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Cassava starch biodegradable films: Influence of glycerol and clay nanoparticles content on tensile and barrier properties and glass transition temperature

Cassava starch biodegradable films: Influence of glycerol and clay nanoparticles content on tensile and barrier properties and glass transition temperature

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ABSTRACT

In this study, glycerol content and its incorporation method on tensile and barrier properties of biodegradable films (BF) based on cassava starch were analyzed. ANOVA showed that the glycerol incorporation method did not influence the results ($P > 0.05$), however the glycerol content influenced significantly the tensile and barrier properties of the films ($P < 0.05$). Films prepared with lower glycerol content presented better tensile and barrier properties than films with higher content. Films were then prepared with addition of clay nanoparticles and their tensile and barrier properties and glass transition temperature were measured. ANOVA indicated that both glycerol and clay nanoparticles influenced significantly the tensile and barrier properties ($P < 0.05$), diminishing film permeability when clay nanoparticles were present, while the glass transition temperature was not influenced ($P > 0.05$).

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1. Introduction

Over the last decades, the use of polymers as food packaging materials has increased considerably due to their advantages over other traditional materials such as glass or tinplate. A great advantage of plastics is the large variety of materials and compositions available, so the most convenient packaging design can be adapted to the very specific needs of each product (López-Rubio et al., 2004).

However, it is widely accepted that the use of long-lasting polymers for short-lived applications (packaging, catering, surgery, hygiene) is not entirely adequate (Avérous, 2004). A huge amount of garbage is generated daily, in which food packaging plays a considerable part. This waste is composed of many different types of material, some of which is not biodegradable and will remain without decomposing for hundreds, sometimes thousands of years. In this context, the development of biodegradable films (BF) for packaging materials that can be used as a substitute for petrochemical polymers is an interesting perspective, since it provides an alternative to non-degradable products, and increases income in the agricultural sector (Souza, Ditchfield, & Tadini, 2010).

Starch has been considered as one of the most promising candidates for the future primarily because of an attractive combination of its large availability and relatively low price (Chivrac, Angellier-Coussy, Guillard, Pollet, & Avérous, 2010).

Cassava starch has been extensively used to produce BF (Alves, Mali, Beléia, & Grossmann, 2007; Chen & Lai, 2008; Chillo et al., 2008; Famá, Flores, Gerschenson, & Goyanes, 2006; Famá, Goyanes, & Gerschenson, 2007; Flores, Famá, Rojas, Goyanes, & Gerschenson, 2007; Henrique, Cereda, & Sarmento, 2008; Kechichian, Ditchfield, Veiga-Santos, & Tadini, 2010; Mali, Grossmann, García, Martino, & Zaritsky, 2006; Müller, Yamashita, & Laurindo, 2008; Paes, Yakimets, & Mitchell, 2008; Parra, Tadini, Ponce, & Lugão, 2004; Shimazu, Mali, & Grossmann, 2007; Teixeira, da Róz, Carvalho, & Curvelo, 2007; The, Debeaufort, Voilley, & Luu, 2009; Veiga-Santos, Oliveira, Cereda, Alves & Scamparini, 2005; Veiga-Santos, Suzuki, Cereda & Scamparini, 2005; Veiga-Santos, Suzuki, Nery, Cereda, & Scamparini, 2008) and the results indicated that these carbohydrates are promising materials in this regard (Müller et al., 2008). Films developed from starch are described as isotropic, odorless, tasteless, colorless, non-toxic and biologically degradable (Flores et al., 2007).

Unfortunately, there are some strong limitations for developing starch based products, since they present poor tensile properties and high water vapor permeability when compared to conventional films derived from crude oil (Souza et al., 2010) on account of their hydrophilic nature and their sensitivity to moisture content, a factor that is difficult to control (Wilhelm, Sierakowski, Souza, & Wypych, 2003).

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Usually, the second major component of a starch based film is the plasticizer, which is used to overcome film brittleness caused by high intermolecular forces. Plasticizing agents commonly used for thermoplastic starch production include water and glycerol (Alves et al., 2007; Famá et al., 2006; Famá et al., 2007; Jangehud & Chinnan, 1999; Mali et al., 2006; Parra et al., 2004), polyethylene glycol (Parra et al., 2004) and other polyols, such as sorbitol, mannitol and sugars (Kechichian et al., 2010; Talja, Helén, Roos, & Jouppila, 2008; Veiga-Santos et al., 2008).

Some authors consider that the glycerol, a polyalcohol found naturally in a combined form as glycerides in animal and vegetable fats and oils, is the best plasticizer for water soluble polymers (Bertuzzi, Castro Vidaurre, Armanda, & Gottifredi, 2007; Jangehud & Chinnan, 1999; Müller et al., 2008). The hydroxyl groups present in glycerol are responsible for inter and intramolecular interactions (hydrogen bonds) in polymeric chains, providing films with a more flexible structure and adjusting them to the packaging production process (Souza et al., 2010).

