Essential Oils of Garlic and Oregano Incorporated in Cellulose Acetate Films:
Antimicrobial Activity and Physical Properties

Óleos Essenciais de Alho e Orégano Incorporados em Filmes de Acetato de Celulose:
Atividade Antimicrobiana e Propriedades Físicas
Aceites Esenciales De Ajo y Oregano Incorporados en Películas de Acetato de Celulosa:
Actividad Antimicrobiana y Propiedades Físicas

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Abstract
Polymers of natural origin and their derivatives are currently used as biomaterials because they are easily available and their properties can be tailored to meet specific requirements. The essential oils are widely used as antimicrobials. The objective of this study was to evaluate the in-vitro antimicrobial efficiency of cellulose acetate (CA) films incorporated with the essential oils of garlic (GR) and oregano (OR) on the microorganisms Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella choleraesuis and Pseudomonas aeruginosa and characterize the films as to their mechanical, optical and structural properties.

Four treatments were evaluated, Control, Film 1 (50 ml OR.100 g⁻¹ CA) Film 2 (50 ml OR + 30 ml GR.100 g⁻¹ CA) and Film 3 (50 ml OR + 50 ml GR.100 g⁻¹ CA). The concentration of oils influenced the mechanical parameters of maximum load, relative deformation at maximum load and elastic modulus, resulting in weaker, less rigid and more flexible films. There was an increase in L* and b* in films incorporated with garlic and oregano essential oil. The films incorporated with a mixture of oregano and garlic essential oils exhibited inhibition against all organisms tested.

Keywords: Listeria monocytogenes; Optical properties; Elastic modulus; Active compounds.

Resumo
Polímeros de origem natural e seus derivados são usados atualmente como biomateriais porque são facilmente disponíveis e suas propriedades podem ser personalizadas para atender a requisitos específicos. Os óleos essenciais são amplamente utilizados como antimicrobianos. O objetivo deste estudo foi avaliar a eficiência antimicrobiana in vitro de filmes de acetato de celulose (CA) incorporados aos óleos essenciais de alho (GR) e orégano (OR) sobre os microrganismos Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella choleraesuis e Pseudomonas aeruginosa e caracterizam os filmes quanto às suas propriedades mecânicas, ópticas e estruturais. Quatro tratamentos foram avaliados, Controle, Filme 1 (50 ml OR.100 g⁻¹ CA) Filme 2 (50 ml OR + 30 ml GR.100 g⁻¹ CA) e Filme 3 (50 ml OR + 50 ml GR.100 g⁻¹ CA). A concentração de óleos influenciou os parâmetros mecânicos de carga máxima, deformação relativa na carga máxima e módulo de elasticidade, resultando em filmes mais fracos, menos rígidos e mais flexíveis. Houve aumento de L* e b* nos filmes incorporados com óleo essencial de alho e orégano. Os filmes incorporados com uma mistura de óleos essenciais de orégano e alho exibiram inibição contra todos os organismos testados.

Palavras-chave: Listeria monocytogenes; Propriedades ópticas; Módulo de elasticidade; Compostos ativos.
Resumen

Los polímeros de origen natural y sus derivados se utilizan actualmente como biomateriales porque están fácilmente disponibles y sus propiedades se pueden adaptar para cumplir requisitos específicos. Los aceites esenciales se utilizan ampliamente como antimicrobianos. El objetivo de este estudio fue evaluar la eficiencia antimicrobiana in vitro de películas de acetato de celulosa (CA) incorporadas con los aceites esenciales de ajo (GR) y orégano (OR) sobre los microorganismos Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Salmonella choleraesuis y Pseudomonas aeruginosa y caracterizar las películas en cuanto a sus propiedades mecánicas, ópticas y estructurales. Se evaluaron cuatro tratamientos, Control, Película 1 (50 ml OR.100 g⁻¹ CA) Película 2 (50 ml OR + 30 ml GR.100 g⁻¹ CA) y Película 3 (50 ml OR + 50 ml GR.100 g⁻¹ CA). La concentración de aceites influyó en los parámetros mecánicos de carga máxima, deformación relativa a carga máxima y módulo elástico, dando como resultado películas más débiles, menos rígidas y más flexibles. Hubo un aumento en L * y b * en las películas incorporadas con aceite esencial de ajo y orégano. Las películas incorporadas con una mezcla de aceites esenciales de orégano y ajo exhibieron inhibición contra todos los organismos probados.

