Polímeros
ISSN 1678-5169 (Online)

http://dx.doi.org/10.1590/0104-1428.11516

ORIGINAL ARTICLE

Microstructure and thermal and functional properties of biodegradable films produced using zein

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Abstract

Research is being conducted in an attempt to produce biodegradable packaging to replace plastic products, thereby reducing solid waste disposal. In this work, zein films were produced from vegetable oils (macadamia, olive and buriti) and from pure oleic acid. The surface of zein-based films made using oleic acid has a good lipid distribution. The high content of oleic acid produced a film with the greatest elongation at break (8.08 ± 2.71%) due to the greater homogeneity of the protein matrix. The different oils did not affect the glass transition temperature (Tg). Tg curves of films with fatty acids showed a reduction in mass at between 50 and 120 °C due to water evaporation. At 120 °C the weight loss was 3-5% and above this temperature further weight loss was observed with the highest loss being seen in the film made using pure oleic acid. In conclusion, although biodegradable films were produced using the four different oils, the film made from pure oleic acid has the best characteristics.

Keywords: *biodegradable films, biomaterial, zein.*

1. Introduction

Around the world, there is great concern about producing renewable materials from biomass. Due to environmental issues and sustainability, biomaterials produced from organic residues are the subject of several studies.

Among the many available raw materials suitable for the production of biodegradable products, starch and proteins are being used to create polymeric matrixes due to their good polymerization properties and because they are totally biodegradable. Both materials, similar to conventional synthetic polymers, are processed with the addition of plasticizers.

The biopolymer zein comprises 50% of the proteins in mature corn kernels[1]; it has hydrophobic characteristics due to its high content of apolar amino acids, in addition to its high degree of polymerization. Zein offers advantages as a raw material in the production of biomaterials, coatings and plastic applications as it is biodegradable and renewable[2-4]. It has been used to develop functional biodegradable packaging[5], packages with antimicrobial agents[6-9], packaging for periodontal biomaterials and substrates for cell culture[10]. Studies with zein have been carried out in respect to the regeneration of osseous tissues[11], with nanoparticles used to improve the mechanical properties of films[12,13] and edible film to increase the shelf life of pears[14] and macadamia[15].

The preparation of a film solution requires high-molecular-weight agents called matrix formers, solvents, plasticizers and, when necessary, a pH adjuster[16]. These components are used in different combinations to give films different characteristics.

Plasticizers are low-molecular-weight organic compounds used to reduce the intermolecular bonding forces, thereby giving high molecular mobility and flexibility to the film[17]. However, an excess of plasticizers results in a reduction of the mechanical properties of the film[18]. Glycerol is most frequently used even though it has hydrophilic properties[19,20]. The greater the amount of glycerol added to produce biopolymer-based films, the lower the stress rupture strength and the Young's modulus and so the elongation at break is higher. Many formulations include fatty acids, such as oleic, linoleic, stearic and palmitic fatty acids[21].

Some edible vegetable oils have large amounts of oleic acid and so potentially, they can be used as plasticizers in the production of zein films. Of the oils containing fatty acids, buriti (*Mauritia flexuosa*), macadamia and olive oil are of great interest as the fatty acid levels are greater than 60%.

Scanning electron microscopy (SEM) is the method most commonly used to evaluate the microstructure of biodegradable films. With this technique, it is possible to see whether the structure of the resulting biomaterial is uniform or if the components do not mix. Another important method to complement SEM in the analysis of the homogeneity of the matrix of films is optical microscopy (OM) which allows the characterization of the components by assessing their color and shape.

Thermal and mechanical analyses are important to determine the conditions under which the film can be used and the types of food that can be packaged. The glass transition temperature (Tg) is of much interest to food

scientists because it helps to explain the chemical and physical behavior of food systems[22]. The Tg of films is important as this characteristic limits the use of the material under extreme conditions, such as at freezing temperatures and during sterilization. The value of the Tg is primarily governed by the chemical composition particularly the presence of plasticizers, and secondarily by structural characteristics such as chain branching, crosslinking and crystallinity[23]. The Tg also provides information on the compatibility of the matrix constituents in a film, especially in the cases of blends/composites[24,25]. Thus, knowledge of the Tg of biodegradable films, specifically edible films, helps in the choice of the best storage conditions.