Sucrose, which is a non-toxic, edible and low cost biodegradable raw material, has shown a higher plasticizing efficiency when compared to sorbitol and glycerol. However, evidence of sucrose crystallization during storage was reported. Some authors have demonstrated the possibility of substituting sucrose by inverted sugar that has a lower tendency to crystallize, increasing film-forming suspension viscosity, making it more difficult for crystals to form (Veiga-Santos et al., 2008).

In this way, the association of cassava starch with plasticizers as glycerol, sucrose, and inverted sugar can promote alterations in the films, justifying the study of these additives to develop a potential and ecological alternative to the synthetic packaging of several food products (Parra et al., 2004).

To overcome high permeability caused by the plasticizer, other additives are used. In this area, the production of bionanocomposites has proven to be a promising option, since polymer composites are increasingly gaining importance as substitute materials due to their superior tensile properties, making them especially suited for transportation and packaging applications.

Mineral clays are technologically important and are mainly composed of hydrated aluminosilicate with neutral or negative charged layers (Wilhelm et al., 2003). Clay is a potential filler; itself a naturally abundant mineral that is toxin-free and can be used as one of the components for food, medical, cosmetic and healthcare products (Chen & Evans, 2005). Moreover, clay is environmentally friendly and inexpensive.

Clay/starch composites have been the most frequently studied, demonstrating a potential for improvement of tensile strength, Young's modulus, water resistance and decrease of the water vapor permeability of starches from many different sources (Avella et al., 2005; Chiou et al., 2007; Cyras, Manfredi, Ton-That, & Vásquez, 2008; Kampeerapappun, Aht-Ong, Pentrakoon, & Srikulkit, 2007; McGlashan & Halley, 2003; Tang et al., 2008). A decrease in water vapor permeability of up to 70% in relationship to pure starch films (corn, wheat, potato, waxy corn and high amylose corn starch) has been reported (Tang et al., 2008). An increase in Young's modulus of up to 500% has also been observed for potato starch/montmorillonite composites (Cyras et al., 2008).

In this paper, the influence of glycerol and nanoclay particles on tensile (tensile strength and percent elongation at break), barrier properties (water vapor permeability and oxygen permeability coefficient) and glass transition temperature of BF based on cassava starch was studied. In the first phase, sucrose, inverted sugar, different glycerol contents and two methods of glycerol incorporation were tested. In the second phase, the effects of different contents of glycerol and clay nanoparticles were evaluated. X-ray diffraction analyses were performed to evaluate the hypothesis of glycerol and starch intercalation into the clay galleries.

2. Materials and methods

2.1. Materials

Native cassava starch, kindly supplied by Cargill Agrícola, Brazil (amylose: 19.7 g/100 g; amylopectin: 80.3 g/100 g; moisture: 12.5 g/100 g) was used as the film-forming component to provide a continuous biodegradable film matrix. Glycerol (Synth, Brazil), liquid inverted sugar from Copersucar, Brazil (inversion: 65 g/100 g) and commercial sucrose from Guarani, Brazil (moisture: 0.2 g/100 g max.) were added to improve their flexibility. Natural -Na montmorillonite clay (commercial product Argel T, used as received, without purification, Bentonit União, Brazil) was used as filler. Distilled water and ethanol (Synth, Brazil) were used as solvents for the filmogenic solutions.

2.2. Film preparation

In the first phase, the filmogenic solution was prepared by dissolving 5.0 g of starch, 0.7 g of sucrose, 1.4 g of inverted sugar, glycerol at different contents ((0.0, 0.17, 0.34, 0.50 and 0.75) g), and distilled water in order to complete 100 g of solution. Glycerol contents were based on preliminary tests. The glycerol incorporation was tested by two different methods.

In the first method, the filmogenic solution was prepared by a simple mixture of all components (cassava starch, glycerol, sucrose, inverted sugar and distilled water) at ambient temperature. Then, this solution was heated in a domestic microwave oven until starch gelatinization which occurred at $(69 \pm 2)\,°C$. According to the casting technique, for each formulation, a specific content of filmogenic solution was poured onto cylindrical acrylic plates ($154\ cm^2$ of area) to obtain a constant thickness of $(100 \pm 10)\ \mu m$, followed by drying at $(35 \pm 2)\,°C$ for approximately 16 h, in an oven with forced air circulation (Nova Ética, series N480, Brazil).

In the second method, glycerol and cassava starch were dried in the oven at $(170 \pm 2)\ °C$ for 45 min and occasionally stirred, allowing diffusion of glycerol into the starch granule. After cooling at ambient temperature, sucrose, inverted sugar and distilled water were added and the film preparation followed the same procedure as the first method.