Palabras clave: Listeria monocytogenes; Propiedades ópticas; Módulo elástico; Compuestos activos.

1. Introduction

Polymers of natural origin and their derivatives are currently widely used as biomaterials because they are available in a variety of compositions and their properties can be tailored to meet specific requirements (Abdel-Naby & Aboubshait, 2013). In addition, there is a growing use of these biopolymers in the development of packaging biodegradable as an alternative to the use of polymers from non-renewable sources for application in food packaging. The most widely studied polymers for this purpose are chitosan, pectin, starch, polylactic acid, whey protein isolate and cellulose (Assis et al., 2020; Carvalho et al., 2019; Cerqueira et a., 2011; de Castro e Silva et al., 2019; de Oliveira et al., 2018; de Oliveira et al., 2019; Porta et al., 2011; Silva et al., 2015).

Cellulose acetate (CA) is a semi-synthetic polymer, being one of the first cellulose derivatives studied and commercialized. Due to its outstanding characteristics of good transparency, high resistance, thermoplasticity, and photostability CA have attractive applications in many industries such as affinity membranes, engineering fabrics, protective
fabric, reinforced nanocomposites, and packaging (Chuai & Zhang, 2014; de Almeida et al., 2020; Felgueiras et al., 2020; Wang et al., 2020).

Antimicrobial packaging is one form of active packaging that can extend the shelf life of the product and provide microbial safety of the consumer (Rooney, 1995). The antimicrobial compound acts to reduce, inhibit, or retard the growth of pathogenic microorganisms in packaged foods (Vermeiren et al., 1999). Furthermore, the antimicrobials agents are slowly released into the food surface, leading the lower diffusion rate of the active compounds, remaining in high concentrations in the packaging material for long periods of time (Biliaderis et al., 1999; Coma et al., 2001; Ouattara et al., 2000).

The growing interest in the incorporation of natural antimicrobials in packaging is based on consumer demand for foods with fewer synthetic additives (Devlieghere et al., 2004). Thus, essential oils are widely used as antimicrobials and their efficiency has been reported by various researchers, the phenolics components being the main responsible for essential oil antibacterial properties (Assis et al., 2020; Carvalho et al., 2017; Cosentino et al., 1999; Dannenberg et al., 2017; Pola et al., 2016).

The essential oils of garlic and oregano are used as antimicrobial additives for packaging (Kırkpınar et al., 2011; Molina-Hernández et al., 2020). Garlic (Allium sativum L.) belongs to the family Liliaceae (Cronquist & Takhtadzhian, 1981) and its biological functions are mainly due to its high content of volatile compounds, such as diallyl sulfide, diallyl disulfide, allicin, and low amounts of nonvolatile water-soluble sulfur compounds. Different studies have demonstrated that garlic oil damages the membrane functions of both gram-negative and gram-positive bacteria (Escherichia coli, Listeria monocytogenes, Bacillus cereus, Salmonella typhimurium, and Staphylococcus aureus) (Kırkpınar et al., 2011; Molina-Hernández et al., 2020).

The oregano (Origanum vulgare) oil possesses the strongest antibacterial properties against foodborne pathogens due to its higher concentrations of phenolic compounds such as carvacrol and thymol. Carvacrol presents a good antibacterial activity because it can cause permeabilization and depolarization of the cytoplasmic membrane (Kırkpınar et al., 2011; Molina-Hernández et al., 2020).

