Antimicrobial zein coatings plasticized with garlic and thyme essential oils

Revestimentos antimicrobianos de zeína plastificados com óleos essenciais de alho e tomilho

Livio Antônio Silva Pereira1*, Priscila de Castro e Silva1, Jorge Pamplona Pagnossa2, Kelvi Wilson Evaristo Miranda1, Eliton Souto Medeiros3, Roberta Hilsdorf Piccoli4, Juliano Elvis de Oliveira5

1Universidade Federal de Lavras (UFLA), Departamento de Engenharia de Biomateriais, Lavras/MG - Brasil
2Universidade Federal de Lavras (UFLA), Departamento de Biologia, Lavras/MG - Brasil
3Universidade Federal da Paraíba (UFPB), Departamento de Engenharia de Materiais (DEMat), João Pessoa/PB - Brasil
4Universidade Federal de Lavras (UFLA), Departamento de Ciência dos Alimentos, Lavras/MG - Brasil
5Universidade Federal de Lavras (UFLA), Departamento de Engenharia, Lavras/MG - Brasil

*Corresponding Author: Livio Antônio Silva Pereira, Universidade Federal de Lavras (UFLA), Departamento de Engenharia de Biomateriais, Av. Doutor Sylvio Menicucci, 1001, Kennedy, Aquenta Sol, CEP: 37200-000, Lavras/MG - Brasil, e-mail: livioanpe@yahoo.com.br

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Abstract

Essential oils with antimicrobial properties are widely used in the food industry. This study aimed to evaluate the influence of a blend of garlic (Allium sativum) and thyme (Thymus vulgaris) essential oils on the antimicrobial and mechanical properties of zein films. Four bacteria (Enteropathogenic Escherichia coli (EPEC), Listeria monocytogenes, Salmonella Enteritidis and Staphylococcus aureus) related to food contamination were chosen to evaluate the antimicrobial properties. The results indicated that the oil blend acted as a plasticizer, decreasing the glass transition temperature and the Young’s Modulus of the films. The addition of the oil blend also resulted in lower solubility and water absorption. The addition of the oil blend (0, 2%, 3% and 5% (v/v)) to the zein films showed inhibitory activity against all the bacteria tested, with inhibitory halos of between 6.5 mm and 8.27 mm. The results showed that the coating could be applied as a support to increase the shelf life of food products.

Keywords: Biopolymer coatings; Food technology; Edible films; Pathogenic bacteria; Biomaterials; Natural preservatives.

Resumo

Óleos essenciais com propriedades antimicrobianas são amplamente utilizados na indústria de alimentos. Neste estudo, objetivou-se avaliar a influência da mistura de óleos essenciais de alho (Allium sativum) e tomilho (Thymus vulgaris) nas propriedades antimicrobianas e mecânicas de filmes de zeina. Quatro bactérias (Escherichia coli enteropatogênica (EPEC), Listeria monocytogenes, Salmonella Enteritidis e Staphylococcus aureus) relacionadas à contaminação de alimentos foram escolhidas para avaliar as propriedades antimicrobianas. Os resultados mostraram que a mistura de óleos atuou como um plastificante, como confirmado por uma diminuição na
temperatura de transição vítrea e pelo Módulo de Young dos filmes. A adição de mistura de óleos também resultou em menor solubilidade e absorção de água. A mistura de óleo 0,2%, 3% e 5% (v/v) adicionada nos filmes de zeina mostrou atividade inibitória contra todas as bactérias testadas com halos inibitórios entre 6,5 mm até 8,27 mm. Os resultados mostram que o revestimento pode ser aplicado como suporte para aumentar o prazo de validade de produtos alimentares.

**Palavras-chave:** Revestimentos biopoliméricos; Tecnologia de alimentos; Filmes comestíveis; Bactérias patogênicas; Biomateriais; Conservantes naturais.

### 1 Introduction

In recent years the interest on biopolymeric packaging has increased, aiming the development of new material that promote low environment impact (Brito et al., 2011). In this context, the package market increased the environmentally friendly materials demand. Moreover, international regulatory agencies have issued guidelines for research into edible coatings, emphasizing the use of materials that can bring health and environmental benefits.