In the second phase of the work, the filmogenic solution was prepared in three steps: first, clay nanoparticles were suspended in distilled water for 1 h, and, after a rest period of 24 h, they were blended with a suspension of 5.0 g of starch, glycerol, and distilled water in order to complete 100 g of solution. The quantities of clay nanoparticles and glycerol were varied from (0.0 to 0.1) g and from (0.75 to 1.25) g, respectively, yielding a total of 6 different formulations elaborated, according to Table 1. After homogenization, this solution was heated in a domestic microwave oven until starch gelatinization, which occurs at $(69 \pm 2)\ °C$. After cooling, this solution was diluted with 14.25 g of ethanol, and, poured onto cylindrical plates and dried at $(35 \pm 2)\,°C$ for $(18–24)$ h, in the same oven with forced air circulation.

After drying, all films (produced in both phases) were stored at a controlled relative humidity of 75% for one week prior to testing. Since starch films have a hydrophilic character, high moisture ambient was chosen in order to evaluate film performance in a typical tropical weather condition (Veiga-Santos et al., 2008).

2.3. Film characterization and tensile properties

The physical appearance of the films was inspected visually and by touch.

Table 1
Glycerol and clay nanoparticles contents used for production of biodegradable films based on cassava starch at the second phase.

	Coded values		Real values[a]	
	Glycerol	Clay nanoparticles	Glycerol	Clay nanoparticles
A	−1	−1	0.75	0.00
B	−1	0	0.75	0.05
C	−1	1	0.75	0.10
D	1	−1	1.25	0.00
E	1	0	1.25	0.05
F	1	1	1.25	0.10

[a] g/100 g of filmogenic solution.

The thickness (t) [mm] was measured using a flat parallel surface micrometer (MITUTOYO SulAmericana Ltda., model 103-137, Brazil, precision 0.002 mm), at five random positions.

Tensile strength (TS) [MPa] and percent elongation at break (E) [%] were evaluated by a tensile test performed on a texture analyzer (TA.XT2i − Stable Micro Systems, UK) using the A/TGT self-tightening roller grips fixture, according to ASTM D882-09 (2009). Filmstrips (130 mm × 25 mm) were cut from each pre-conditioned sample and mounted between the grips of the equipment. Initial grip separation and test speed were set to 50 mm and 0.8 mm s^{-1}, respectively. Tensile strength (nominal) was calculated dividing the maximum load by the original minimum cross-sectional area of the specimen (related to minimum thickness). Percent elongation at break (nominal) was calculated by dividing the extension at the specimen break point by its initial gage length and multiplying by 100. All specimens were evaluated in triplicate.

2.4. Barrier properties

Water vapor transmission (WVT) was determined by a gravimetric method based on ASTM E96/E96M-05 (2005), using the Desiccant Method. This property was reported as water vapor permeability (WVP) that is the rate of water vapor transmission (WVT) through a unit area of flat material of unit thickness induced by unit vapor pressure difference between two surfaces, under specified humidity condition of 75%. Each film sample was sealed with paraffin over a circular opening of 44 cm^2 at the permeation cell (PVA/4, REGMED, Brazil) that was stored, at room temperature, in a desiccator. To maintain 75% of relative humidity (RH) gradient across the film, silica gel was placed inside the cell and a sodium chloride saturated solution (75% RH) was used in the desiccator. Two cells without silica gel were prepared and submitted to the same conditions to account for weight changes occurring in the film, since it is a hydrophilic material. The RH inside the cell was always lower than the outside, and water vapor transport was determined from the weight gain of the permeation cell. After steady state conditions were reached (about 2 h), ten weight measurements were made over 48 h. WVP was calculated according to Equation (1):

$$WVP = (w/\theta) \times [(24 \times t)/(A \times \Delta p)] \tag{1}$$

wherein:
WVP is the water vapor permeability [g mm m^{-2} d^{-1} kPa^{-1}]; w is the weight gain (from the straight line) [g]; θ is the time during which w occurred [h]; t is the average film thickness [mm]; A is the test area (cell top area) [m^2] and Δp is the vapor pressure difference [kPa]. All specimens were evaluated in triplicate.

Oxygen transmission rate (OTR) [cm^3 m^{-2} d^{-1}] of the films was measured at 23 °C and 75% RH on a 50 cm^2 circular films using an oxygen permeation system (OXTRAN 2/21, MOCON, USA), in accordance with ASTM F1927-07 (2007). A starch based film was sealed between two chambers (each one with two channels), the lower one supplied with O$_2$ at a controlled flow rate (20 mL min^{-1}) to keep the pressure constant in that compartment, and the other one was purged by a stream of nitrogen carrier gas (0.98 part of nitrogen and 0.02 part of hydrogen), at controlled flow rate (10 mL min^{-1}). A colorimetric sensor determined the amount of oxygen transmitted through the film into the carrier gas. The oxygen transmission rate was determined for all specimens in duplicate. The permeance (PO_2) of the films was calculated according to Equation (2):

$$PO_2 = OTR/p \tag{2}$$

wherein:
PO_2 is the permeance of the films [cm^3 m^{-2} d^{-1} Pa^{-1}]; OTR is the oxygen transmission rate [cm^3 m^{-2} d^{-1}]; and p is the partial pressure of oxygen, which is the mol fraction of oxygen multiplied by the total pressure (nominally, one atmosphere), in the test gas side of the diffusion cell. The partial pressure of O$_2$ on the carrier gas side is considered zero.

