

Development of Biodegradable Films of Cassava Starch and Poly (Butylene Adipate Co-Terephthalate): Effects of Oregano Essential Oil and Potassium Sorbate in Films Characterization

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Four samples of thermoplastic starch (STP) and poly (butylene adipate co-terephthalate) (PBAT) films added with the preservatives oregano essential oil (OEO) and potassium sorbate (PS), were prepared from the extrusion blow moulding technique in order to evaluate the mechanical properties, water vapour permeability (WVP) and microstructural characteristics so as to develop active films with antimicrobial activity. The samples FC (film without preservative) and F1 (1% OEO) showed higher water sorption than the samples F2 (0.5% OEO and 2.5% PS) and F3 (5% PS) essentially in relative humidity (RH) higher than the 60%. Samples water vapour permeability containing OEO and PS, showed no significant difference from FC. However, the presence of OEO in samples F1 and F3 decreased the solubility coefficient (β) in 99.5% and 93.8% respectively, compared to FC. The presence of PS in sample F2 increased the diffusion coefficient (D_w) in 4.7 times related to sample FC, so the presence of small molecule PS gave higher porosity to the film. The sample F1 showed an increase in tension at rupture (TR), elongation (E) and elastic modulus (EM) when compared to FC, while F2 and F3 samples revealed a reduction in TR and EM, and increased E. From Scanning Electronic Microscopy, it was possible to observe greater uniformity in FC and F1 compared to F2 and F3 samples. Thus, OEO presence contributes to improve the polymer mechanical properties and the increased homogenization. As well as, the presence of PS contributed to the film higher elasticity, less resistance and less homogeneity. Therefore, the presence of OEO in STP and PBAT films gives the best feature for the development of active films related to the mechanical properties, microstructural and of barriers.

1. Introduction

Plastic packaging is widely used because its chemically and mechanically resistant characteristics, thermally weldable, printability and large quantities availability at low cost (Souza et al., 2013). However, the fact that these materials are not biodegradable and highly consumed, make them an environmental impact problem.

Searching for alternatives to reduce environmental pollution, it is noticed an interest in the use of biodegradable polymers. Synthetic polymers are derived from non-renewable resources such as petroleum, while natural polymers, polysaccharides and proteins, are annually available in large quantities from renewable sources (Zullo & Iannace, 2009; Souza et al., 2013). Starch transformation into thermoplastic starch, i.e. into flexible material, involves heating and/or shearing together with a plasticizer, being glycerol commonly used. However, the major challenge in biodegradable films use is its low mechanical resistance and hygroscopicity (Mali et al., 2005).

Materials produced from thermoplastic starch blends with other biodegradable polymers have become an option for the development of lower cost biodegradable packages with better mechanical and barrier properties (Brandelero et al., 2012; Ren et al., 2009). Poly (butylene adipate co-terephthalate) (PBAT) is a biodegradable polyester, derived from petroleum, which has mechanical properties like those of polyethylene films, is resistant to grease, moisture and temperature variations (BASF, 2013).

Packaging, besides being applied as a means of transport and foods protection, may also interact with the product and the environment that surrounds it, minimizing, for example, microbiological contamination risks. In most solid or semi-solid foods, microbial growth occurs primarily on the surface and, thus, preservatives are mixed directly with the product and may result in overuse. The active antimicrobial packaging concept arises in response to consumers' demand for healthy food, without preservatives, or as an alternative to reduce the food preservatives intakes maintaining product quality (Mezule and Juhna, 2016; Kuplennik, 2015).

Among the antimicrobials employed in food pellicule and active films are the essential oils (EO), being aromatic oily liquids obtained from plants and are generally mixtures of various components. Inherent aroma and antimicrobial activity of EO are usually related to its components chemical structure, the concentration at which the components are present, and the interactions between them that affect bioactive properties. Some studies reported that essential oils have higher antibacterial activity than their separately employed components. Within the wide range of EO, oregano essential oil (OEO) extracted from *Origanum vulgare* is well known for its antioxidant and antimicrobial activity (Botsoglou et al., 2003). Carvacrol, thymol, γ -terpinene and p-cymene are the most OEO active constituents, with a wide spectrum of antimicrobial and antioxidant properties (Lambert et al., 2001; Rocha-Guzman et al., 2007).

Low-cost traditional commercial food preservatives with wide use in the food industry and high thermal stability, become attractive antimicrobial agents for active packaging. Among them, it includes potassium sorbates, widely used to inhibit the development of filamentous fungi, yeasts and some bacterial strains in different foods, including cheeses, breads, fruits, vegetables, compotes and certain meats (Kuplennik et al., 2015).

