier to further water permeation, avoiding rapid film solubilization in the packaging of low-moisture materials.

3.5 Film degradability tests

The degradability test showed that the samples containing thyme essential oil degraded entirely after 24 days in contact with the standard soil used in the test. The evolution of film degradation in this period is shown in Figure 6.

Norcino et al.[59] reported a degradation period of 28 days for pectin films containing copaiba oil (zero to 6.0 wt.%) and exposed to soil. Mendes et al.[60], producing thermoplastic starch films containing pectin and lemongrass essential oil, observed that the produced films took 20 days to decompose when exposed to soil. Leonardelli et al.[15], evaluating the biodegradability of whey-based films by adding chitosan as an antimicrobial agent, reported a degradation time of 8 days for films when disposed of in soil. Film degradability was similar to those of other biopolymeric films and superior to commercial films, considering that plastic films have degradation times longer than one year, even when discarded in landfills[61].

The rapid degradation observed for the formulation tested makes it an interesting alternative considering the current environmental problems the synthetic polymers pose and their inherent resistance to degradation. Furthermore, pectin, a common agroindustrial material sometimes regarded as waste, makes this material even more interesting from an environmental standpoint. In this sense, pectin and other polysaccharides can be considered viable and potential alternatives to non-biodegradable polymers as packaging materials, being further studies needed to make this type of material more competitive economically and with standardized feedstock parameters and production procedures for large-scale production.

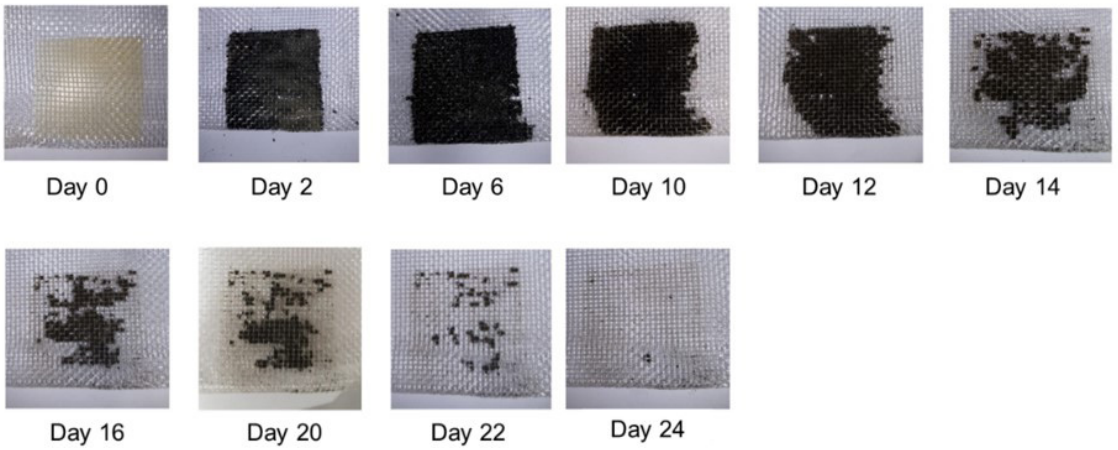

Figure 6. Evolution of the degradation of the film composed of pectin, glycerol, and thyme essential oil (Pec/Gly/EO formulation) during 24 days of exposure to standard soil.

4. Conclusions

The films produced from the filmogenic solution containing 2.0 wt.% pectin and 0.6 wt.% glycerol (30 wt.% relative to pectin mass) had better properties among the formulations tested. Adding thyme essential oil in contents above 1.0 wt.% conferred antimicrobial activity to the film against *E. coli*. However, the presence of essential oil reduced the barrier properties and made the films more hydrophilic, causing the reduction of the mechanical properties and thickening of the films. All produced films showed a high degradability, indicating the potential use of these materials as sustainable alternatives to the development of more environmentally friendly packaging materials, being further studies necessary to optimize and standardize the production parameters and feedstock specifications.