Several studies focusing on edible coatings have demonstrated the possibility of production of biopolymer packaging from various materials, such as polysaccharides, lipids and proteins. However, there is a need for hydrophobic materials, which support contact with water without undergoing major changes for coatings applied on foods exposed to moisture. Therefore, zein may be a promising material for this situation. The application of zein adds value to the little known and exploited maize. Zein has been used in the films preparation for food applications (Colzato et al., 2011; Corradini et al., 2006; Lawton, 2002, 2004).

Zein corresponds to 50% maize endosperm proteins (Zea mays). It is extracted from the corn gluten meal, being therefore a byproduct. The presence of nonpolar aminoacids in the zein is responsible for its high hydrophobic propensity, which makes it soluble in nonpolar solvents, such as the ethanol. The zein application as a raw material from natural and renewable sources is relevant for scientific advances. As a raw material for the packaging production, it contributes to the market diversification through the corn protein fraction, adding more economic value to the milling industries and grain producers. However, the fragility nature of zein can compromise its application in many situations, being necessary to add plasticizers, such as glycerol and poly (ethylene glycol), to improve the films mechanical properties (Lawton, 2004). Although there are studies about the plasticizers application in zein films, the essential oils capacity as plasticizers has not yet been evaluated. Besides the action as a plasticizer, the addition of essential oils can improve the film antimicrobial properties (Forato et al., 2013).

Essential oils can be regarded as natural food preservatives, due to their antimicrobial activity. Therefore they can help in the foodborne pathogens control (Muriel-Galet et al., 2012). Among these oils are the garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) oils (Bilenler et al., 2015; Emiroğlu et al., 2010; Teixeira et al., 2014). The use of antimicrobial coatings containing these essential oils can be effective in food protection against several microorganisms, such as the *Escherichia coli, Listeria monocytogenes, Salmonella Enteritidis, and Staphylococcus aureus* (Moradi et al., 2016). Thus, this study aimed is to prove the plasticizer and antimicrobial effect of this essential oils blend in the zein coating.

The major constituents of the garlic essential oil are divided into two groups: sulfur containing substances such as the cysteine derivatives (84%), diallyl trisulfide (PubChem CID: 16315) (37.3%-45.9%), diallyl disulfide (PubChem CID: 16590) (17.5%-35.6%), allyl methyl trisulfide (PubChem CID: 61926) (7.7%-10.4%), 2-vinyl-1,3-dithiane (PubChem CID: 521985) (3.9%-5.9%); and terpenes such as the γ-cadinene (PubChem CID: 92313) (4.3%-6.8%) and bisabolene (PubChem CID: 3033866) (2.1%-2.5%) (Dziri et al., 2014). The major constituents of the thyme (*Thymus vulgaris*) essential oil are: thymol (PubChem CID: 6989) (54%-58%), p-cymene (PubChem CID: 7463) (17%-21%), γ-terpinene (PubChem CID: 7461) (6%-7%)
and carvacrol (PubChem CID: 10364) (2%-11%) (Daferera et al., 2000). The antimicrobial activity of thyme essential oil appears to be related to the presence of thymol and carvacrol phenolic compounds. Phytochemical components may vary in relation to the harvest time, climate and season (Burt, 2004; Jakiemiu et al., 2010).

In this context, edible coating films of zein containing an essential oils blend composed by garlic and thyme oils were investigated in order to assess the blend effectiveness as a plasticizer in zein films and as an antimicrobial agent against the Enteropathogenic Escherichia coli (EPEC), Listeria monocytogenes, Salmonella Enteritidis and Staphylococcus aureus - bacteria related to food poisoning.

2 Materials and methods

2.1 Materials

Zein from maize (Sigma-Aldrich, Saint Louis, Missouri, USA), ethanol (Vetec Fine Chemicals Ltd., Duque de Caxias, Rio de Janeiro, Brazil), essential garlic oil (Sigma-Aldrich, Saint Louis, Missouri, USA) and thyme essential oil (Ferquímica, Vargem Grande Paulista, São Paulo, Brazil) were used without further purification.

2.2 Zein films preparation

Zein solutions were prepared adding 20 g of zein in 100 mL of ethanol (90 vol. %) at 50 °C under vigorous stirring for ten minutes. After the solution was cooled to room temperature. Garlic and thyme (1:1 volume ratio) essential oils were added in this solution and mixed using a magnetic stirrer for 1 h to form a homogenous mixture. The zein solutions were prepared following the 0, 2%, 3%, and 5% (v/v) essential oils proportions (Pankaj et al., 2014). Zein and zein/essential oils blend films were produced by casting the solutions into 50×10 mm Teflon plates and the solvent was evaporate at room temperature for 24 h. The films were immediately used for further analysis.