Good mechanical properties are also required and therefore, the elongation percentage and the tensile strength of the film need to be studied. The aim of this study was to produce biodegradable films of zein using pure oleic acid and edible vegetable oils (macadamia, buriti and olive oil) and to determine their microstructures and functional properties.

2. Materials and Methods

2.1 Biodegradable films

Zein (Freeman Industries, Inc. NY, USA) was used at a ratio of 20% (w/v) in 75% ethanol solution. Other components were used in the following proportions in respect to 100g of zein: 70g edible vegetable oil (macadamia, olive or buriti) purchased at local shops (Vital Atman, La Violeteira and Rio Essências, respectively) or 70g of oleic acid (VETEC, Brazil) and 30g of glycerol (Merck, Brazil) as plasticizers and 5g of Emustab® emulsifier (Duas Rodas Industrial Ltda., Brazil) to facilitate emulsification.

After combining the components, the filmogenic solution was heated to 62 °C while stirring at 250 rpm to be subsequently cast on rectangular acrylic plates and maintained at 25 °C for 24 hours to dry. The films were peeled off the acrylic plates and stored inside a desiccator at 58% relative humidity until analyses.

2.2 Composition analysis of the fatty acids of vegetable oils

The fatty acid composition of oils was investigated at the Instituto de Tecnologia de Alimentos (ITAL), Campinas[26-29].

2.3 Scanning electron microscopy

SEM was performed on 12-mm round samples of film fixed under stubs using double-sided adhesive tape with conductive copper and covered with 35 nm of gold (EMITEC K550, UK). Samples were examined in duplicate by electronic microscopy (LEO 435 VP, UK) at 15 kV in a climate room.

2.4 Optical microscopy

Optical Microscopy (OM) was used to identify the compounds of the films. Duplicate samples were stained with *Xylidine Ponceau* (pH = 2.5) directly and dehydrated at 37 °C for 24 hours (Odontobras ECB 1.2 Digital, Brazil). The samples were analyzed at room temperature using an optical microscope (Olympus BX 60, USA) with an image capture system (Olympus DP 71, USA). Different points in the sample were analyzed at 10× magnification.

2.5 Analysis of differential scanning calorimetry

Measurements were made using a differential scanning calorimeter (TA Instruments - TA Q100). Approximately 6-mg samples were subjected to pre-heating from 25 °C to 120 °C to eliminate the thermal history. They were then reheated to 120 °C or 200 °C at a rate of 10 °C/minute under a nitrogen flow of 50 mL/minute. The Tg and melting temperature (Tm) were obtained from the minimum of the first derivative and minimum peak on the differential scanning calorimetry (DSC) curve, respectively.

2.6 Thermogravimetric analysis

The thermal stability of the materials was studied using a thermogravimetric analyzer (TGA - TA Instruments, Model Q500). All tests used a mass of approximately 7.0 mg under a nitrogen flow of 50 mL/minute and heating rate of 10 °C/minute within the temperature range of 25 to 900 °C.

2.7 Mechanical properties

Tensile strength tests were performed with a Universal-Instron device (Model 5569, Instron Engineering Corp., Canton, MA) following the standard testing methods ASTM D882-91[30]. Tensile strength at break (σr), elongation at break (εr) and the Young's modulus (E) were determined.

3. Results and Discussion

3.1 Appearance and thickness of the films

After drying, the films were peeled off the rectangular acrylic plates and visual and tactile analyses were carried out in order to analyze only homogeneous samples in respect to thickness and without cracking. The color and thickness of the material should be uniform, there should be no brittle areas, and the material should be easy to handle. Films that did not have these characteristics were discarded.