5. Author's Contribution

- **Conceptualization –** Greice Ribeiro Furlan; Camila Baldasso.
- **Data curation –** Greice Ribeiro Furlan.
- **Formal analysis –** Wendel Paulo Silvestre.
- **Funding acquisition –** NA.
- **Investigation –** Greice Ribeiro Furlan.
- **Methodology –** Wendel Paulo Silvestre; Camila Baldasso.
- **Project administration –** Camila Baldasso.
- **Resources –** Camila Baldasso.
- **Software –** NA.
- **Supervision –** Camila Baldasso.
- **Validation –** Wendel Paulo Silvestre.
- **Visualization –** Wendel Paulo Silvestre.
- **Writing – original draft –** Greice Ribeiro Furlan; Wendel Paulo Silvestre.
- **Writing – review & editing –** Camila Baldasso.

6. References

1. Platt, D. (2006). *Biodegradable polymers: market report*. Shawbury: iSmithers Rapra Publishing.
2. Pirsa, S., & Hafezi, K. (2023). Hydrocolloids: structure, preparation method, and application in food industry. *Food Chemistry*, *399*, 133967. http://dx.doi.org/10.1016/j.foodchem.2022.133967. PMid:35998495.
3. Vargas, M., Pastor, C., Chiralt, A., McClements, D. J., & González-Martínez, C. (2008). Recent advances in edible coatings for fresh and minimally processed fruits. *Critical Reviews in Food Science and Nutrition*, *48*(6), 496-511. http://dx.doi.org/10.1080/10408390701537344. PMid:18568856.
4. Falleh, H., Ben Jemaa, M., Saada, M., & Ksouri, R. (2020). Essential oils: a promising eco-friendly food preservative. *Food Chemistry*, *330*, 127268. http://dx.doi.org/10.1016/j.foodchem.2020.127268. PMid:32540519.
5. Batista, J. A. (2004). *Development, characterization and applications of biofilms based on pectin, gelatin and fatty acids in bananas and broccoli seeds* (Master's dissertation). Universidade Estadual de Campinas, Campinas.
6. Espitia, P. J. P., Du, W.-X., Avena-Bustillos, R. J., Soares, N. F. F., & McHugh, T. H. (2014). Edible films from pectin: physical-mechanical and antimicrobial properties - a review. *Food Hydrocolloids*, *35*, 287-296. http://dx.doi.org/10.1016/j.foodhyd.2013.06.005.
7. Miranda, M., Sun, X., Ference, C., Plotto, A., Bai, J., Wood, D., Assis, O. B. G., Ferreira, M. D., & Baldwin, E. (2021). Nano- and micro- carnauba wax emulsions versus shellac protective coatings on postharvest citrus quality. *Journal of the American Society for Horticultural Science*, *146*(1), 40-49. http://dx.doi.org/10.21273/JASHS04972-20.
8. Parris, N., Coffin, D. R., Joubran, R. F., & Pessen, H. (1995). Composition factors affecting the water vapor permeability and tensile properties of hydrophilic films. *Journal of Agricultural and Food Chemistry*, *43*(6), 1432-1435. http://dx.doi.org/10.1021/jf00054a004.
9. Rojas-Graü, M. A., Avena-Bustillos, R. J., Olsen, C., Friedman, M., Henika, P. R., Martín-Belloso, O., Pan, Z., & McHugh, T. H. (2007). Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *Journal of Food Engineering*, *81*(3), 634-641. http://dx.doi.org/10.1016/j.jfoodeng.2007.01.007.

