

Effect of glycerol and amylose enrichment on cassava starch film properties

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Abstract

Cassava starch films were produced by casting with the objective to investigate the effect of glycerol and amylose enrichment on its properties. The effects of different amylose quantities (6.3, 15.6 and 25.0 g/100 g of starch) and glycerol contents (20.0, 32.5 and 45.0 g/100 g of starch) on filmogenic solution were evaluated in barrier and mechanical properties of films. Enrichment of amylose in films formulation produced starches in filmogenic solutions with amylose contents ranging between 25.5% and 36.6%. Mechanical and barrier properties of cassava starch films were influenced by glycerol and amylose contents (Tukey test, $p \leq 0.05$). The enrichment of filmogenic solutions with amylose solution originates stronger and more permeable films. Glycerol behaved as a typical plasticizer in starch films; with increasing glycerol concentration, water vapor permeability, strain at break and puncture deformation increased, and stress at break, Young's modulus and puncture strength decreased.

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1. Introduction

The excessive amount of disposables used in modern society has led to serious demands for biodegradable plastic materials made from renewable source and starch is a biopolymer that is an attractive alternative for packaging material (Bengtsson, Koch, & Gatenholm, 2003). Several studies have been done to analyze the properties of starch-based films (Arvanitoyannis, Billiaderis, Ogawa, & Kawasaki, 1998; García, Martino, & Zaritzky, 1999, 2000; Lourdin, Della Valle, & Colonna, 1995; Mali & Grossmann, 2003; Mali, Grossmann, García, Martino, & Zaritzky, 2002, 2004, 2005). Edible and/or biodegradable films are not meant to totally replace synthetic packaging films, however, they do have potential to replace the conventional packaging in some applications. The use of a biopolymer such as starch can be an interesting solution

because this polymer is quite cheap, abundant, biodegradable and edible.

Starch consists primarily of branched and linear chains of glucose molecules, named as amylopectin and amylose, respectively. Amylose is essentially a linear molecule with a few branches, whereas amylopectin is a highly branched molecule. Preponderance of amylose in starches gives stronger films. Branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (Tharanathan, 2003).

Common amylose sources are inappropriate to food industry purposes because of the chemical residues left by the fractionating methods. To overcome this restraint, some alternatives could be used, such as genetic varieties of high amylose starches, however, these starches were expensive and not readily available in every country (Garcia, Martino, & Zaritzky, 1995). The more common techniques for amylose separation include selective leaching or lixiviation, complex formation and fractional precipitation by salts (Young, 1984). Amylose purification

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is costly and generally none of the products available in market meet food standards because amylose is usually isolated by complexing with low molecular weight alcohols (Garcia et al., 1995).

According to Ellis et al. (1998), amylose can be extracted from starch by leaching in hot (50 °C) water; when a starch suspension in excess water is heated at or above its gelatinization temperature, starch granules become disordered and swell to many times their original size. At this point, amylose molecules diffuse more easily than amylopectin molecules, which remain by hydrogen unions or crystallized inside the granule (Noel, Ring, & Whittman, 1992).

Thus, the objectives of our work were to investigate the effect of amylose rich aqueous solution and glycerol on barrier and mechanical properties of cassava starch films.

2. Materials and methods

2.1. Raw materials

Cassava starch was provided by Indemil Industry (Paranaíba, PR, Brazil). Amylose and amylopectin content of cassava starch were determined simultaneously by the Landers, Gbur, and Sharp (1991) method; ash, protein and fat were determined according to the standard AOAC (1995) methods.

2.2. Preparation of amylose solution

Amylose solution was prepared as follows: an aqueous cassava starch dispersion (4 g starch/100 g water) was heated in a Brabender Viscograph Pt 100 (Duisburg, Germany) from 30 to 60 °C, maintained at 60 °C for 20 min and then cooled to 50 °C, with regular shaking (75 rpm) and constant heating/cooling rates (3 °C/min). The gelatinized starch solution was centrifuged at 25 °C for 10 min at 9000 g and the supernatant was collected. Amylose and amylopectin content of supernatant were determined simultaneously by the Landers et al. (1991) method and total solids were determined drying the solution until constant weight at 105 °C. The amylose solution was added on filmogenic solution for film preparation considering that the filmogenic solution had 5.5 mg solids/mL and that the solids were 100% amylose.