2.3 ATR-FTIR

Attenuated total reflectance Fourier transform infrared spectra of the essentials oils, oils blend and films with and without oils were recorded between 4000 and 500 cm\(^{-1}\) on a Shimadzu-IRAffinity (Shimadzu Co., Kyoto, Japan) FT-IR spectrophotometer. Spectra were calculated from a 16 scans at a resolution of 4 cm\(^{-1}\). Few drops of the oil sample were positioned in contact with the attenuated total reflectance (ATR) on a multi-bounce crystal plate at room temperature (Erdogan et al., 2015).

2.4 Thermal analyses (TGA and DSC)

The materials thermal stability was investigated using a DTG-60AH thermogravimetric analyzer, TGA (Shimadzu Co., Kyoto, Japan). Samples were heated at a rate of 10 °C min\(^{-1}\) from 25 to 600 °C in a platinum crucible under a nitrogen flow rate of 50 mL min\(^{-1}\). Differential scanning calorimetry (DSC) was carried out for the films with different oils concentrations on a DSC-60 calorimeter (Shimadzu Co., Kyoto, Japan). Each sample (3-5 mg) was heated in a crimped aluminum pan at a scanning rate of 5 °C min\(^{-1}\) from 25 to 120 °C under a nitrogen atmosphere at a flow rate of 40 mL min\(^{-1}\) (Li et al., 2013). Reproducibility was checked by running the samples in triplicate.
2.5 Scanning Electron Microscopy (SEM)

Fracture surface was analyzed on a LEO EVO 40 XVP scanning electron microscope (Carl Zeiss, Jena, Germany) equipped with a secondary electron detector. Cross-section images were taken from cryogenic fractured samples, which were previously immersing in a liquid nitrogen for 2 min. Samples were mounted into stubs using a double-sided adhesive carbon tape and sputtered with gold using a plasma sputter coater (Balzer, SCD 050) (Pena-Serna & Lopes-Filho, 2013). Images were taken at 15-20 kV at the magnifications of 1000 and 5000X. Pore size was measured using a J 1.50i Image (Wayne Rasband, National Institute of Health, USA).

2.6 Water Solubility (WS)

Film solubility in water was analyzed (Parris et al., 1997), determining the film solubilized percentage after 24 h of immersion in water at 23 ± 2 °C. Briefly, 3 cm film discs were weighed, dried at 50 °C for 24 h, and immersed in a petri dish containing 50 mL of water. The discs were removed from water and dried at 50 °C for 24 h. The solubility in water (Ws) was calculated by Equation 1:

\[ W_s(\%) = \frac{W - W_0}{W} \times 100 \]  

where Ws is the solubility percentage, W and W0 are, respectively, the disk weight before and after immersion in water.

2.7 Water Absorption (WA)

Water absorption was determined according to ASTM D-570 (ASTM International, 1998). The analysis was carried out in triplicate. Discs of 3 cm in diameter were conditioned in an oven at 50 °C for 24 h; after they were cooled in a desiccator and weighed. Conditioned specimens were entirely immersed in 50 mL of distilled water at 25 °C. Specimens were removed from the soaking water at several time intervals. The surface water was wiped off with a dry cloth, and the specimens were weighed. Water absorption (WA) was calculated by Equation 2:

\[ WA(\%) = \frac{m_w - m_c}{m_w} \times 100 \]  

where mw and mc are, respectively, the wet and conditioned mass of the samples. Values reported were calculated as an average of three determinations.

The water absorption kinetics by the zein films was determined according to Shi et al. (2012) (Equation 3):

\[ y(t) = M_s(t - e^{-t/T}) \]  

where \( y(t) \) is the water absorption rate of the films (g min\(^{-1}\)), \( M_s \) is the water absorption capacity, \( t \) is the immersion time in minutes, and \( T \) is the temperature in Kelvin.