The oxygen permeability coefficient ($P'O_2$) was calculated as follows:

$$P'O_2 = PO_2 \times t \tag{3}$$

wherein:
$P'O_2$ is the oxygen permeability coefficient [cm^3 m^{-1} d^{-1} Pa^{-1}]; and t is the average thickness of the specimen [mm].

2.5. Glass transition temperature

Glass transition temperature (T_g) of BF was determined by differential scanning calorimetry, using a DSC TA 2010 controlled by a TA 4000 module (TA Instruments, New Castle, USA), with a quench cooling accessory, operated with nitrogen at 150 mL min^{-1}. Temperature and melting enthalpy calibrations were performed with indium and bidistilled water and Milli-Q. Samples of about (2–5) mg were weighed in a precision balance (Scientech, SA210, USA), conditioned in hermetic aluminum pans (20 μL), and submitted to a temperature program, under inert atmosphere (100 mL min^{-1} of N$_2$). In the first scan, after cooling the sample at −10 °C min^{-1} up to −60 °C, it was submitted to heating at 10 °C min^{-1} until 100 °C. The second scan was between −60 °C and 250 °C, at the same cooling and heating rates. The glass transition temperature (T_g) [°C] was calculated using the software Universal Analysis 2.6 (TA Instruments, New Castle, USA) as the inflection point of the base line, caused by the discontinuity of sample specific heat, in the second scan. All aluminum pans were weighed before and after tests to verify that no material was lost during the experiment.

2.6. X-ray diffraction

X-ray diffraction (XRD) measurements were performed, using θ−2θ reflection geometry, on a PHILIPS X-PERT MPD diffractometer using CuKα radiation ($\lambda = 1.5406$ Å), operated at a generator voltage of 40 kV, a current of 40 mA, and goniometer speed of 0.02°(2θ) s^{-1}.

2.7. Statistical analyses

Analysis of variance (ANOVA) was applied on the results using the statistical program Statgraphics Centurion v.15.0 (StatPoint®, Inc., USA) and the Tukey test was used to evaluate average differences (at a 95% of confidence interval).
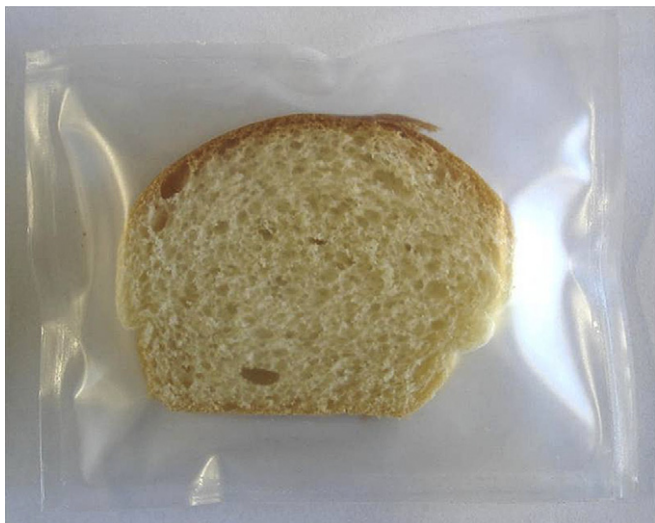
Fig. 1. Toast packaged with biodegradable film based on cassava starch formulated with glycerol, sucrose and inverted sugar as plasticizers.

3. Results and discussion

Most formulations produced transparent, homogeneous and flexible films, and their surfaces were smooth, continuous and homogeneous, without pores and cracks, or insoluble particles (Fig. 1).

3.1. Films preparation: selection of glycerol incorporation method

Tensile strength, elongation at break and water vapor permeability results obtained from films produced in the first phase according to the different glycerol incorporation methods were analyzed by ANOVA (data not shown) and the results indicated there were no significant differences between the two methods tested ($P > 0.05$). Although the results were satisfactory, the films produced by the second method did not present homogeneous appearance, especially those produced with lower glycerol content. Therefore, the first method of glycerol incorporation was chosen, because it supplied films with a better appearance and was also easier to carry out.

3.2. Tensile properties

Tensile properties may vary with specimen thickness, method of preparation, speed of testing, type of grips used and manner of measuring extension. Consequently, it is difficult to compare with literature data.

Tensile strength of films produced at the first phase, according to the first method of glycerol incorporation, varied from (1.85 ± 0.34) MPa to (6.06 ± 1.04) MPa. The use of glycerol, independent of its content, lowered the TS of the films. The average specimen thickness was (85.59 ± 13.57) μm and their values according to the glycerol content were very similar (Table 2).