The aim of this work was to develop and characterize active biodegradable films made by extrusion and blowing from cassava starch, poly (butylene adipate co-terephthalate) (PBAT), glycerol, oregano essential oil (OEO) and potassium sorbate (PS), evaluating the effects of oregano and potassium sorbate on the mechanical properties, water vapor permeability and films microstructural characteristics.

2. Material and Methodology

2.1 Material

For films preparation, the following materials were used: native cassava starch (Indemil Com. Ind. Ltda, Brazil), glycerol (Synth PA, Brazil), poly ((butylene adipate co-terephthalate) (Ecoflex®, BASF Chemical Company, Brazil), potassium sorbate, oregano essential oil (*Origanum vulgare* - Fermiquímica, Ind. And Com. Ltda, Brazil), whose composition according to the supplier corresponds to 71% carvacrol, 3% thymol, 4.5% γ -terpinene, 3.5% p-cymene and 4% beta-caryophyllene.

2.2 Biodegradable films production

Films formulations containing starch, PBAT, glycerol, potassium sorbate and oregano essential oil were prepared by keeping constant the ratio PBAT and glycerol 40/13 (w/w) and varying starch and preservatives proportions (Table 1).

Table 1. Biodegradable films formulation with oregano essential oil and potassium sorbate as preservatives.

Films	Films componentes (w/w/w/w)			
	PBAT + Glycerol	Starch	Oregano essential oil	Potassium sorbate
FC	53	47	-	-
F1	53	46	1	-
F2	53	42	-	5
F3	53	44	0.5	2.5

Films was produced at Technology Laboratory, Department of Food Science and Technology, State University of Londrina (UEL - Brazil). Preservative compounds were mixed in glycerol. Initially the pellets were produced in a twin-screw extruder (BGM, model D-20, Brazil), equipped with screws 20 mm in diameter and 680 mm in length. Screw speed was set at 100 rpm and the temperature profile in the 5 heating zones was 90/120/120/120/120 °C. Films were produced in a single-screw pilot extruder (BGM, model EL-25, Brazil),

equipped with a 250 mm diameter screw. The speed was maintained at 35 rpm, and the temperature profile in the 4 heating zones and in the balloon forming plate was 90/120/120/130/130 °C.

2.3 Films characterization

2.3.1 Thickness and density

Films thickness was determined using a digital micrometer (0.001 mm resolution, Mitutoyo, Japan). Twelve random points of each film area were evaluated. For the density, the mean of 10 square samples (25 mm x 25 mm), previously conditioned in a desiccator with anhydrous calcium chloride for 20 days, was calculated.

2.3.2. Water vapour permeability (WVP)

Permeability of the films was carried out according to ASTM E 96-95 (ASTM, 1995).

2.3.3. Sorption isotherms

Sorption isotherms of the films were determined according to the methodology described by Fidelis et al. (2015) using the following equilibrium relative humidity at 25 °C: 11.3%, 33%, 43.2%, 52.9%, 64.5%, 75.3%, 84.3% and 90.2%.

The isotherms were modeled by the Guggenheim-Anderson-de-Boer (GAB) model (Eq.1) using Quasi-Newton method of Statistica 10.0 software.

$$X_w = \left(\frac{m_0 \cdot C \cdot K \cdot a_w}{[(1 - K \cdot a_w) \cdot (1 - K \cdot a + C \cdot K \cdot a_w)]} \right) \quad (1)$$

Where: X_w = equilibrium relative humidity (g water / g dry matter), m_0 = water content in the monolayer (g water / g solids), a_w = water activity, C and K = constant GAB model representing respectively sorption heat in the first layer and the sorption heat of the multilayers.

2.3.4. Coefficients of solubility (β) and diffusion (D_w)

The method used to calculate the solubility coefficient (β) was proposed by Larotonda et al. (2005) from the first order derivative of the GAB model. This model is correlated with the water activity divided by the water vapor pressure (P_s) at 25 °C according to Equation 2 (Eq. 2).

$$\beta = \frac{CKm_0}{P_s} \left[\frac{1}{(1 - ka_w)(1 - ka_w + Cka_w)} - \frac{a_w}{[(1 - Ka_w)(1 - ka_w + Cka_w)]^2} [-k(1 - ka_w + Cka_w) + (1 - ka_w)(-k + Ck)] \right] \quad (2)$$

Where: β = solubility coefficient, C, k, and m_0 = parameters of the GAB model, P_s = water vapor pressure at 25° C. The a_w used was the mean of the relative humidity gradient used in the WVP (item 2.3.2).