The thickness was obtained by the arithmetic mean of six random points in different segments of the film using a digital micrometer (Digimess) at a resolution of 0.001 mm. All films had uniform thicknesses from 0.19-0.20 ± 0.02 mm.

3.2 Characterization of edible vegetable oils

The content of fatty acids of edible oils and the oleic acid used in the formulations are shown in Table 1.

Table 1. Fatty acid composition of plasticizing oils used in the formulation of zein films.

Plasticizers	Oleic (%)	Linoleic (%)	Palmitic (%)	Palmitoleic (%)
Oleic acid	73.2	4.0	4.1	3.7
Buriti oil	35.1	53.6	5.5	0.1
Macadamia oil	61.0	1.7	7.9	16.8
Olive oil	64.7	17.1	11.3	0.7

Fatty acid composition of edible oils and oleic acid are in accordance with the literature regarding the concentration of oleic acid (above 60%) except for buriti oil. Buriti oil has less than 60% of oleic acid (35.1%), but has a concentration of 53.6% of linoleic acid. Both acids produce materials with good flexibility and functional properties[31].

3.3 Scanning electron microscopy

Micrographs (Figure 1) show that the films made with macadamia and buriti oil had similar structures with the homogeneous presence of rounded components that look like pores on the surface of the biomaterials. The lipid volume fraction in the dry film and the size of lipid aggregates are the main factors involved in the optical heterogeneity of the film matrix[32]. Films produced by the casting method show different morphological shapes due to drying with the side in contact with air presenting an irregular and opaque appearance and black globular deposits[33].

The presence of globular deposits or pores was verified by the presence of rounded structures, showing that a continuous filmogenic matrix did not form. This observation was also reported in zein films[12,19,34-36], chitosan film[37] and pectin[38].

The films produced with oleic acid (Figure 1c) and olive oil (Figure 1d) showed a smaller number of rounded forms and a significant reduction in the size of the fat globules thus demonstrating that a more homogenous matrix had been created.

3.4 Optical microscopy

Optical microscopy was used to complement the SEM analysis of the homogeneity of the matrix of films in respect to the pore-like rounded forms similar to globular deposits. The *Xylidine Ponceau* technique provides two types of staining; red represents protein (zein) and white represents lipid globules.

Images of each sample are shown in Figures 2 and 3, with the scales of the micrographs being 4× (10 μm) and 10× (500 μm), respectively.

The micrographs show that zein film produced from oleic acid has greater homogeneity (Figure 3c, d) supporting the results of the SEM analysis. Moreover, the film made using macadamia oil (Figure 2c, d) was more homogeneous than the films of buriti (Figure 2a, b) and olive oil (Figure 3a, b). The arrangements of the fat globules in the films made with buriti and olive oil are more heterogeneous and larger, suggesting that a higher oleic acid content produces a more homogeneous material.

In addition, there is much streaking suggesting aligned fibrillar proteins which demonstrates that the incorporation of the plasticizer was not uniform in the matrix[34,39]. The homogeneity of the material seems to be correlated with the quantity (%) of oleic acid in the oils because pure oleic acid produced the most uniform film with the best structural and functional characteristics and buriti oil the