In this context, studying the antimicrobial effects of the addition of essential oils of oregano and garlic in acetate films is innovative, as it associates the antimicrobial action of the phenolic compounds in oregano with the antimicrobial action of the volatile compounds of garlic. Thus, the aim of this study was to evaluate the in-vitro efficiency of cellulose acetate antimicrobial films incorporated with essential oils of garlic and oregano on
**2. Material and Methodology**

**2.1. Material**

Cellulose acetate was from Rhodia (Germany), acetone PA was from Isofar (Brazil), essential oils of garlic and oregano were from Petite Marie (Brazil), and the Mueller-Hinton agar was from Merck (Brazil). The microorganisms *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella choleraesuis* (ATCC 10708) and *Listeria monocytogenes* (ATCC 15313) were acquired of Oswaldo Cruz Foundation (Brazil).

**2.2. Preparation of films**

The films were prepared by the casting method (Silveira et al., 2007). Cellulose acetate (CA) and the essential oil were solubilized in acetone (10% w/v CA/acetone). The solution was allowed to stand for 24 hours and then homogenized (magnetic stirrer - 150 rpm) until complete visual CA solubilization in the acetone. Four coded treatments were developed according to Table 1. The 100 ml of the film solution mixture was spread onto 30 x 20 cm rectangular glass plates. After acetone evaporation at room temperature (25 ºC) for 48 hours, the films were removed from the plates and stored in sealed multi-layered packaging for essential oil preservation.

The preparation and analysis of the films were carried out in the Department of Food Science of the Federal University of Lavras and in the Department of Food Technology of the Federal University of Viçosa.
**Table 1.** Composition of films.

<table>
<thead>
<tr>
<th>Film</th>
<th>Oregano (ml of oil. 100 g(^{-1})CA)</th>
<th>Garlic (ml of oil. 100 g(^{-1})CA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

Source: Authors.

2.3. Characterization of antimicrobial films

2.3.1. Film conditioning and thickness

All the films were stored at a controlled temperature of 23 ± 2 °C and 50 ± 5 % relative humidity for 48 h before analysis according to the (ASTM, 2000). The average film thickness was measured by reading at ten distinct points, randomly selected in each test body, using a Mitutoyo digital micrometer (0.01 mm precision, Mitutoyo Sul Americana, Brazil).

2.3.2. Scanning electron microscopy (SEM)

The Morphological characterization of films was performed by Scanning Electronic Microscopy (SEM) on equipment HITACHI TM 3000 (Japan). Three 1 cm diameter samples of each treatment were assembled in aluminum stubs with double carbon tape and metalized in a gold evaporator. Pictures were obtained with an acceleration voltage of 20 kV.

2.3.3. Mechanical properties

The films were subjected to mechanical tests according to the ASTM (2002) method using an Instron Universal Testing machine model 3367 (Instron Corporation, USA). For tensile tests, maximum load (N), relative deformation at maximum load (%) and elastic modulus (MPa) were evaluated. The test bodies were cut into strips (150 x 25mm) with an initial separation between grips of 100mm, 1kN load cell and a velocity of 50 mm/min.

2.3.4. Optical Properties

The opacity of the films was determined using a Cary 50 UV - Visible spectrophotometer (Varian, Australia) (Giménez et al., 2009). The films were cut (3x3 cm)
and a control film was used as the reference in the measurements. The absorbance spectra of the films were recorded at 600 nm and the opacity calculated by Equation 1:

\[
\text{Opacity} = \frac{\text{Abs}_{600}}{X}
\]  

(1)

where, \(\text{Abs}_{600}\) is absorbance value at 600 nm, and \(X\) is film thickness (mm).

CIELab color parameters \((L^*, a^*, b^*)\) were obtained using illuminant D65 and 10° observer in Konica Minolta CM 700D equipment (Konica Minolta Sensing Americas, USA). The total color difference \((\Delta E)\) was calculated according to Equation 2 (Ramos & Gomide, 2017):

\[
\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}
\]  

(2)

where, \(\Delta L^* = L_{*2} - L_{*1}^*\); \(\Delta a^* = a_{*2}^* - a_{*1}^*\); and, \(\Delta b^* = b_{*2}^* - b_{*1}^*\).

