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WATER VAPOR PERMEABILITY, CONTACT ANGLE AND WATER SOLUBILITY OF ZEIN BIOFILMS

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ABSTRACT Zein is a corn protein with a degree of polymerization twice what is needed to produce linear polymers, thus, along with plasticizers, it has been considered to produce biomaterials. To determine the behaviour of these materials it is necessary to know their characteristics. Among those, water vapour permeability (WVP) in films is a factor that interferes with the quality and shelf life of foods because quality and conservation of the product could be affected. Water contact angles are indicators of the surface hydrophobicity of the film and solubility is an important property because some applications require insolubility in water to maintain the integrity of the product. The objectives of this work were to produce zein-based biofilms with three types of edible oils (buriti, macadamia and olive oils) and determine the behaviour of the water interactions of the films and their compositions. As a control, pure oleic-acid zein biofilms were used and WVP, contact angle and water solubility were obtained. The WVP for films with buriti and macadamia oils have the highest values of 0.76 to 0.82 gmm/m2dKPa, respectively but with no statistical differences (p <0.05) between them. Lipid particles dispersed on the surface of the films, observed by optical microscopy, could interfere on these results. The wettability characteristic could not be measured because of instantaneous absorption of the water droplets by those films. The initial value of contact angle for zein-oleic acid was 66.3±4.7° and 33.9±2.1° for zein-olive oil. The solubility of zein-olive oil film was also lower and accounted for 5.04±1.58%. Control films showed the lowest values for WVP, wettability and solubility, which implies that they were more hydrophobic than the others produced with edible oils.

Keywords: zein biofilm, biomaterial, water vapor permeability, wettability, water solubility.

INTRODUCTION Food quality can deteriorate during storage due to losses/gains of moisture and volatile aromas; however, this can be prevented by an adequate selection of food packaging and edible coatings. Research on biobased industrial polymeric materials, driven by agricultural economics and environmental concerns, require more studies, mainly when they are formulated with alternative materials as zein and edible oils. Edible and/or biodegradable films are not meant to totally replace synthetic packing films; however they do have the potential to replace the conventional packaging in some applications (MALI and GROSSMANN, 2003).

Coating composition and morphology play an important role in controlling water affinity. Compositional factors include addition of plasticizers; polymer properties such as saturation degree, presence of lateral chains, polymer cross-linking; and those that involve polymer heterogeneity such as crystalline and amorphous regions (GENNADIOS and WELLER, 1994). Lipid-based films are moisture resistant, but their mechanical properties are inferior to protein, and polysaccharide-based films. Since the 80's years, edible films have been of great interest to the food industry as carriers of nutrients and as biodegradable films (CAO *et al*, 2007). Natural biopolymers, polysaccharides and protein based show the highest potential, mainly because they are renewable, abundant, economic to produce and can form a continuous matrix.

Previous works indicated that zein, a major corn protein, is potentially useful as a biodegradable polymeric material. Its degree of polymerization is twice as much needed to produce linear polymers of polyamide/polyester (SUKLA, 1992). According Cuq *et al.* (1997), in general, the protein films are poor moisture barriers due to the hydrophobic nature of proteins.

Water vapor permeability (WVP) in films is an example of a factor that interferes with the quality and shelf life of foods. Because of the entry of water vapor through the packaging, the quality and conservation of the product could be affected.

Water contacts angles are another indicator of the surface hydrophobicity because show the wettability of a polymer (PÉROVAL *et al.*, 2002). When a drop of liquid is placed on a solid surface, the liquid spread across the surface to form a thin, approximately uniform film or spread to a limited extend but remain as a discrete drop on the surface. The final condition of the wettability of the surface is taken as an indication of the wettability of the surface by the liquid or the wetting ability of the liquid on the surface, depending on one point of view. The wetting process is evaluated by measuring the contact angle (θ) that the edge of the drop makes with the solid (MAYER, 1999).