10. Butler, B. L., Vergano, P. J., Testin, R. F., Bunn, J. M., & Wiles, J. L. (1996). Mechanical and barrier properties of edible chitosan films as affected by composition and storage. *Journal of Food Science*, *61*(5), 953-956. http://dx.doi.org/10.1111/j.1365-2621.1996.tb10909.x.
11. Cervera, M. F., Karjalainen, M., Airaksinen, S., Rantanen, J., Krogars, K., Heinämäki, J., Colarte, A. I., & Yliruusi, J. (2004). Physical stability and moisture sorption of aqueous chitosan-amylose starch films plasticized with polyols. *European Journal of Pharmaceutics and Biopharmaceutics*, *58*(1), 69-76. http://dx.doi.org/10.1016/j.ejpb.2004.03.015. PMid:15207539.
12. Kokoszka, S., Debeaufort, F., Hambleton, A., Lenart, A., & Voilley, A. (2010). Protein and glycerol contents affect physico-chemical properties of soy protein isolate-based edible films. *Innovative Food Science & Emerging Technologies*, *11*(3), 503-510. http://dx.doi.org/10.1016/j.ifset.2010.01.006.
13. Seydim, A. C., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Research International*, *39*(5), 639-644. http://dx.doi.org/10.1016/j.foodres.2006.01.013.
14. Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science*, *82*(3), 338-345. http://dx.doi.org/10.1016/j.meatsci.2009.02.004. PMid:20416718.
15. Leonardelli, C., Silvestre, W. P., & Baldasso, C. (2020). Effect of chitosan addition in whey-based biodegradable films. *Brazilian Archives of Biology and Technology*, *63*, e20200178. http://dx.doi.org/10.1590/1678-4324-2020200178.
16. Macleod, G. S., Fell, J. T., & Collett, J. H. (1997). Studies on the physical properties of mixed pectin/ethylcellulose films intended for colonic drug delivery. *International Journal of Pharmaceutics*, *157*(1), 53-60. http://dx.doi.org/10.1016/S0378-5173(97)00216-0.
17. Meydanju, N., Pirsa, S., & Farzi, J. (2022). Biodegradable film based on lemon peel powder containing xanthan gum and TiO_2–Ag nanoparticles: investigation of physicochemical and antibacterial properties. *Polymer Testing*, *106*, 107445. http://dx.doi.org/10.1016/j.polymertesting.2021.107445.
18. Imeson, A. (2010). *Food stabilisers, thickeners, and gelling agents*. Singapore: Blackwell Publishing Ltd.
19. May, C. D. (1990). Industrial pectins: sources, production and applications. *Carbohydrate Polymers*, *12*(1), 79-99. http://dx.doi.org/10.1016/0144-8617(90)90105-2.
20. Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., Gorris, L. G. M., & Von Wright, A. (1998). Characterization of the action of selected essential oil components on gram-negative bacteria. *Journal of Agricultural and Food Chemistry*, *46*(9), 3590-3595. http://dx.doi.org/10.1021/jf980154m.
21. Zheng, Z. L., Tan, J. Y. W., Liu, H. Y., Zhou, X. H., Xiang, X., & Wang, K. Y. (2009). Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). *Aquaculture*, *292*(3-4), 214-218. http://dx.doi.org/10.1016/j.aquaculture.2009.04.025.
22. Hosseini, M. H., Razavi, S. H., & Mousavi, M. A. (2009). Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and cinnamon essential oils. *Journal of Food Processing and Preservation*, *33*(6), 727-743. http://dx.doi.org/10.1111/j.1745-4549.2008.00307.x.
23. Gómez-Estaca, J., López de Lacey, A., López-Caballero, M. E., Gómez-Guillén, M. C., & Montero, P. (2010). Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*, *27*(7), 889-896. http://dx.doi.org/10.1016/j.fm.2010.05.012. PMid:20688230.