2.3.5. In-vitro antimicrobial activity of the films

Mueller-Hinton agar was prepared and sterilized according to the instructions of the manufacturer. In a laminar flow hood, Mueller-Hinton agar was poured into 9 cm internal diameter Petri dishes with a volume from 25 to 30 ml. The lids of the plates were ajar to prevent moisture formation on their inner surface. After solidification, all plates were covered and placed in an incubator 35 °C for 24 hours.

The suspension (0.1 ml of 108 CFU ml\(^{-1}\)) of the microorganisms \(Staphylococcus aureus\), \(Escherichia coli\), \(Pseudomonas aeruginosa\), \(Salmonella choleraesuis\) and \(Listeria monocytogenes\) were seeded on solidified Mueller-Hinton agar in a Petri dish. A disc of the produced films (diameter of 2.1 cm) was then aseptically placed in the center (Appendini & Hotchkiss, 2002). The discs were previously subjected to sterilization under UV/nm light (Prodicil, 110V, 254 nm) for 15 minutes. Three replicates in triplicate were conducted. The plates were incubated at 35 °C for 24 hours and evaluated as to the measure of the inhibition halo formation with a 6" pachymeter model Mitutoyo (Mitutoyo Sul Americana, Brazil).
2.4. Statistical Analyses

The results were analyzed in the SISVAR software (Ferreira, 2014) employing the Univariate Statistical Analysis (ANOVA) and Tukey test considering 5% significance level.

3. Results and Discussion

3.1. Scanning electron microscopy (SEM)

In general, cellulose acetate films incorporated with the essential oils of oregano and garlic presented insoluble points of dispersion (Figure 1).

Figure 1. Scanning electron microscopy (SEM) micrographs of cellulose acetate film (CA) incorporated with essential oils of garlic (GR) and oregano (OR) (A) Control (B) Film 1 (C)Film 2 (D) Film 3.

In Figure 1A and 1B cellulose, acetate agglomerates can be observed. Figure 1B shows points that possibly refer to globules of oregano essential oil that were not fully dispersed in the film matrix. Figures 1C and 1D show an alteration in the film matrix,

Source: Authors.
characteristic of porosity with the addition of garlic essential oil. The presence of agglomerates can be due to partial solubilization of cellulose acetate and the essential oils (Moreira Gonçalves et al., 2020) since they were solubilized in acetone, a hydrophilic solvent with low solubility for lipidic compounds. The amount of lipid globules increases as the oil concentration rises in the treatments. Films containing a higher oil concentration showed an uneven lipid distribution in the polymeric matrix which results from the limited dispersibility of the oil (Pereda et al., 2010).

3.2. Mechanical Properties

Film thickness did not differ significantly (p > 0.05), with a mean value of 40 μm ± 3. The mechanical properties of biodegradable films are associated with their behavior during the handling and storage of food. The mechanical strength and extensibility are necessary for the film to support the external stress and maintains its integrity (de Castro e Silva et al., 2019). The maximum load measures the maximum force the film supports up to rupture, deformation upon maximum load refers to the ability to stretch before breaking and the elastic modulus defines the stiffness of the film (ASTM, 2002).

The variation of the oil concentration factor significantly (p ≤ 0.05) influenced the parameters maximum load, relative deformation upon maximum load, and elastic modulus (Table 2). The control film showed a higher maximum load than the active films, i.e. with the addition of essential oils, the films became less resistant. In contrast, there was an increase in the relative deformation upon maximum load. This indicates that the addition of oils provided the film with a higher stretch capacity compared to the control. It was observed that the treatment with the mixture of oils resulted in decreased stiffness (p = 0.05) and control treatments and Film 1 have significantly the same stiffness.
Table 2. Maximum load (ML), relative deformation at maximum load (D) and elastic modulus (EM) of the films.