Solubility in water is another important property related to the functionality of a film that can limit its use. According to Perez-Gago e Krochta (2001) some applications require insolubility in water to maintain the integrity of the product, while others, a total solubility is required, as for example to cook the product with its coating or pack.

The objectives of this work were to produce zein-based biofilms with three types of edible oils with high content of oleic-acid and to determine the water interactions behavior of the films as affected by their compositions. Water vapor permeability (WVP); contact angle (wettability) and water solubility were the characteristics determined.

MATERIAL AND METHODS

Materials Corn zein was acquired from Freeman Industries Inc., Tuckahoe, NY; ethanol 75% (Synth, Brazil) was used as solvent; oleic acid (VETEC, Brazil) or edible oils (macadamia or olive or buriti oil) used as plasticizers; emulsifier (EMUSTAB- Duas Rodas Industrial Ltda, Brazil) as stabilizer; and glycerol (Merck, Brazil) as assistant agent of plasticizer.

Preparation of zein biofilms The films were prepared by dissolving granular zein in 75% ethanol solution, to a concentration of 20% (w/v) at room temperature (~25ºC). Oleic acid or edible oils were added at a ratio of 70 g/100 g zein (w/w), glycerol at a ratio of 30 g/100 g zein (w/w) and emulsifier at a ratio of 5 g/100 g zein (w/w). The mixture was stirred in a water bath at 60 to 65°C for 10 min. Filmogenic solutions were casted on rectangular supports of acrylic plates and maintained at room temperature (25°C) for 48 hours to dry (KLEEN et al., 2002 with adaptations). After drying, the films were peeled out and stored inside desiccator at 58% relative humidity (RH) before analyses. The thickness of films was determined by the arithmetic mean of six values measured in six randomized points of each sample using a digital micrometer with 0.001 mm resolution (Digimess, Brazil). Six random positions around the film were taken for each sample (PEREZ-GAGO and KROCHTA, 2000; HSU et al., 2005).

Biofilms characterization, visual aspect Visual and tactile analyses were carried out in to choose only homogenous film sample for the tests. Samples with absence of insoluble particles, uniform color, without rupture or brittle zones and easy to remove from the support were considered in the experiment. The others samples were discarded.

Water vapor permeability (WVP) It was determined gravimetrically according to the ASTM E96-95 desiccant method (ASTM, 1995). Each film sample was sealed over a circular opening of 9.08cm^2 in a permeation cell and stored at 25ºC inside a desiccator. Silica-gel was used to maintain 0% RH atmosphere inside the cells. Distilled water at the bottom of desiccator maintained 100% RH atmosphere outside the cells. Weight changes of test cells at each 24h over 8 days were measured and the equilibrium was considered when the weight remained constant.

Contact angle (Wettability) Wettability of the biofilms were evaluated as static water contact angle using a contact-angle goniometer (homemade equipment assembled in EMBRAPA/CNPDIA- São Carlos, Brazil). Briefly, a droplet of deionized water (2μL) was put over the surfaces of the material and images were taken at 0, 30, 60, 120 and 300 seconds at constant temperature (25°C) according to SCRAMIM et al., 2007 and WANG et al., 2009. The angle between the baseline of the drop and the tangent at the drop boundary was measured using the Software FTA32 Image Software (First Tem Angstroms). Measurements were performed at six different locations for each sample.

Water Solubility Solubility of biofilms in water was determined according to Gontard *et al.* (1994). Initially, three discs of each film sample with 2.0cm diameter were cut, dried for 24h at 105°C and subsequently weighed. The samples were immersed into 50 mL distilled water and constantly stirred at low speed (72rpm) at 25°C in a shaker (MA-410, Marconi, Brazil). After 24h the samples were removed from the solution and oven dried at 105°C for more 24h. The difference in weight was used to calculate the water soluble matter as a percentage of initial weight.

Statistical analysis Analyses of Variance (ANOVA) was performed considering a randomized experimental design and Tukey tests applied to compare data means at 5% probability using a computational program ESTAT, version 2.0, according to Banzatto and Kronka (2006).