The presence of glycerol changed the percent elongation at break of the films: a decrease of this property was observed as the glycerol content increased from (0.17 to 0.75) g/100 g. This fact is probably due to the antiplasticizing effect caused by the high plasticizer content, already reported by other authors (Shimazu et al., 2007), indicating stronger interactions between plasticizer and biopolymer that induce a loss of macromolecular mobility. Moreover, the use of sucrose and inverted sugar contributed to this effect because they also acted as plasticizing agents. Veiga-Santos et al. (2005) showed that sucrose addition in BF based on cassava starch can increase E until 2900% when compared to films without sucrose. According to Teixeira et al. (2007), this particular behavior can be attributed to the fact that sucrose has a higher number of OH groups than other sugars and, therefore, is more hydrophilic and is a more efficient plasticizing agent.

In the second phase of the work, as can be observed in Table 3, an increase of clay and glycerol contents caused a decrease of TS. For each content of clay nanoparticles, an increase of glycerol content from 0.75 g to 1.25 g caused a significant decrease of TS ($P < 0.05$). The same tendency can be observed for each glycerol content: an increase of clay nanoparticles content decreases significantly the TS of the films ($P < 0.05$). The regression analysis applied on results, using the response surface methodology, indicated that both glycerol (G) and clay nanoparticles (C) content, as well as their interaction, influenced significantly this property and the fitted model, in real values is ($r^2 = 72\%$):

$$TS = (5.62 - 1.83 \times G + 21 \times C - 28 \times G \times C) \pm 0.59$$
$$(0.75 \leq G \leq 1.25) \quad (0.00 \leq C \leq 0.10) \tag{4}$$

wherein TS is the tensile strength of films [MPa]; G is the glycerol content [g/100 g of filmogenic solution]; and C is the clay nanoparticles content [g/100 g of filmogenic solution].

As can be observed in Fig. 2(a), higher contents of glycerol and clay yield films with lower TS.

For films formulated without clay nanoparticles, the E decreased as glycerol content increased, as expected, confirming the results obtained in the first phase. An opposite effect was observed for films produced with clay nanoparticles, i.e., for each clay content, with increasing glycerol content, E of the films increased. This effect was more pronounced and significant ($P < 0.05$) with higher clay nanoparticles content (0.10 g/100 g).

3.3. Barrier properties

ANOVA applied on results of water vapor permeability and oxygen permeability coefficient indicated that the glycerol content influenced significantly these properties ($P < 0.05$), since a rise in glycerol content caused an increase in both permeabilities (Table 2).

Table 2
Thickness (t), tensile strength (TS), percent elongation at break (E), water vapor permeability (WVP) and oxygen permeability coefficient ($P'O_2$) of biodegradable films based on cassava starch as a function of glycerol content, formulated at the first phase.

Glycerol content [g/100 g of solution]	t [μm]	TS [MPa]	E [%]	WVP [g mm m^{-2} d^{-1} kPa^{-1}]	$P'O_2 \times 10^{-9}$ [cm^3 m^{-1} d^{-1} Pa^{-1}]
0.00	86.33 ± 12.02[ab]	6.06 ± 1.04[c]	158.78 ± 26.35[b]	3.36 ± 0.51[a]	4.28 ± 0.05[a]
0.17	89.58 ± 11.96[ab]	2.37 ± 0.16[b]	213.43 ± 34.52[d]	4.00 ± 0.34[a,b]	8.80 ± 0.04[b]
0.34	76.67 ± 14.23[ab]	1.85 ± 0.34[a]	191.24 ± 35.46[c]	4.79 ± 0.66[b]	11.22 ± 0.31[c]
0.50	82.81 ± 12.38[ab]	2.72 ± 0.23[b]	168.64 ± 30.96[b]	4.92 ± 0.75[b]	21.19 ± 0.41[d]
0.75	91.84 ± 12.94[ab]	2.25 ± 0.13[a,b]	89.85 ± 15.15[a]	7.08 ± 0.33[c]	42.38 ± 0.98[e]

Means in the same column with the same letter are not significantly different ($P > 0.05$).

Table 3
Tensile strength (*TS*), percent elongation at break (*E*), water vapor permeability (*WVP*), oxygen permeability coefficient (*P'O$_2$*), 1st glass transition temperature (*T$_{g1}$*) and 2nd glass transition temperature (*T$_{g2}$*) of biodegradable films based on cassava starch as a function of glycerol and clay nanoparticles contents, formulated at the second phase.