2.8 Water Vapor Permeability (WVP)

The water vapor permeability (WVP) of the films was determined gravimetrically at 25 °C using the standard method ASTM E-96-95 desiccant method (ASTM International, 1995). The samples of each film were sealed in hermetically sealed acrylic permeation cells containing desiccant (silica gel) in the interior (0% RH, 0 mmHg of water vapor pressure). These cells were conditioned in desiccators at 25 °C with saturated solutions of calcium nitrate or distilled water (100% RH and 23.76 mmHg of water vapor pressure). The diffusion of moisture through the biofilm led to the increase in cell weight due to the absorption of moisture by the silica gel, which was recorded on analytical balance (0.0001 g) at 24-hour intervals for
7 to 8 days stabilization). The determinations were performed in triplicate. The permeability rate was calculated by the Equation 4:

\[ WVP = \frac{w.e}{t.A.\Delta p} \]  

(4)

where PVA is water vapor permeability (g mm h\(^{-1}\) m\(^{-2}\) kPa\(^{-1}\)), w is weight difference (amount of permeant through the film), e is film thickness, t is time at which mass gain occurs, A is exposed film area and \(\Delta p\) is partial pressure difference of water vapor at 100% RH and 0% RH, both at 25 \(^\circ\)C, corresponding to 2646 KPa.

### 2.9 Mechanical properties

The methodology used to assess the mechanical properties was based on the works of Giménez; Montero, 2013 and Zivanovic et al. (2005) and carried out according to the ASTM D882 (ASTM International, 2012). Tensile tests were determined using a TA-XT2i texture analyzer (Stable Micro Systems, United Kingdom). 20 mm\(\times\)100 mm samples were tested using a load of 1 kN and a crosshead speed of 125 mm sec\(^{-1}\). Tensile strength and elasticity modulus were calculated as an average of 10 measurements. The films were cutted into squares of 9 cm\(^2\) and fixed in a holder with central opening for puncture testing. A spherical probe of 5.0 mm diameter was perpendicularly displaced to the film surface at a constant speed of 0.8 mm s\(^{-1}\) until the tube passed through the film. The puncture strength was calculated by dividing the load at the breaking point by the film thickness. Results were calculated as an average of 10 measurements.

### 2.10 Microbiological analyzes

The microbiological tests were performed for Enteropathogenic *Escherichia coli* (EPEC INCQS 00181), *Listeria monocytogenes* (ATCC 19117), *Salmonella Enteritidis* (S64) and *Staphylococcus aureus* (GL 5674).

The stock cultures were stored in freezing media (15 mL of glycerol; 0.5 g of bacteriological peptone; 0.3 g of yeast extract; 0.5 g of NaCl and 100 mL of distilled water at pH 7.0). Cultures were reactivated according to the Clinical and Laboratory Standards Institute (CLSI) method, inoculating 100 uL aliquots into tubes containing 10 mL of Tryptone Soya Broth (TSB) (Clinical and Laboratory Standards Institute, 2003). The cultures were incubated at 37 \(^\circ\)C for 24 h. A growth curve was performed in order to standardize the inoculum using the TSB. After the strains reactivation, 50 μL of inoculum was transferred to 300 mL of TSB and incubated at 37 \(^\circ\)C. One-hour interval (12 times points) spectrophotometrical readings were performed followed by the TSA plating and incubation at 37 \(^\circ\)C for 24 h. Standard cultures were set to 108 CFU mL\(^{-1}\).

### 2.11 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentrations (MBC) of the essential oils were determined using the microdilution technique according to CLSI with adaptations (Clinical and Laboratory Standards Institute, 2003). EOs solutions at concentrations of 5.00%, 3.75%, 3.0%, 1.0%, 0.5%, 0.25% and 0.125% (v/v) were prepared using TSB and 0.5% Tween 80. Subsequently, aliquots of 150 uL of these solutions were transferred for amicrrotiter plate and 10 uL of each standard culture were inoculated into each culture media. Microplates were sealed and incubated at 37 \(^\circ\)C for 24 h. After, 10 uL aliquots of each culture media were plated on TSA and incubated at 37 \(^\circ\)C for 24 h. The lowest non-grown concentration was determined as MBC. The analysis was carried out in triplicate with three replicates for each replica, using a positive control (TSB with 0.5% Tween 80) and culture for each repetition.