Water vapor diffusion coefficient (D_w) was calculated according to Equation 3 (Eq.3), using the values of β and WVP determined in 2.3.2 item, and where ρ is the film density.

$$D_w = \frac{PVA}{\rho \cdot \beta} \quad (3)$$

2.3.5. Mechanical properties

Mechanical properties (maximum tension at rupture, flow elongation and elastic modulus) were evaluated according to ASTM D 882-91 (ASTM, 1996) using texturometer (Stable Micro Systems, model TA.TX2i, England), with distance between the jaws of 30 mm and a traction speed of 500 mm/min. Samples were cut (70 mm long and 6 mm wide) and conditioned under a relative humidity of 64.5% at 25 °C for 7 days prior to analysis. For each formulation, 10 replicates were performed.

2.3.6 Microstructural evaluation

Evaluations was performed using scanning electron microscope (Shimadzu SS-550N, Japan) based on the methodology described by Fidelis et al (2015).

3. Results and Discussion

The blends STP / PBAT / OEO / PS were capable of producing films by pilot scale blowing extrusion which continuously flowed through the extruder barrel under conditions set for the process.

3.1 Sorption isotherms

All the films presented water sorption practically constant up to 60% RH and, above 60%, there was an sorption increment (Figure 1) due to the hydrophilic character of the starch. According to Talja et al. (2008), in relative humidities superior 60%, substitution of the interactions starch-starch and starch-glycerol for starch-water and water-glycerol may occur, justifying the water sorption increase from such moisture.

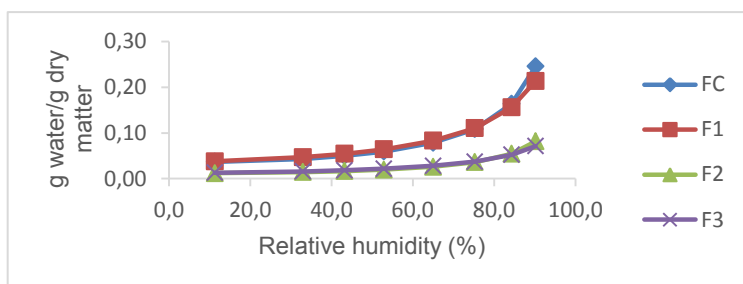


Figure 1: Water sorption isotherms of biodegradable films at 25 °C

Sample F1 film, 1% OEO, showed a very similar behavior to control, in other words, the oil presence practically did not interfere in blends hydrophilicity when compared to FC. Although, those contain potassium sorbates in their composition presented more hydrophobic character. Sorption behavior changes caused by PS presence, may be related to potassium sorbates ability to form complexes of inclusions with the carbon hydrate reducing interactions between polymers networks that compound the film. This may be correlated to F2 film lower value of m_0 (Table 2) as this is the one containing the lowest PS percentage. These complexes formation may hinder the water molecule interaction with the monolayer sorption sites (Ofmana et al., 2004). Data were modeled by the GAB model (Eq.1), adjusting satisfactorily, since the coefficients of determination (R^2) ranged from 0.96 to 0.98 (Table 2). The value of C, Guggenheim constant, is related to the enthalpy difference between the monolayer and the multilayers, for this issue, all samples presented similar behavior. In relation to K values, multilayers water sorption heat measurement, F2 film was the closest to FC, however, all values were close to the ones reported by authors that studied cassava STP films (Chang et al., 2006; Fidelis et al., 2015; Mali et al., 2005).

Table 2. GAB equation parameters at 25 °C of biodegradable films.

GAB model parameters	Film formulation			
	FC	F1	F2	F3
m_0	0.016	0.049	0.006	0.014
C	0.98	0.99	0.99	0.99
K	0.57	1.54	0.67	1.27
R^2	0.96	0.98	0.96	0.98

3.2. Water vapour permeability (WVP), solubility coefficient (β), diffusion coefficient (Dw), thickness (δ) and density (ρ)

One of food package main functionality is to avoid or, at least, reduce the moisture transfer between the food and surrounding atmosphere, water vapor permeability (WVP) should be as low as possible (Shen et al., 2010).

Table 3. Water vapour permeability (WVP), thickness (δ), density (ρ), solubility coefficient (β) and diffusion coefficient (Dw) of biodegradable films.

Formulations	WVP ($\times 10^{-6}$) (g/m.Pa.day)	δ ($\times 10^{-2}$) (mm)	ρ ($\times 10^6$) (g/m ³)	β ($\times 10^{-6}$) (g/g Pa)	D ^w ($\times 10^{-7}$) (m ² /day)
FC	1.35 \pm 0.14 ^{ab}	1.83	2.05	5.69	1.16
F1	0.51 \pm 0.01 ^b	1.02	1.33	0.30	1.30
F2	2.44 \pm 0.28 ^a	8.64	1.56	2.88	5.42
F3	2.46 \pm 0.42 ^a	7.99	1.62	0.35	0.44

Same lower case letters signify samples in a column that do not differ based on Tukey's test ($p < 0.05$).