	Glycerol content [g]	Clay content [g]	*TS* [MPa]	*E* [%]	*WVP* [g mm m^{-2} d^{-1} kPa^{-1}]	*P'O$_2$* × 10^{-9} [cm^3 m^{-1} d^{-1} Pa^{-1}]	*T$_{g1}$* [°C]	*T$_{g2}$* [°C]
A	0.75	0.00	4.51 ± 0.69[b,B]	113.60 ± 19.09[a,B]	5.18 ± 0.42[b,A]	30.80 ± 0.41[b,A]	36.15 ± 1.66[a,A]	56.62 ± 4.92[a,A]
B		0.05	4.30 ± 0.68[ab,B]	165.23 ± 26.21[b,A]	4.43 ± 0.65[ab,A]	22.88 ± 1.63[a,A]	36.03 ± 3.38[a,A]	56.77 ± 0.57[a,A]
C		0.10	3.96 ± 0.60[a,B]	123.61 ± 19.57[a,A]	3.81 ± 0.58[a,A]	22.51 ± 0.79[a,A]	36.63 ± 1.00[a,A]	53.86 ± 3.91[a,A]
D	1.25	0.00	3.49 ± 0.55[b,A]	88.80 ± 14.14[a,A]	7.81 ± 0.58[b,B]	111.64 ± 4.86[b,B]	38.71 ± 1.92[a,A]	62.89 ± 0.04[a,A]
E		0.05	2.16 ± 0.35[a,A]	167.12 ± 27.70[b,A]	6.14 ± 0.78[b,B]	101.37 ± 6.63[ab,B]	37.28 ± 0.54[a,A]	59.70 ± 4.34[a,A]
F		0.10	2.07 ± 0.33[a,A]	200.24 ± 33.50[c,B]	5.38 ± 0.80[a,B]	94.00 ± 3.90[a,B]	35.93 ± 2.45[a,A]	54.99 ± 5.31[a,A]

Means in the same column, at same glycerol content, with the same lower case letter are not significantly different ($P > 0.05$).
Means in the same column, at same clay nanoparticles content, with the same capital letter are not significantly different ($P > 0.05$).

As mentioned earlier, glycerol is a relatively small hydrophilic molecule, which can be entrapped between adjacent polymeric chains, decreasing intermolecular attractions and increasing molecular mobility, facilitating migration of water vapor and oxygen molecules (Rodríguez, Osés, Ziani, & Maté, 2006).

Similar tendencies have been reported for BF based on potato starch (Rodríguez et al., 2006; Talja, Helén, Roos, & Jouppila, 2007), yam starch (Mali, Grossmann, García, Martino, & Zaritzky, 2004), corn starch (Bertuzzi et al., 2007) and cassava starch (Alves et al., 2007; Chillo et al., 2008).

In Table 3, the positive influence caused by the addition of clay nanoparticles on water vapor permeability and oxygen permeability coefficient can be noticed. Again, for films formulated without clay nanoparticles these barrier properties increased with increasing glycerol content and presented values comparable to those obtained in the first phase.

Addition of glycerol significantly affects *WVP* and *P'O$_2$* ($P < 0.05$). Since the main function of a food packaging is often to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere, *WVP* should be as low as possible (Mali et al., 2006).
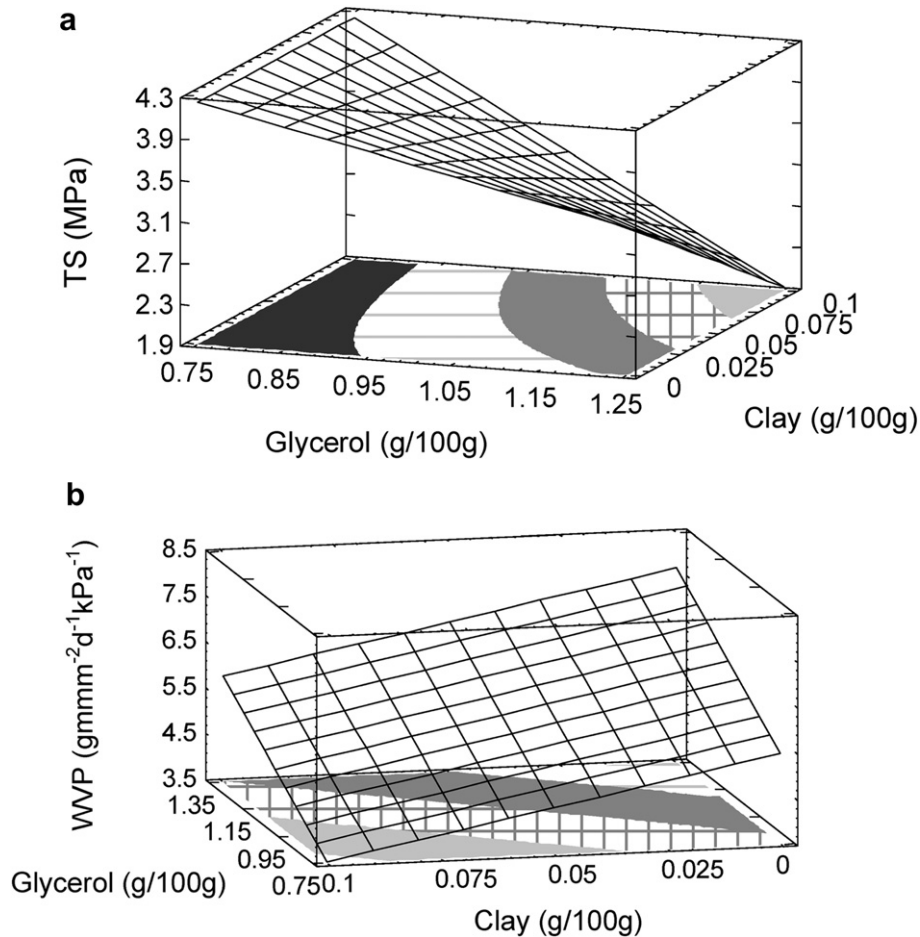