### 2.12 Films antimicrobial activity

The antimicrobial activity of the zein films without and with the oils blend against the selected microorganisms was studied using the agar diffusion method with adaptations (Bahram et al., 2014;
Gómez-Estaca et al., 2010). This method was chosen to simulate the application and antimicrobial effects of the zein/oils blend solution directly on the food surface. Bacterial strains were cultured in TSB at 37 °C and seeded in a Petri dish with 0.1 mL of the inoculum containing approximately 10⁸ CFU mL⁻¹ of the bacteria. 100 uL of the different film-forming solutions were poured into 6 mm diameter TSA culture medias, and the plates were incubated at 37 °C for 48 h. After that, the diameter of the zone of inhibition was calculated. A filmogenic solution without EOs was used as a positive growth control and a filmogenic solution containing chloramphenicol 1% (v/v) was used as a negative control. Measurements were performed in triplicate.

2.13 Statistical analysis

One-way ANOVA and Tukey Test at a significance level of \( p < 0.05 \) were used to analyze the differences among the groups, using the Statistica 8.0 (Dell Statistica, Hoklahoma, USA) software. The data were presented by the means ± standard deviations.

3 Results and discussion

3.1 ATR-FTIR

FTIR results are shown in Figure 1. Infrared spectra detected the presence of the main functional groups of the garlic and thyme essential oils, in accordance with the results founded by Wang et al. (2011). Major functional groups founded in the garlic and thyme oils was also detected in the garlic/thyme blend, indicating some interaction degree between these essential oils. It was noted a symmetry stretching vibration of CH = CH₂ at 3008 cm⁻¹. There were intense peaks attributed to C = C at 1423 cm⁻¹, -CH₂ at 1634 cm⁻¹, CSC at 918 cm⁻¹ and 985 cm⁻¹ and SC peak at 723 cm⁻¹, assigned to the garlic essential oil components. The presence of thyme essential oil components was indicated by a long band at 3399 cm⁻¹ due to the axial deformation of O-H on intermolecular bonds. The metinic ring groups (-CH) bonds were shown by an aromatic axial deformation at 2960 cm⁻¹. Bands combination are observed at 1667-1800 cm⁻¹. The axial deformation of the C=C aromatic ring was noted at the range of 1470-1617 cm⁻¹, the axial deformation of the linkage (C-O) occurred at 1225 cm⁻¹, and the metinic angular deformation out of plane was observed at 720-810 cm⁻¹.
The associated hydroxyl (O-H) effecting hydrogen bonding was detected in the films at 3250 cm⁻¹ region bond. Aliphatic compounds (C-H) for the chain elongation generated multiple bands with peaks at 2918 cm⁻¹ region and extending with the addition of oils blend. This can also be observed from the changes at 1640-1537 cm⁻¹, referring to the peaks of the amides present in the zein structure. These interactions were confirmed by the -OH peak at 1120 cm⁻¹, related to the zein connections - oils blend. After insertion of the oils blend, new peaks are observed in regions between 800 cm⁻¹ and 500 cm⁻¹ related to aromatic compounds. Similar results about the interactions among proteins and plasticizers were reported in the literature. Wongsasulak et al. (2010) noted similar interactions between zein and glycerol.

From 1100 cm⁻¹ region, it was noted the appearance of new peaks, which intensities increased with the oils blend content increasing. This can be related to the thyme compounds and garlic sulfur compounds presence. Oil blend compounds detected in the zein films can be considered as an evidence of retention effectiveness of the oils by the zein matrix, once it is expected that the presence of these compounds interferes directly in the coating structural and functional properties.

### 3.2 Thermal analyses (TGA and DSC)

Thermogravimetric analyses were carried out to evaluate and compare the thermal stability and decomposition of the zein films incorporated with the different essential oils blend concentrations, as observed in Figure 2. The weight loss started at room temperature, caused by the oil blend volatile compounds with significant weight loss beginning at 50 °C with 5 wt% and to thermal degradation at 180 °C. The film showed a similar weight loss profile until 175 °C, subsequently, the thermal decomposition of the different profiles was observed among the neat film and the films with essential oils blend at this temperature. The loss
of residual water and volatiles (dehydration reactions) from the films at 120 °C was also observed. The neat film showed a loss of 7% by weight and the 2%, 3% and 5% (v/v) blend films lost 6, 5.2 and 5 wt. % respectively. The films with essential oils blend had a weight loss of 9% at 175 °C. This can be related to the essential oils volatilization and thermo-oxidative degradations.

Figure 2. TGA analyses of the zein films.