<table>
<thead>
<tr>
<th>Film</th>
<th>ML (N)</th>
<th>D (%)</th>
<th>EM (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1995 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>19 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 ± 6</td>
<td>825 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>16 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300 ± 70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>16 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194 ± 33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with the same letters in the same column do not differ statistically (p ≤ 0.05). Source: Authors.

The change in the film matrix with lipid agglomerates and porosity was observed in the micrographs (Figure 1), which may have affected the mechanical properties of the films. As Moreira Gonçalves et al. (2020), it is believed that essential oils and their mixture modified the microscopic structures of the films, and increased mobility of the polymer chains, resulting in weak (less maximum load) less rigid (lower elastic modulus) and more flexible films (more deformation). In this work and work of Moreira Gonçalves et al. (2020), the possible non-miscible characteristics of the material may explain the variations in the mechanical behavior of the films, which, in turn, proved to be partially heterogeneous, as shown in Figure 1. The mechanical properties depend on the intra and intermolecular forces of the polymer chains and how they interact within the film network. The addition of essential oils interrupted the network (Han Lyn & Nur Hanani, 2020).

3.3. Optical Properties

The visual aspect is related to the color and transparency of the films, attributes that impact the appearance of the packaging, and product acceptability by the consumer. The active films showed a sharp increase in opacity and the control film presented the highest transparency (Table 3). In addition, it was observed that opacity increased with increased essential oil concentrations. Yang and Paulson (2000), Han Lyn and Nur Hanani (2020), and Shojaee-Aliabadi et al. (2013) report that increased film opacity arises from the dispersion of light produced by the lipid droplets dispersed in the emulsion, causing a milky/whitish film appearance. Thus, the different opacities observed in Table 3 can be justified by the presence of non-miscible material, forming heterogeneous films, since the hydrophobicity of essential
oils can hinder their interaction with the CA matrix, as also evidenced in the previous analyzes.

Table 3. Opacity, color parameters (L*, a*, b*) and the total color difference (ΔE) of the films.

<table>
<thead>
<tr>
<th>Film</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
<th>Opacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.1 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.14 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>2.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>54.0 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.2 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.20</td>
<td>18.0 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>87.0 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.82 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.15 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.48</td>
<td>81.0 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>93.0 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-1.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.72</td>
<td>99.0 ± 3.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with the same letters in the same column do not differ statistically (p ≤ 0.05). Source: Authors.

There was a significant increase in L*, approaching white, in the films with incorporated essential oils of garlic and oregano. The L* increase can be observed by a color change (white to transparent) compared to the control film (Figure 2).

Figure 2. Cellulose acetate film (CA) incorporated with essential oils of garlic (GR) and oregano (OR)(A) Control (B) Film 1 (C)Film 2 (D) Film 3.

Source: Authors.
This change was due to the addition of the oils essentials. The value of a * gives the color value between red and green; the b * value, which gives the color value between yellow and blue. Film 3 was the only one that differed significantly (p ≤ 0.05) in relation to the control for parameters a* and b*. With increasing concentration of the essential oil of the garlic in the film the color tends towards yellow, as indicated by an increase in the b* value. This result is possibly due to the yellowing arising from the combination of essential oils used for film production. This result was consistent with Ghasemlou et al. (2013), Shojaee-Aliabadi et al. (2013), and Han Lyn and Nur Hanani (2020), where the incorporation of plant essentials oils into films significantly increased the intensity of yellow colour in the films.

It is generally recognized that ΔE values greater than 5.0 can be easily detected by the human eye and values above 12.0 imply an absolute color difference, easily noticeable by untrained people. However, differences of 3.0 may be barely noticeable and values less than 1.0 are not noticeable to the human eye (Ramos & Gomide, 2017). Thus, the difference between Film 1 and Control can be easily detected. The difference between Films 2 and 3 and Control are noticeable. But, the difference between Film 2 and 3 may not be detectable to the human observer.