Fig. 2. Tensile strength [MPa] (a) and water vapor permeability [g mm m^{-2} d^{-1} kPa^{-1}] (b) response surfaces of biodegradable films as a function of glycerol and clay nanoparticles content. Guide for tensile strength [MPa]: 1.9–2.4, 2.4–2.9, 2.9–3.4, 3.4–3.9, 3.9–4.4. Guide for water vapor permeability [g mm m^{-2} d^{-1} kPa^{-1}]: 3.8–4.8, 4.8–5.8, 5.8–6.8, 6.8–7.8.

The regression analysis, using response surface methodology, was applied on results of WVP and $P'O_2$ of the films indicating that both components glycerol (G) and clay nanoparticles (C) influenced significantly WVP and $P'O_2$, however only for WVP, expressed by Equation (5), in real values, was obtained with good correlation ($r^2 = 77\%$). As can be observed in Fig. 2(b), biodegradable films produced with lower contents of glycerol and higher contents of clay nanoparticles presented lower WVP.

$$WVP = (2.65 + 3.77 \times G - 19.5 \times C) \pm 0.71 \quad (5)$$
$$(0.75 \leq G \leq 1.25) \quad (0.00 \leq C \leq 0.10)$$

wherein WVP is the water vapor permeability [g mm m^{-2} d^{-1} kPa^{-1}]; G is the glycerol content [g/100 g of filmogenic solution]; and C is the clay nanoparticles content [g/100 g of filmogenic solution].

In order to compare these results with those of classic materials, cellophane water vapor permeability was obtained with assays using the same conditions of the tests performed with BF and the result ((0.49 ± 0.02) g mm m^{-2} d^{-1} kPa^{-1}) was 10 times lower than for the BF.

Comparable results of WVP were shown by commercial materials produced by Cargill Dow (USA) under the Natureworks® trade mark and by Solvay (Belgium) under the CAPA® trade mark (Avérous, 2004).

3.4. Glass transition temperature

Glass transition temperatures obtained from DSC experiments are reported in Table 3. The results showed the same behavior for all samples of BF elaborated, independent of glycerol and clay contents. Two distinct glass transition temperatures, associated with two heat capacity changes in the samples, were observed in all formulations produced, the first varying from (35 to 39) °C and the second one from (53 to 63) °C. Similar values were observed in other polymeric materials. Polylactic acid (PLA), a biodegradable polyester commonly used for trays, cups, bottles and films, has been industrially produced by Cargill Dow (USA) under the Natureworks® trade mark, with a similar glass transition temperature: 58 °C (Avérous, 2004). Tang et al. (2008) fabricated biodegradable nanocomposites from corn starch and montmorillonite nanoclays by melt extrusion processing, with T_g varying from (50.71 ± 2.76) °C to (54.74 ± 1.21) °C, when water content of starch-clay nanocomposite decreased from (13.06 ± 1.73) g/100 g to (9.75 ± 0.21) g/100 g. Specimens fabricated by injection molding using pellets produced with wheat starch (74 g/100 g), glycerol (10 g/100 g) and water (16 g/100 g) presented T_g of 43 °C (Avérous, Fauconnier, Moro, & Fringant, 2000). Arvanitoyannis, Psomiadou, and Nakayama (1996) observed a decrease on glass transition temperature of edible films based on corn starch and plasticized with glycerol from (88.8 ± 3.4) °C to (33.0 ± 1.2) °C, when glycerol content increased from (5 to 15) g/100 g.

It has been reported that increasing glycerol content decreases T_g because the polymer matrix becomes less dense and the mobility of polymer chains is facilitated with the addition of plasticizer (Mali et al., 2006). This fact was not observed in the present work, since a significant effect was not found ($P > 0.05$) at T_g in relation to glycerol content. This fact can be related to the same equilibrium water content presented in all formulations elaborated, (14.11 ± 0.12) g water/100 g of film, since it is well known that water content of a material influenced its glass transition temperature.