24. Zivanovic, S., Chi, S., & Draughon, A. F. (2005). Antimicrobial activity of chitosan films enriched with essential oils. *Journal of Food Science*, *70*(1), M45-M51. http://dx.doi.org/10.1111/j.1365-2621.2005.tb09045.x.
25. Santos, V. S., Aouada, F. A., & Moura, M. R. (2018). *Incorporation of polymeric nanoparticles and garlic essential oil in pectin-based films for edible packaging*. In *23º Congresso Brasileiro de Engenharia e Ciência dos Materiais* (pp. 8442-8452). São Paulo: Metallum Congressos Técnicos e Científicos.
26. Pirouzifard, M., Yorghanlu, R. A., & Pirsa, S. (2020). Production of active film based on potato starch containing Zedo gum and essential oil of *Salvia officinalis* and study of physical, mechanical, and antioxidant properties. *Journal of Thermoplastic Composite Materials*, *33*(7), 915-937. http://dx.doi.org/10.1177/0892705718815541.
27. Igarashi, M. C. (2010). *Development of a film based on alginate incorporated from the antimicrobial agent essential oil of clove: application in food* (Master's dissertation). Universidade de São Paulo, São Paulo.
28. Souza, A. C. (2011). *Development of active biodegradable cassava starch packaging and natural antimicrobial agents* (Doctoral thesis). Universidade de São Paulo, São Paulo.
29. Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry*, *122*(1), 161-166. http://dx.doi.org/10.1016/j.foodchem.2010.02.033.
30. Pavlath, A. E., Voisin, A., & Robertson, G. H. (1999). Pectin-based biodegradable water insoluble films. *Macromolecular Symposia*, *140*(1), 107-113. http://dx.doi.org/10.1002/masy.19991400112.
31. Slavutsky, A. M., Gamboni, J. E., & Bertuzzi, M. A. (2018). Formulation and characterization of bilayer films based on Brea gum and Pectin. *Brazilian Journal of Food Technology*, *21*, e2017213. http://dx.doi.org/10.1590/1981-6723.21317.
32. McHugh, T. H., & Krochta, J. M. (1994). Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry*, *42*(4), 841-845. http://dx.doi.org/10.1021/jf00040a001.
33. Du, W.-X., Olsen, C. W., Avena-Bustillos, R. J., McHugh, T. H., Levin, C. E., & Friedman, M. (2009). Effects of allspice, cinnamon, and clove bud essential oils in edible apple films on physical properties and antimicrobial activities. *Journal of Food Science*, *74*(7), M372-M378. http://dx.doi.org/10.1111/j.1750-3841.2009.01282.x. PMid:19895483.
34. Sánchez-González, L., González-Martínez, C., Chiralt, A., & Cháfer, M. (2010). Physical and antimicrobial properties of chitosan-tea tree essential oil composite films. *Journal of Food Engineering*, *98*(4), 443-452. http://dx.doi.org/10.1016/j.jfoodeng.2010.01.026.
35. Bona, E. A. M., Pinto, F. G. S., Fruet, T. K., Jorge, T. C. M., & Moura, A. C. (2014). Comparison of methods for evaluating antimicrobial activity and determining the minimum inhibitory concentration (MIC) of aqueous and ethanolic plant extracts. *Arquivos do Instituto Biológico*, *81*(3), 218-225. http://dx.doi.org/10.1590/1808-1657001192012.
36. Bierhalz, A. C. K. (2010). *Production and characterization of active biofilms based on pectin and pectin/alginate crosslinked with calcium* (Master's dissertation). Universidade Estadual de Campinas, Campinas.
37. Tong, W. Y., Rafiee, A. R. A., Leong, C. R., Tan, W.-N., Dailin, D. J., Almarhoon, Z. M., Shelkh, M., Nawaz, A., & Chuah, L. F. (2023). Development of sodium alginate-pectin