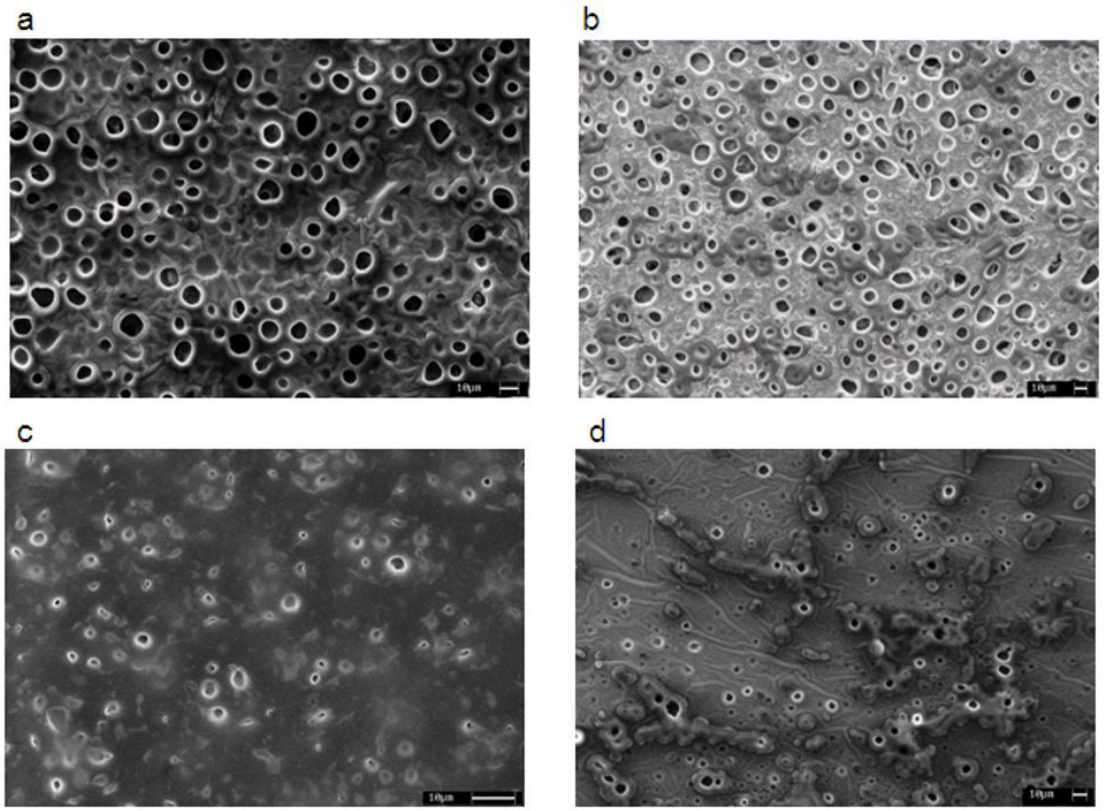

Figure 1. SEM micrographs of surfaces of: (a) zein- buriti oil; (b) zein-macadamia oil; (c) zein-oleic acid and (d) zein-olive oil. The increase indicative bar corresponds to 10 μm.