RESULTS AND DISCUSSION

Water vapor permeability (WVP) Table 1 presents mean thickness, WVP and permeance of zein-edible oils films. WVP values were highest for buriti and macadamia oils films and were not statistically different between them. This can be due to the higher thickness of these films compared with the others. However, analyzing the permeance values, that is the permeability divided by the film thickness, the results shown no influence of the thickness because the values are similar and close to observed by Wolf (2007) to collagen films (4.15g.mm/h.m2.kPa).

Table 1. Thickness, WVP and permeance of biofilms at 25°C.

Biofilms	Thickness (mm)	WVP (gmm/m^{2}d.KPa)	Permeance (g/h.m^2.KPa)
Zein + Oleic acid	0.12 ± 0.01	0.51 ± 0.07^b	4.21 ± 0.58^{ab}
Zein + Buriti oil	0.17 ± 0.02	0.82 ± 0.09^a	4.84 ± 0.56^{ab}
Zein + Macadamia oil	0.15 ± 0.05	0.76 ± 0.05^a	5.07 ± 0.32^a
Zein + Olive oil	0.12 ± 0.02	0.45 ± 0.02^b	3.78 ± 0.13^b

^{a,b} – Means followed by the same letter in each column are not different according to Tukey´s test ($p<0.05$)

Lower WVP values of zein-oleic acid and zein-olive oil films were caused by the formation of a more structured and homogeneous matrix, having, therefore, less OH-groups available. According to Li et al. (2008), the WVP depend on the number of free OH-groups on the polymer matrix.

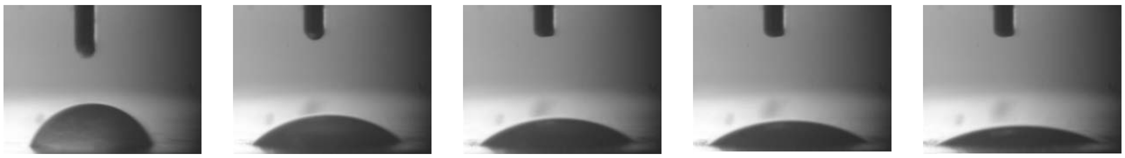
Despite of zein and plasticizer be hydrophobic, the highest WVP in the films with buriti oil or macadamia oil can be explained by the presence of a large number of spherical particles uniformly dispersed in the matrix. This dispersion increases the distance traveled by water molecules diffusing through the film, making easy the migration of moisture by preference channels, so, these films are more permeable to the water vapor (SZTUKA e KOŁODZIEJSKA, 2009; PÉROVAL *et al*., 2002; PERAZ GAGO and KROCHTA, 2001; DEBEAUFORT et al., 2000).

According to Gontard et al. (1994), other possibility of this highest WVP, is that, if lipid components, generally spherical, are not able to associate with the protein chain, the structure of the protein matrix can be broken resulting in wastage of moisture barrier properties. According to these authors, the organization of lipid-protein complex and the interaction between these two components appears to be a critical factor in the permeability of the films. The lipid dispersion was observed in the present study by Scanning Electronic Microscopy (SEM) and Optical Microscopy (OM) analyses showing heterogeneity of the material as fat globules dispersed in the matrix.

Contact angle (Wettability) The hydrophibicity of the materials was illustrated by the kinetics of water droplet absorption determining the contact angle formed on the surface of biofilms (°/second). The pictures showing water absorption along time by zein-oleic acid and zein-olive oil films are presented in Figures 1 and 2. It is important to observe that all films presented similar thickness between 0.12±0.01 and 0.13±0.03mm.