biodegradable active food packaging film containing cinnamic acid. *Chemosphere*, *336*, 139212. http://dx.doi.org/10.1016/j.chemosphere.2023.139212. PMid:37315854.

38. Silva, W. A., Pereira, J., Carvalho, C. W. P., & Ferrua, F. Q. (2007). Determination of color, topographic superficial image and contact angle of the biofilms of different starch sources. *Ciência e Agrotecnologia*, *31*(1), 154-163. http://dx.doi.org/10.1590/S1413-70542007000100023.
39. Melo, P. T. S., Aouada, F. A., & Moura, M. R. (2017). Fabricação de filmes bionanocompósitos à base de pectina e polpa de cacau com potencial uso como embalagem para alimentos. *Química Nova*, *40*(3), 247-251. http://dx.doi.org/10.21577/0100-4042.20160188.
40. Camargo, L. A., Moreira, F. K. V., Marconcini, J. M., & Mattoso, L. H. C. (2013). *Avaliação do efeito de plastificante induzido pelo glicerol em filmes de pectina reforçados com nanopartículas de Mg(OH)$_2$*. In *VII Workshop de Nanotecnologia Aplicada ao Agronegócio* (pp. 340-342). São Carlos: Embrapa Instrumentação.
41. Antonioli, G., Fontanella, G., Echeverrigaray, S., Delamare, A. P. L., Pauletti, G. F., & Barcellos, T. (2020). Poly(lactic acid) nanocapsules containing lemongrass essential oil for postharvest decay control: in vitro and in vivo evaluation against phytopathogenic fungi. *Food Chemistry*, *326*, 126997. http://dx.doi.org/10.1016/j.foodchem.2020.126997. PMid:32422511.
42. Gorjian, H., Mihankhah, P., & Khaligh, N. G. (2022). Influence of tween nature and type on physicochemical properties and stability of spearmint essential oil (*Mentha spicata* L.) stabilized with basil seed mucilage nanoemulsion. *Journal of Molecular Liquids*, *359*, 119379. http://dx.doi.org/10.1016/j.molliq.2022.119379.
43. Sousa, C. P. (2006). Food safety and food-borne diseases: using the coliform group as an indicator of food quality. *Revista de APS*, *9*(1), 83-88.
44. Almasi, H., Azizi, S., & Amjadi, S. (2020). Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (*Origanum majorana* L.) essential oil. *Food Hydrocolloids*, *99*, 105338. http://dx.doi.org/10.1016/j.foodhyd.2019.105338.
45. Lorevice, M. V. (2019). *Nanoemulsions of essential oils: stability mechanisms and interactions with pectin in bionanocomposites for use as active packaging* (Doctoral thesis). Universidade Federal de São Carlos, São Carlos.
46. Caetano, K. S. (2006). *Use of starch, orégano oil and pumpkin waste extract in the development of active biodegradable films* (Master's dissertation). Universidade Federal do Rio Grande do Sul, Porto Alegre.
47. Aitboulahsen, M., El Galiou, O., Laglaoui, A., Bakkali, M., & Zerrouk, M. H. (2020). Effect of plasticizer type and essential oils on mechanical, physicochemical, and antimicrobial characteristics of gelatin, starch, and pectin-based films. *Journal of Food Processing and Preservation*, *44*(6), e14480. http://dx.doi.org/10.1111/jfpp.14480.
48. Syafiq, R., Sapuan, S. M., Zuhri, M. Y. M., Ilyas, R. A., Nazrin, A., Sherwani, S. F. K., & Khalina, A. (2020). Antimicrobial activities of starch-based biopolymers and biocomposites incorporated with plant essential oils: a review. *Polymers*, *12*(10), 2403. http://dx.doi.org/10.3390/polym12102403. PMid:33086533.
49. Braskem. (2002). *Propriedades de referência dos compostos de PVC*. São Paulo: Braskem. Retrieved in 2023, September 1, from https://www.braskem.com.br/Portal/Principal/Arquivos/html/boletm_tecnico/Tabela_de_Propriedades_de_Referencia_dos_Compostos_de_PVC.pdf