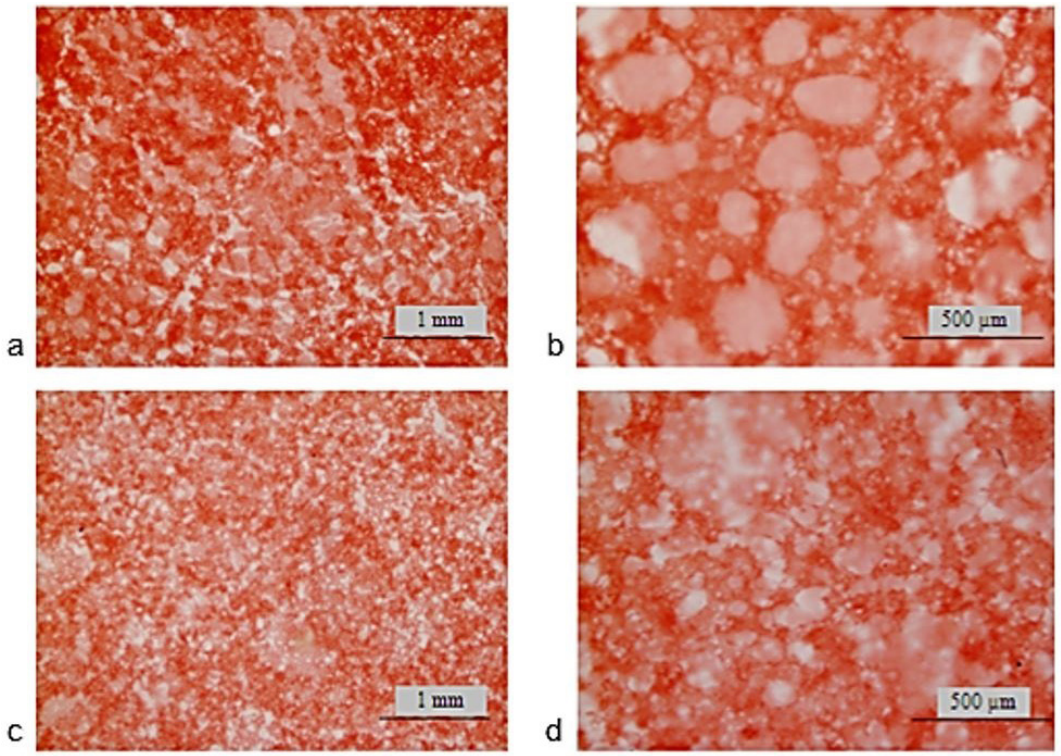

Figure 2. Image Optical Microscopy with Xylidine Ponceau for films: (a, b) zein-buriti oil and (c, d) zein-macadamia oil. The increase indicative bar corresponds 4× (10 µm) and 10× (500 µm).

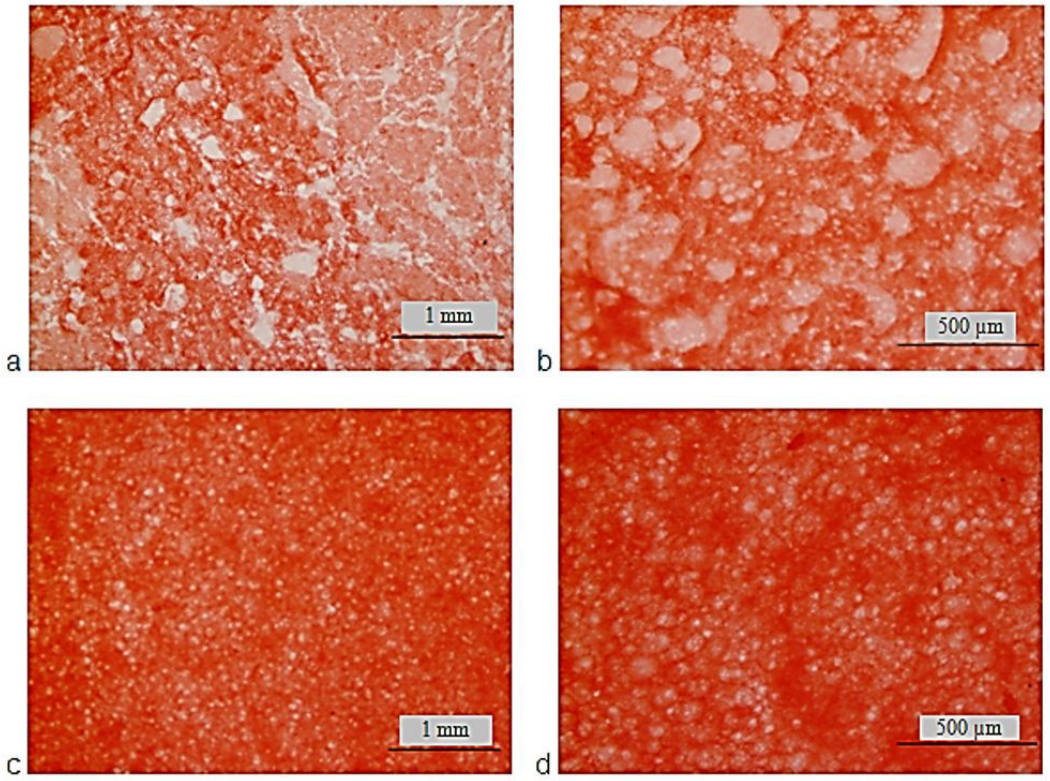

Figure 3. Image Optical Microscopy with Xylidine Ponceau for films: (a, b) zein-olive oil and (c, d) zein-oleic acid. The increase indicative bar corresponds 4× (10 µm) and 10× (500 µm).

least uniform; Buriti oil has the lowest content of oleic acid (Table 1).