The pure film has marked its thermal decomposition at 245 °C, showing 13% weight loss. This difference can be explained by the oils blend volatile and constituents loss. Intermolecular interactions occurred among the biopolymer matrix and the essential oils reducing the film thermal resistance of the film. This indicates the blend plasticizer action efficiency (Mallardo et al., 2016).

The films thermal decomposition finished at 373 °C, it was observed different thermal degradation profiles in this temperature range. The film showed 44% pure thermally stable mass, and the 2%, 3% and 5% oils blend films had 31%, 29% and 27% of residual mass respectively. The oils addition in the blend films decreased their thermal stability, as observed by Altiok et al. (2010). They noted a decrease in the thermal stability of chitosan films incorporated with the thyme essential oil.

DSC curves are shown in Figure 3. The inset showed a decrease in the glass transition temperatures – Tg, with increasing the oil content. Tg values for 0, 2%, 3% and 5% films were 160.5, 158, 153 and 148 °C respectively. This indicated the essential oils action as plasticizers, reducing the intermolecular interactions among the zein chains and improving the films flexibility. Similar phenomena were observed in several studies. Xu et al. (2012) concluded that the supramolecular structure was changed by the plasticizers, decreasing the Tg. Wang et al. (2003) observed a similar influence on lipid and zein protein interactions.
3.3 Scanning Electron Microscopy (SEM)

Figure 4 shows the SEM micrographs of the zein films cross-sections. The zein films surface (Figure 4a) displayed a brittle nature with several cracks. However, with the addition of garlic/thyme oils blend, there was a reduction in the number of cracks until a crack-free surface was observed in the 3% and 5% oils blend samples (Figure 1c, d). It occurred due to the essential oils plasticizing effect (Forato et al., 2013).

It can also be observed that all films were porous due to the oils presence their solubility limit, the presence of fatty acids residues in the zein, and due to the solvent evaporation during the drying process.
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Moreover, increasing the oils content, a gradual increase in the films pores was noted. The 0, 2, 3 and 5% oils blend films presented diameters of 2.9, 6.0, 4.8 and 5.7 μm, was observed respectively. Similar results were founded by Del Nobile et al. (2008) for zein films plasticized by thymol.

3.4 Water Solubility (WS), Water Absorption (WA) and Water Vapor Permeability (WVP)

Table 1 shows the zein films water absorption, solubility and water vapor permeability. It can be observed that with increasing oils blend concentration there was a decrease in the solubility, water absorption and water vapor permeability. Pure zein films showed the highest solubility 4.00% ± 0.20%, water absorption 1.76 ± 0.52 and WVP 2.15 ± 0.13. These parameters decreased consecutively until 0.54 ± 0.03, 0.84 ± 0.15 and 1.58 ± 0.05 (5% (v/v) oils blend). A similar behavior was noted for the absorption rate. Both solubility, absorption rate and WVP are dependent of the water molecules diffusion into the zein films. As the amount of oils blend increases, the films become more hydrophobic. The ultimate result is a reduction in their solubility and water molecules diffusion (Biswas et al., 2009).

| % Oils blend | Solubility (%) | Water absorption at 120 min (%) | Absorption Rate (g min⁻¹) | WVP x 10⁻³ (g mm m⁻² h⁻¹ kPa⁻¹) |
|--------------|----------------|-------------------------------|--------------------------|---------------------------------|
| 0            | 4.00 ± 0.05abc | 1.76 ± 0.02abc               | 0.62 ± 0.08abc            | 2.15 ± 0.13abc                 |
| 2            | 2.11 ± 0.07abc | 1.59 ± 0.06abc               | 0.56 ± 0.10abc            | 1.89 ± 0.02abc                 |
| 3            | 0.83 ± 0.04abc | 1.33 ± 0.05abc               | 0.47 ± 0.03abc            | 1.85 ± 0.14abc                 |
| 5            | 0.54 ± 0.03abc | 0.84 ± 0.15abc               | 0.30 ± 0.05abc            | 1.58 ± 0.05abc                 |

Values followed by different letters are significantly different each other (p ≤ 0.05) using the Tukey test followed by the standard deviations.

The solubility, water absorption and WVP evaluation has a fundamental importance in food coatings. In many cases, when this properties presenting low values, becomes a prerequisite to enhance the product integrity, to confer better barrier properties to the moisture and to increase the shelf life. This is required, especially in high-moisture foods applications, such as meat products, cheeses and sausages (Sandoval et al., 2014).