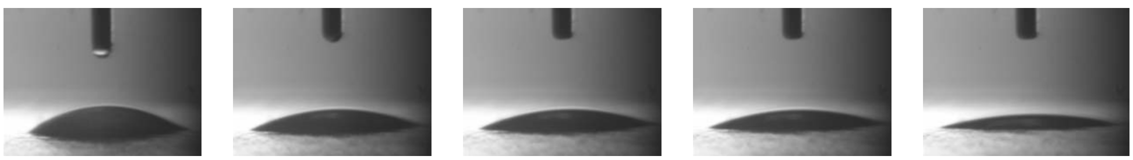
Contact angles for zein-macadamia oil and zein-buriti oil films could not be measured because water droplets were instantaneously absorbed due to the highly hydrophilic nature of these materials. This is consistent with the WVP results where these biofilms showed the highest values, which demonstrates that they are more hydrophilic.

The results for zein-oleic acid and zein-olive oil films (Figs. 1 and 2) show that the initial values of contact angle for zein-oleic acid starts at 66.32 ± 4.7°. The value agrees with result reported by Scramim *et al.*, (2007) and Ghanbarzadeh *et al.* (2007). It turns clear in the pictures a decreasing in the angle as the water is absorbed in the surface of the film from 0 to 300s.



0 second 30 seconds 60 seconds 120 seconds 300 seconds

Figure 1. Contact angle of water droplet over zein-oleic acid biofilm surface during time.



0 second 30 seconds 60 seconds 120 seconds 300 seconds

Figure 2. Contact angle of water droplet over zein-olive oil biofilm surface during time.

For zein-olive oil biofilms, the initial value started at 33.97°±2.1° and also decreases with time (Fig. 2). Comparing Figures 1 and 2 it is evident that zein-olive oil has more water affinity as the angle starts with lower value and decreases rapidly. The contact angle is an indication of the water absorption capacity. Scramim *et al.* (2007) determined the contact angle for zein-oleic acid films and also observed a hydrophilic nature of the films. They found a similar initial contact angle of 68±3° which decreases with time.

According Wang *et al.* (2009), zein is a hydrophobic protein due to a high amount of the hydrophobic amino acids such leucine, proline, alanine and phenylalanine. This is the main reason for zein films present water contact angles over 50°. Scramim *et al.* (2007) reported that same peptides could vary from non-polar to polar when they changed from an air/water interface to a solid/air interface, and the zein protein surface could also restructure or reorient in response to different environments. It means that, zein would orient its polar chemical components toward the interface when the film surface is exposed to an aqueous or highly polar environment if there is sufficient mobility condition. Therefore, zein films should show hydrophilic behavior.

Solubility Table 2 presents solubility in water at 25°C for all film samples.

Table 2. Solubility in water of biofilms at 25°C.

Biofilms	Solubility (%)
Zein + Oleic acid	5.04 ± 1.58[c]
Zein + Macadamia oil	14.34 ± 4.19[b]
Zein + Olive oil	23.19 ± 2.85[a]
Zein + Buriti oil	17.25 ± 3.78[ab]

[a,b,c] – Means followed by the same letter in each column are not different according to Tukey´s test ($p<0.05$)

All films samples used for tests presented similar thickness from 0.13±0.01 to 0.14±0.03mm and presented no injuries after 24h when they were removed from the solution. It means that protein chain remained intact after the test.

Only sample film of zein-oleic acid shown different result ($p<0.05$) from the others films (Table 2). It had the lowest solubility in water (5.04 ± 1.58%) showing that it is the more hydrophobic than the others. The less hydrophobicity of the others films can be because of plasticizers used contain fat heterogeneity which facilitates the interaction among their groups.

CONCLUSION It was possible to produce biofilms composed by zein-oleic acid and zein with three types of edible oils as plasticizers. All films selected for characterization shown homogeneous visual aspect, uniform thickness and good ductility. Biofilms prepared with pure oleic acid had the lowest values of WVP, contact angle (wettability) and solubility, therefore, are more hydrophobic than the others produced with edible oils: macadamia, buriti, and olive. Plasticization with macadamia oil and buriti oil increased WVP, contact angle and solubility of the zein based films. Thus, the choice of plasticizers is a major factor in determining water adsorption of zein films because water interactions in zein products are affected by composition, structure and morphology of materials.

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