The optical microscopy analysis showed that the artifacts identified by SEM and believed to be pores are in fact fat globules in the film matrix. Almeida et al.[36] also observed this in films produced with xanthan gum and zein.

3.5 Differential scanning calorimetry

Thermograms of the films show exothermic and endothermic peaks that characterize transitions or reactions that occurred during analysis, such as the Tg, Tm and crystallization (Tc), among others. Figure 4 shows the DSC curves with the Tm and Tc and Figure 5 shows the expanded region of the DSC curves between 25 °C and 100 °C of the materials studied.

The small peaks in the direction of exothermic heat flow occurring between -55 °C and -35 °C, clearly seen in the films produced with buriti and macadamia oil, are attributed to the crystallization of the residual water (Tc) in the matrices[40]. Moreover, the peaks observed for all materials in the direction of endothermic heat flow, between -30 °C and 3 °C, are explained by the Tm of the oils.

The external factor that changes the Tg and Tm of a film is the presence of plasticizers in the liquid form[41]. By analyzing the composition of the fatty acids, it was found

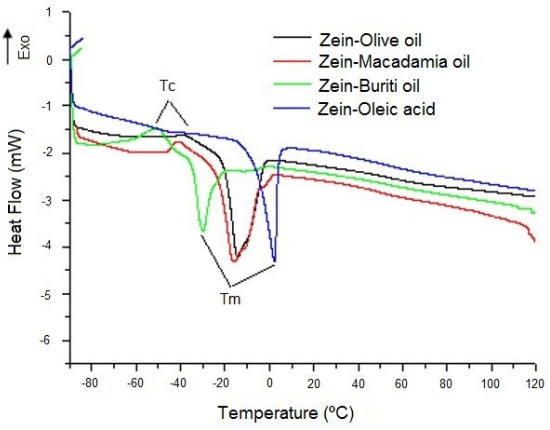

Figure 4. DSC curves of four zein biomaterials in temperature range of -90 to 90 °C.

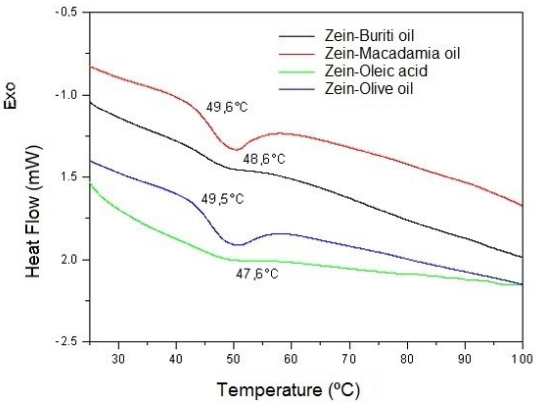

Figure 5. DSC curves showing Tg of zein films.

that buriti oil contained a higher concentration of linoleic acid (53.6%) than oleic acid (35.1%). The Tm of oleic acid is -2.2 °C, while buriti oil has a lower Tm (-29.8 °C). This is due to the lower content of oleic acid present in buriti oil; the film made from this oil has a different chemical structure.

These results confirm observations in literature who reported that oleic acid, which is the major component of the oils studied, presents a solid-solid phase transition of the order-disorder type ($\gamma \rightarrow \alpha$) at -2.2 °C[42].

The Tg of pure zein powder is in the range of 150 to 180 °C; this decreases to 50 °C to 80 °C when fatty acids or polyols are added[43,44]. In this study, the Tg of the zein films were in the range of 47 °C to 50 °C (Figure 5). The lowest value (47.6 °C) was for the zein film made with oleic acid and the highest temperatures were obtained for the films produced from macadamia and olive oil (49.6 °C and 49.5 °C, respectively).