For the absorption, it was noted that the films became slightly opaque after a period of 120 min, indicating an excessive water absorption. The same phenomenon was also observed by Shi et al. (2012) in zein films incorporated with triethyl citrate. Significant differences (p ≤ 0.05) among the pure film and coated films with 5% oils blend were founded. The other treatments showed no significant differences. However, there was a strong tendency in reducing water absorption with the oil blend content increasing.

The 3 and 5% films presented the lowest absorption capacity and WVP (1.74% and 1.63%, WA; 1.85% and 1.58%, WVP, respectively). These differences are related to the spatial arrangement of the hydrophilic and hydrophobic groups of zein. The exposed hydrophilic groups of the protein are likely to interact with the water molecules. With the essential oils concentration increasing, there is less availability of the hydrophilic groups and then, the hydrophobic groups repel the water. The addition of hydrophobic plasticizers (such as oils and fats) help maintain the film integrity even in high humidity conditions, indicating therefore that such films can be potentially used as coating applications even where moisture is present. A similar behavior was funded by Santos & Padua (1999). In the water absorption of zein films was modified by the oleic and linoleic acids addition in their study. WVP depends on the structure of the zein film WVP depends on the structure of the zein film. In this case, with addition of the oils blend there is a successive reduction in cracks and pores, increase hydrophobicity and a progressive decrease of the WVP results. Ghanbarzadeh et al. (2007) in zein films plasticized with sugars, related similar results. In yours studies was detected increasing of WVP with increase of sugars plasticisers.
3.5 Mechanical properties

The mechanical properties of the films are shown in Table 2. The films mechanical properties were affected by the essential oil addition, corroborating the plasticizing effect already discussed above. Young’s modulus (E), elongation at break, tensile strength and puncture resistance were affected by the plasticizer presence. Despite the plasticizing effect of the garlic/thyme oils blend that promoted the Young’s modulus and tensile strength decreasing, the elongation at break decreased due to the pores presence. These results are in agreement with the work of Altiok et al. (2010). They detected a decrease in the elongation percent of zein films with the thymol addition due to the pores presence.

Table 2. Mechanical Properties of the zein films.

| % Oils blend | Young’s Modulus (E) (MPa) | Elongation at break (%) | Tensile strength (MPa) | Puncture resistance (MPa) |
|--------------|--------------------------|-------------------------|------------------------|--------------------------|
| 0            | 7.57 ± 0.15             | 1.37 ± 0.10             | 6.20 ± 0.03            | 0.74 ± 0.08             |
| 2            | 4.30 ± 0.07             | 0.80 ± 0.04             | 4.83 ± 0.10            | 2.73 ± 0.05             |
| 3            | 4.38 ± 0.03             | 0.76 ± 0.04             | 4.23 ± 0.15            | 3.27 ± 0.04             |
| 5            | 3.41 ± 0.06             | 0.42 ± 0.03             | 3.26 ± 0.03            | 2.67 ± 0.06             |

Values followed by different superscript letters are significantly different eachother (p ≤ 0.05) using the Tukey test followed by the standard deviations.

The puncture resistance results were significantly affected by the oils addition 3% oil blend film showed the highest value (3.27 MPa) in agreement with the SEM results, i.e., it presented a reduce in the pore size, absence of cracks and a better dispersion. In general, the films mechanical properties depend on a number of different factors. The polymer Naturef, plasticizer type and its concentration, crystallinity, chemical cross-linking, aging effects and microstructure are important factors that can modify the film strength (Xu et al., 2012).

3.6 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the thyme and garlic essential oils and its blend were shown in Table 3. The garlic essential showed a better inhibition bacteriological at 3.0 vol. % against Listeria monocytogenes. Inhibition occurred at 3.75% against Staphylococcus aureus and Salmonella Enteritidis, and at 5.0% against Enteropathogenic Escherichia coli (EPEC). This fact can be explained by the existence of an outer membrane surrounding the Gram-negative bacteria cell wall that restricts the hydrophobic compounds diffusion through the cell wall. The thyme essential oil presented a minimum bactericidal concentration of 0.5% against all bacteria. These results are similar to those founded in the literature (Aureli et al., 1992).