3.5. X-ray diffraction

Fig. 3 shows XRD patterns of BF produced during the second phase. Graph peaks represent inter-layer spacing values and,
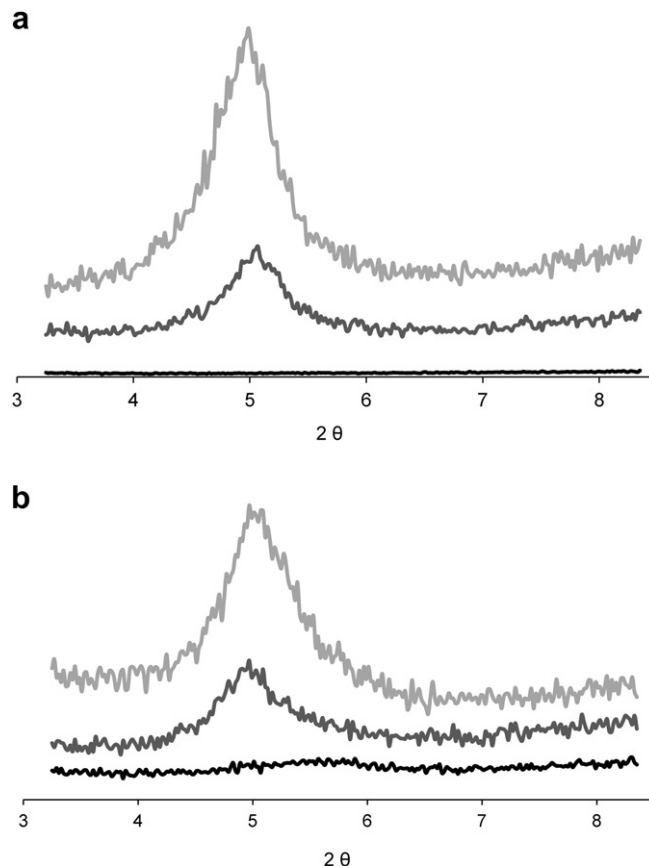

Fig. 3. . X-ray diffraction patterns of biodegradable films formulated with 0.50 g (a) and 0.75 g (b) of glycerol at three levels of clay nanoparticles (0.00 g, 0.05 g and 0.10 g). Guide for 0.50 g: ━━A ━━B ━━C; Guide for 0.75 g: ━━D ━━E ━━F.

therefore, they yield information about the crystalline structure of the analyzed material. It is generally thought that during the intercalation process, the polymer enters into clay spaces and forces apart the platelets, thus increasing the gallery spacing (Tang et al., 2008). The distance d_{001} of pure clay (1.44 nm) is typical of hydrated Na-montomorillonite and is lower than the distance observed in the peaks of BF ((1.76 ± 0.01) nm), indicating the uptake of glycerol and/or starch into clay galleries. According to Chen and Evans (2005), many polymers when taken up by montmorillonite produce an expanded structure with $d_{001} \sim 1.8$ nm, therefore it is not clear if starch and glycerol have entered into the clay galleries or just glycerol.

Since the BF containing clay presented peaks, the clay was not completely delaminated, indicating that starch did not enter into all clay inter-layer spacing, which is further supported by lower results for TS when clay was used. Nevertheless, the reduction of water vapor and oxygen permeability values can also indicate a partial delamination of the clay, which was not detected by XRD. The stacks of clay lamellae (not delaminated) did not contribute significantly to improve tensile properties and could initiate film fracture, which could explain the lower values of TS.

The adsorption and intercalation of glycerol into clays is known and has been studied for many years (Hoffmann & Brindley, 1961). In fact, the glycerol used as plasticizer in the BF formulations, could have prevented the entry of starch molecules in the interlamellar spaces of clay and may have covered the entire inter-layer space. However, a non-volatile plasticizer is essential for processing useful starch based materials; without it the mixture of starch and clay

powders cannot cohere after the evaporation of water (Chen & Evans, 2005).

4. Conclusions

Glycerol and sugars are plasticizers compatible with starch, improving film flexibility, facilitating its handling and preventing cracks, but it was demonstrated in this study that their presence greatly affected film barrier properties. To overcome this problem, clay nanoparticles were used successfully, since permeability values decreased significantly.

The results establish that films based on plasticized cassava starch reinforced with clay nanoparticles can be considered as an interesting biodegradable alternative packaging material. Nevertheless, further research is necessary to improve their mechanical and barrier properties since adequate tensile strength and extensibility are generally required for a packaging film to withstand external stress and maintain its integrity as well as barrier properties during applications in food packaging.

These issues should be focused in future studies. A direction of the investigation will be the development of complementary approaches to give further insight into the molecular structure of biodegradable films based on cassava starch.

Moreover, the elaboration of biodegradable films by extrusion is the main point to explore in a next future, representing an evolution of this research, since a twin screw extruder, equipment conventionally used in flexible packaging industries, yields films with better mechanical and barrier properties due to the complete delamination of clay nanoparticles.

Finally, as a natural biopolymer, besides its biodegradable character, starch would be a promising alternative for the development of new food packaging materials because of its attractive combination of availability and price, supporting the continuity of this study.

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