Although there was no significant difference between the temperatures of the films, this result may be due to the purity and content of the oleic acid in the plasticizers as according to Lucas et al.[45], larger amounts of plasticizer will reduce the Tg.

3.6 Thermogravimetric analysis

Thermal stabilities of zein in its native form (powder), zein film without plasticizer and plasticized films were determined by thermogravimetry (TG). The TG curves and the first derivative or derivative thermogravimetry (DTG) for zein powder and the zein film prepared without plasticizers are shown in Figures 6a and 6b, respectively.

The thermal behaviors of zein powder and zein film are quite similar. Initially, weight loss occurs in the temperature range of 25 °C to 120 °C due to the evaporation of water. Between 120 °C and 200 °C, there is a slight weight loss probably because of the evaporation of fatty acids. The main thermal degradation for zein powder and film occurs in the range of 270 °C to 415 °C. These results are in accordance with the literature[43,46]. There is also a slight shift to higher temperatures at the peak of the decomposition curve (DTG) obtained for the zein film compared to the peak of zein powder decomposition, which may be due to structural differences between zein powder and zein film.

Figures 7a and 7b show the TG and DTG curves, respectively, for the plasticized zein films made with different vegetable oils. TG curves for all films showed a weight loss at 50 °C to 120 °C due to water evaporation. The materials also exhibited a weight loss of 3-5% at 120 °C; these values are similar to those obtained by Corradini[47]. Successive weight losses are noted above 120 °C for all materials.

Major thermal degradation of the films occurs in the range of 250 °C to 405 °C. For all materials, the slight weight loss seen in the temperature range from 110 °C to 200 °C is probably due to the evaporation of fatty acids or glycerol because glycerol plasticizer, analyzed in isolation, has a decomposition temperature of around 213 °C[48]. Note that the weight loss started at a lower temperature in the film made with pure oleic acid. These results are similar to those found in the literature for zein powder[43,46,47]. Small changes in the peaks at higher temperatures were also observed. This is

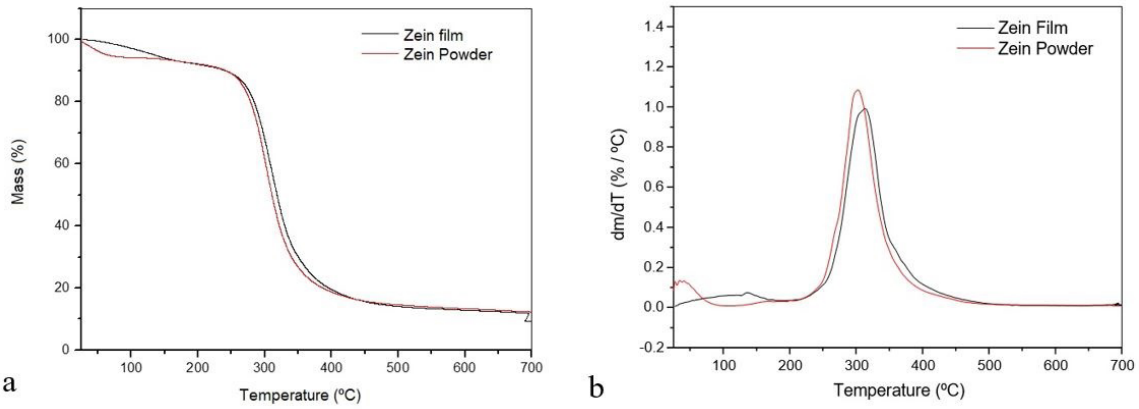

Figure 6. (a) TG curves for zein powder and zein film without plasticizer; (b) Curves of the first derivative (DTG) of Figure 7a.