Table 3. inhibition Concentration of the garlic and thyme essential oils against the Enteropathogenic Escherichia coli, Listeria monocytogenes, Salmonella Enteritidis and Staphylococcus aureus microorganisms.

| Microorganism          | Garlic oil (%) | Thyme oil (%) | Oils blend (%) |
|------------------------|----------------|---------------|----------------|
|                        | MBC (Minimum Bactericidal Concentration) |
| E. coli (EPEC)         | 5.00           | 0.5           | 1.0            |
| L. monocytogenes       | 3.00           | 0.5           | 0.5            |
| Salmonella Enteritidis | 3.75           | 0.5           | 1.0            |
| S. aureus              | 3.75           | 0.5           | 1.0            |

The essential oils blend (Table 3) at concentrations as low as 1.0% inhibited Enteropathogenic Escherichia coli, Salmonella Enteritidis and Staphylococcus aureus growth. A synergistic effect of the essential oils blend was noted by the Listeria monocytogenes inhibition at 0.5%. The garlic oil addition intensified the antibacterial effect against the Listeria monocytogenes, due to the sulfite constituent action. Although their synergistic effect is not clearly elucidated, it is understood that only physicochemical factors such as the pH, temperature and water activity can increase or decrease the antimicrobial essential oils blend effect no synergistic effect was noted against the other bacteria.
3.7 Films antimicrobial activity

The inhibition zones in millimeters noted for the different essential oils concentrations applied in zein are shown in Table 4.

Table 4. Diameters of the inhibition zone of the zein films produced with garlic/thyme essential oils blend.

| % Oils blend in zein | E.coli EPEC | L.monocytogenes | Salmonella Enteritidis | S. aureus |
|---------------------|-------------|----------------|------------------------|-----------|
| 0                   | n/i         | n/i            | n/i                    | n/i       |
| 2                   | 6.46 a ± 0.03b, a1 | 6.75 ± 0.02a, a1 | 6.62 ± 0.015a, a1 | 6.74 ± 0.01a, a1 |
| 3                   | 7.13 b ± 0.015a, a2 | 7.36 ± 0.02b, b2 | 7.10 ± 0.04b, a2 | 6.95 ± 0.01b, c2 |
| 5                   | 7.36 c ± 0.04c, a1 | 8.27 ± 0.01c, b3 | 8.18 ± 0.02c, c3 | 8.20 ± 0.005c, c3 |
| PC*                 | 20.06 ± 0.03 | 21.02 ± 0.04 | 19.34 ± 0.07 | 19.48 ± 0.05 |

*Test carried out in triplicate. PC = Positive Control - Chloramphenicol. Inhibition Zone in mm. n/i = not inhibited. Values followed by different letters are significantly different each other, where the first letter represents the results displayed in the column and the second letter corresponds to the results displayed in the row (p ≤ 0.05) using the Tukey test, followed the standard deviations.

Results showed that there were significant differences (p ≤ 0.05) among the treatments. The antimicrobial activity was directly proportional to the essential oils concentration. All concentrations tested showed an inhibition zone below the positive control (PC). The films containing 2% showed no significant difference for any of the bacteria tested. It is known that the essential oils antimicrobial effect is somewhat reduced when added to a biopolymer matrix due to the different oils concentrations with the zein, oil release rate and microstructure developed during the film processing (Hosseini et al., 2009). However, significant differences were observed between the films with 3% and 5% oil blend, in which the Listeria monocytogenes showed the largest inhibition zones, confirming the synergistic effect found in the minimum bactericidal concentration test. These differences in the antimicrobial growth inhibition can be explained by the differences in the cell wall composition and/or inheritance of genes in plasmids which can be easily transferred among the bacterial strains (Springfield et al., 2003).

4 Conclusions

Results showed the garlic and thyme essential oils blend effectiveness as plasticizers and as antimicrobial agents applied to zein films. With the incorporation the oils blend, through thermal analysis (TGA and DSC) was observed a thermal stability reduction of the film indicating plasticizing action and reduction in the glass transition temperature due to polymer blend-interaction. The cracks elimination and a puncture resistance increasing were observed with the oils addition. Zein films produced with essential oils blend above 2% content inhibited the bacteria Enteropathogenic Escherichia coli (EPEC), Listeria monocytogenes, Salmonella Enteritidis and Staphylococcus aureus growth. This study demonstrated the great potential of the essential oils in improving the biopolymers coating properties, contributing to the food quality and shelf life improvement.

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