50. Ngo, T. M. P., Nguyen, T. H., Dang, T. M. Q., Tran, T. X., & Rachtanapun, P. (2020). Characteristics and antimicrobial properties of active edible films based on pectin and nanochitosan. *International Journal of Molecular Sciences*, *21*(6), 2224. http://dx.doi.org/10.3390/ijms21062224. PMid:32210135.
51. Isotton, F. S. (2013). *Development and characterization of corn starch films etherified with the plasticizers glycerol, sorbitol, and poly(vinyl alcohol)* (Master's dissertation). Universidade de Caxias do Sul, Caxias do Sul.
52. Nisar, T., Wang, Z.-C., Yang, X., Tian, Y., Iqbal, M., & Guo, Y. (2018). Characterization of citrus pectin films integrated with clove bud essential oil: physical, thermal, barrier, antioxidant and antibacterial properties. *International Journal of Biological Macromolecules*, *106*, 670-680. http://dx.doi.org/10.1016/j.ijbiomac.2017.08.068. PMid:28818729.
53. Ezati, P., & Rhim, J.-W. (2020). pH-responsive pectin-based multifunctional films incorporated with curcumin and sulfur nanoparticles. *Carbohydrate Polymers*, *230*, 115638. http://dx.doi.org/10.1016/j.carbpol.2019.115638. PMid:31887862.
54. Sriamornsak, P., Wattanakorn, N., Nunthanid, J., & Puttipipatkhachorn, S. (2008). Mucoadhesion of pectin as evidence by wettability and chain interpenetration. *Carbohydrate Polymers*, *74*(3), 458-467. http://dx.doi.org/10.1016/j.carbpol.2008.03.022.
55. Law, K.-Y. (2014). Definitions for hydrophilicity, hydrophobicity, and superhydrophobicity: getting the basics right. *The Journal of Physical Chemistry Letters*, *5*(4), 686-688. http://dx.doi.org/10.1021/jz402762h. PMid:26270837.
56. Pagno, C. H. (2016). *Effect of the addition of nanostructures, essential oils, and chitosan on the development of films and biodegradable coatings with antimicrobial properties* (Doctoral thesis). Universidade Federal do Rio Grande do Sul, Porto Alegre.
57. Siracusa, V., Romani, S., Gigli, M., Mannozzi, C., Cecchini, J. P., Tylewicz, U., & Lotti, N. (2018). Characterization of active edible films based on citral essential oil, alginate and pectin. *Materials*, *11*(10), 1980. http://dx.doi.org/10.3390/ma11101980. PMid:30326558.
58. Kamnev, A. A., Colina, M., Rodriguez, J., Ptitchkina, N. M., & Ignatov, V. V. (1998). Comparative spectroscopic characterization of different pectins and their sources. *Food Hydrocolloids*, *12*(3), 263-271. http://dx.doi.org/10.1016/S0268-005X(98)00014-9.
59. Norcino, L. B., Mendes, J. F., Natarelli, C. V. L., Manrich, A., Oliveira, J. E., & Mattoso, L. H. C. (2020). Pectin films loaded with copaiba oil nanoemulsions for potential use as bio-based active packaging. *Food Hydrocolloids*, *106*, 105862. http://dx.doi.org/10.1016/j.foodhyd.2020.105862.
60. Mendes, J. F., Norcino, L. B., Martins, H. H. A., Manrich, A., Otoni, C. G., Carvalho, E. E. N., Piccoli, R. H., Oliveira, J. E., Pinheiro, A. C. M., & Mattoso, L. H. C. (2020). Correlating emulsion characteristics with the properties of active starch films loaded with lemongrass essential oil. *Food Hydrocolloids*, *100*, 105428. http://dx.doi.org/10.1016/j.foodhyd.2019.105428.
61. Grisa, A. M. C., Sirena, M. C., Zini, A., Brancher, L. R., Zeni, M., & Nunes, M. F. O. (2019). Characterization of non-structural poly (vinyl) chloride, rock wool and medium density fiberboard waste composites. *Material Science & Engineering International Journal*, *3*(6), 201-203. http://dx.doi.org/10.15406/mseij.2019.03.00114.

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