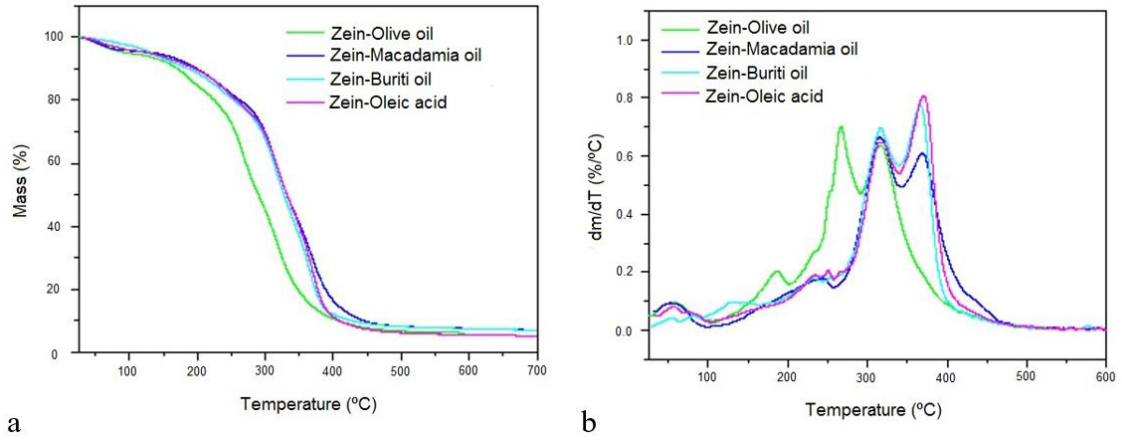

Figure 7. (a) TG curves; (b) DTG curve for zein films with plasticizers.

related to the decomposition curve obtained for zein films produced using buriti, macadamia and olive oil in relation to the peak of the control film decomposition. This result may be due to structural differences between oleic acid and other fatty acid components of the oils.

3.7 Mechanical properties

The mechanical properties of the films were affected by the type of plasticizer added. Table 2 shows the values found for tensile strength (σ_r), elongation at break (ε_r) and Young's modulus (E).

Films made with oleic acid had the highest percentage of elongation (8.08% ± 2.71) which is related to the elasticity of the materials. Similar results were reported[49]. The films made with macadamia oil had the lowest percentage of elongation at break (0.93 ± 0.24%) due to the lower homogeneity of the protein matrix. The percent of elongation decreases when fatty acids are not incorporated into the matrix[50,51]. This tendency is explained by the fact that lipids are unable to form a cohesive and continuous matrix.

The addition of lipids also affects the tensile strength and Young's modulus of the films; these values are the lowest when the concentration of lipids in the matrix is optimized[50,52,53]. Thus, the tensile results indicate that the control film (pure oleic acid) was the most homogeneous as the cohesion between the components of the matrix was the highest.

Table 2. Tensile Strength (σ_r), Elongation at Break (ε_r) and Young's modulus (E) for the zein films.

Film	Tensile strength (σ_r) (MPa)	Elongation at break (ε_r) (%)	Young's modulus (E) (MPa)
zein-oleic acid	0.64 ± 0.27	8.08 ± 2.71	48.6 ± 16.8
zein-buriti oil	1.66 ± 0.29	1.19 ± 0.38	278.28 ± 17.67
zein-macadamia oil	1.30 ± 0.45	0.93 ± 0.24	278.86 ± 28.06
zein-olive oil	1.02 ± 0.26	1.20 ± 0.46	202.59 ± 13.71

4. Conclusions

It was possible to produce biomaterials of zein using edible oils as plasticizers. Although all formulations formed biodegradable films, the film made from pure oleic acid had the best characteristics. Other techniques should be tested, such as ultrasonic frequency during the preparation

of the filmogenic solution, to improve the formation of the matrix of the films and, consequently, improve the thermal and mechanical properties of the films.

5. Acknowledgements

The authors express their sincere thanks to the CAPES – "Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil" for the postgraduate fellowship and EMBRAPA – "Brazilian Agricultural Research Corporation" (São Carlos/Brazil) for analysis support.

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Received: Nov. 30, 2016
Accepted: Apr. 02, 2017