Films with preservatives did not present significant difference in relation to the control. As for WVP (Table 3) all films presented similar magnitude to other biodegradable films (Fidelis et al., 2015; Jouki et al., 2014; Souza et al., 2013). However, F1 (1% OEO) presented lower permeability than PS content films.

Films mass transfer was evaluated based on the solubility coefficient (β) and the diffusion coefficient (Dw). The first relates the water affinity of the material, while the second relates the water molecules permeation through the polymeric material. The OEO presence reduced F1 solubility coefficient by 94.7% and F3 by 93.8% in relation to FC, this fact is due to some components hydrophobic character that compose it. As for the

diffusion coefficient, it can be seen an expressive increase in F2, which presented a D_w 4.7 times greater than the FC.

3.3 Mechanical properties

Regarding mechanical properties (Figure 2), films were evaluated at 64.5% relative humidity. It was apparent the significant influence of preservatives on the films mechanical properties.

Sample F1, containing 1% OEO, compared to FC obtained higher tension at rupture, an increase of 85.2%, greater elongation, an increase of 273.1% and larger elastic modulus, an increase of 92.8%. As for F2 and F3 films, which contained potassium sorbates in its composition, similar behaviours were observed, both when compared to FC, showed a reduction in tension at rupture of 58.6% and 61.6%, an increase in elongation of 362.1% and 446.9% and elastic modulus reduction of 63.1% and 73.8% respectively.

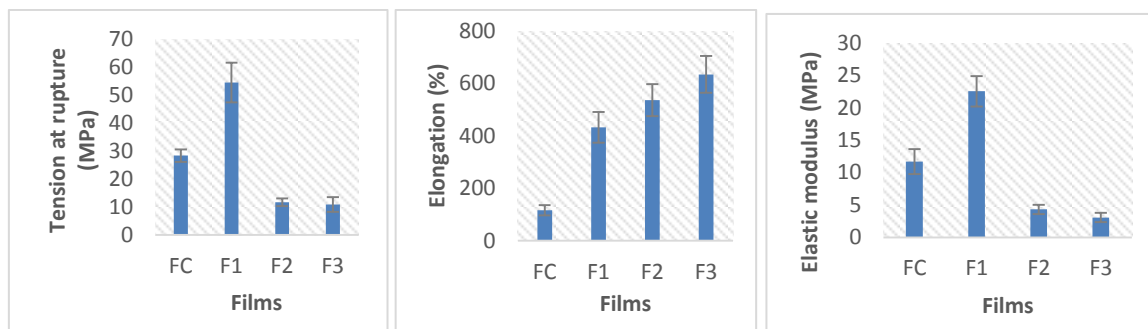


Figure 2. Tension at rupture, elongation and elastic modulus analysis for biodegradable films.

Researches that studied potassium sorbates incorporation in biodegradable starch films reported similar results to this study, attributing the fact to the film crystallinity reduction by the presence of the potassium sorbates molecule that promotes alteration of the starch network making them less resistant and rigid, yet more flexible (Fama et al., 2005; Flores et al., 2007; Shen et al., 2010).

Studies that applied OEO in films obtained similar results regarding the elongation behaviour. The oil presence increased flexibility in relation to sample control. As to rupture stress and elastic modulus, films presented opposite behaviour, i.e., these studies obtained resistance and stiffness reduction with OEO, whereas the results found in the present work, show that oil presence promoted greater resistance to the film (Jouki et al., 2014; Wu et al., 2014). This behaviour may have been triggered by crosslinking reactions between thermoplastic starch and PBAT with OEO.

3.4 Microstructure

In the micrographies of the films surface (not showed), no evident pores were detected and some granules of non-gelatinized starch could be detected in FC and F1. However, the films FC and F1 presented more homogeneous characteristics, no phase separation, showing good interaction between the components.

4. Conclusion

Biodegradable starch / PBAT / glycerol films containing antimicrobial agents (oregano essential oil and potassium sorbate) have good mechanical and barrier properties to the water vapor and can be tested as active food packaging. The oregano essential oil and potassium sorbate influence the mechanical and water vapor barrier properties of the films. Apparently, the potassium sorbate acts as a plasticizer, reducing mechanical resistance and increasing both the water vapor permeability as well as the solubility and diffusion coefficient of the water. On the other hand, the components of the oregano essential oil improve the interaction between the starch and the polyester, making the films more resistant and rigid.

Acknowledgments

The authors are grateful to the COMCAP/UEM and Fundação Araucária for funding the research.

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