3.4. In-vitro antimicrobial activity of the films

No inhibition zone against the microorganisms *Escherichia coli*, *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus* was observed in the control film. Films 2 and 3 presented inhibition against all the test organisms (Table 4). Figure 3 presents the bacterial inhibitory halos on Film 2.

Film 1 presented inhibition zones for *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella choleraesuis*. Du et al. (2009) reported that tomato puree films incorporated with essential oil of garlic have no activity against *E. coli O157, H7* and *Salmonella enterica*, however, they were effective against *L. monocytogenes*. In the same study, oregano essential oil was added in the tomato puree films and there was inhibition for the test microorganisms *Escherichia coli O157, H7*, *Salmonella enterica* and *Listeria monocytogenes* (Du et al., 2009). Seydim and Sarikus (2006) observed that with increasing concentration of oregano and garlic oil, the inhibition zone also significantly increased against *Salmonella enteritidis, Escherichia coli O157, H7*, *Staphylococcus aureus*, and *Listeria monocytogenes*. These results are similar to those found in the present study.
Of the test microorganisms *Escherichia coli*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa* are gram-negative and presented, on average, smaller inhibition zones compared to the gram-positive organisms *Listeria monocytogenes* and *Staphylococcus aureus*. The studies that have investigated the action of essential oils on foodborne spoilage and pathogenic microorganisms agreed that the essential oils are slightly more active against gram-positive bacteria than gram-negative bacteria (Harpaz et al., 2003; Pelissari et al., 2009; Pintore et al., 2002).

**Figure 3.** Bacterial inhibitory halos on Film 2:(A) *Staphylococcus aureus*, (B) *Escherichia coli*, (C) *Pseudomonas aeruginosa*, (D) *Salmonella choleraesuis* and (E) *Listeria monocytogenes*.

![Bacterial inhibitory halos on Film 2](image-url)
Table 4. Antimicrobial activity of cellulose acetate films incorporated with essential oils against the test microorganisms.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Film</th>
<th>Inhibition zone (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1</td>
<td>10.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.32</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18.71</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>10.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.06</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.32</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pseudomonasaeruginosa</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11.59</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Salmonella choleraesuis</td>
<td>1</td>
<td>11.95</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.94</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.99</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>1</td>
<td>10.84</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.82</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>19.38</td>
</tr>
</tbody>
</table>

Source: Authors.

The mechanism proposed by Zivanovic et al. (2005) for the antimicrobial activity of essential oil phenolic compounds is the attack on the phospholipid membrane, causing an increase in permeability and cytoplasm loss or causing interaction with enzymes located in the cell membrane. Thus, the resistance of gram-negative bacteria to essential oils probably lies in protective role of cell wall liposaccharides or outer membrane proteins, which restricts the diffusion of hydrophobic compounds through the lipopolysaccharide membrane (Burt, 2004).

An additive effect is seen when the combined effect is the sum of the individual effects. Antagonism is observed when the effect of one or both compounds is lower when
they are applied in combination than when applied individually. Synergism is observed when the combined effect of the substance is greater than the sum of the individual effects (Davidson, 1989). In this study, when the oregano essential oil was associated with garlic essential oil inhibition of all test microorganisms was obtained. An additive effect was observed between the oils, since the inhibition zone was enhanced when compared to inhibition zone of oregano oil. A synergistic effect was also observed, since oregano oil separately in no presented antimicrobial effect on the microorganism *Pseudomonas aeruginosa*.

**4. Conclusion**

The films incorporated with the mixture of essential oils of oregano and garlic exhibited inhibition against all organisms tested. There was a synergistic and an additive effect between oils since the inhibition zone was enhanced when compared to the inhibition zone of oregano oil. Thus, films with cellulose acetate and oregano and garlic essential oil showed efficient antimicrobial activities that associated with good colorimetric and mechanical properties with potential applications in the food industry, especially in perishable foods such as fruits. In addition, this study allows studies with other biopolymers and essential oils in order to also achieve efficient active packaging with potential applications in food.

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**References**


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