2.3. Films preparation

Cassava starch films were prepared by casting employing 2 g of starch/100 g of filmogenic solution using different amylose quantities (6.3, 15.6 and 25.0 g/100 g of starch) and glycerol contents (20.0, 32.5 and 45.0 g/100 g of starch) (Table 1). Starch, amylose solution and glycerol were directly mixed with distilled water to make batches with a total weight of 400 g. The film-forming solutions were transferred quantitatively to the viscograph cup of a Brabender Viscograph Pt 100 (OHG, Duisburg, Germany) and were heated from 30 to 95 °C and maintained at 95 °C for

Table 1
Cassava starch films formulation^a

Run	Glycerol (g/100 g starch)	Amylose (g/100 g starch)
1	45.0	15.6
2	20.0	15.6
3	32.5	25.0
4	32.5	15.6
5	32.5	6.3
6	–	6.3
7	–	–

^a All films prepared with 2 g of starch/100 g of filmogenic solution.

1 min, with regular shaking (75 rpm) and constant heating rate (3 °C/min). Gelatinized suspensions were immediately poured on rectangular acrylic plates (20 × 20 cm). For each experiment, the quantity of filmogenic solution poured onto the plate was calculated to obtain a constant weight of dried matter for films with 100 µm thickness. Starch suspensions were dried at 40 °C in a ventilated oven model TE-394-3 (Tecnal, Piracicaba, SP, Brazil) to constant weight (about 20 h). The result was translucent films, which could be removed from the plate. All films were conditioned for at least 2 days at 58% relative humidity before measurements.

2.4. Mechanical properties of starch films

The tensile properties were determined using a TA.TX2i Stable Micro Systems texture analyzer (Surrey, England) in accordance with ASTM D-882-91 method (1996). Twelve film specimens (100 mm × 25 mm) of each formulation were clamped between tensile grips. Force (N) and deformation (mm) were recorded during extension at 50 mm min⁻¹, with an initial distance between the grips of 50 mm. The parameters determined were: stress at break (MPa), strain at break (%) and Young's modulus (MPa). For each test eight samples were analyzed.

Puncture tests were made to determine puncture strength (N) and deformation (mm) using the same equipment. Samples with diameters of 40 mm were fixed on the plate of the equipment with a hole of 20 mm diameter with help of a tape (3M Scotch, Brazil). A cylindrical probe of 5 mm diameter was moved perpendicularly to the film surface at a constant speed of 1 mm/s until the probe passed through the film. Force–deformation curves were recorded. At rupture point force and deformation were determined. For each test eight samples were analyzed.

2.5. Water vapor permeability (WVP)

WVP tests were conducted using ASTM method E96 (1996) with some modifications. Each film sample was sealed over a circular opening of 0.00181 m² in a permeation cell that was stored at 25 °C in a desiccator. To maintain a 75% RH gradient across the film, anhydrous calcium chloride (0% RH) was placed inside the cell and a sodium chloride saturated solution (75% RH) was used in the

dessicator. 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), eight weight measurements were made over 24 h. Changes in the weight of the cell were recorded to the nearest 0.0001 g and plotted as a function of time. The slope of each line was calculated by linear regression ($r^2 > 0.99$) and the water vapor transmission rate (WVTR) was calculated from the slope of the straight line (g/s) divided by the cell area (m^2). After the permeation tests, film thickness was measured and WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated as $\text{WVP} = [\text{WVTR}/S(R_1 - R_2)]d$; where S is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R_1 , the RH in the dessicator, R_2 , the RH in the permeation cell and d is the film thickness (μm). Under these conditions, the driving force [$S(R_1 - R_2)$] was 1753.55 Pa.

2.6. Statistical analysis

Two control samples, one without glycerol and other without glycerol and amylose were tested to evaluate the effect of these components on film properties. Analysis of Variance (ANOVA), Tukey mean comparison test ($p \leq 0.05$) and regression analysis were performed employing Statistica software (Statsoft, Oklahoma, USA).

3. Results and discussion

3.1. Chemical composition of starch and characterization of amylose solution

On dry basis, the chemical composition of cassava starch was: ash ($0.22 \pm 0.01\%$), protein ($0.28 \pm 0.01\%$), lipids ($0.11 \pm 0.02\%$) and carbohydrate ($99.39 \pm 0.05\%$). The amylose and amylopectin contents of native cassava starch were 20.8 ± 0.6 and $79 \pm 9\%$, respectively. These data agreed with other authors (Hoover, 2001; Li & Yeh, 2001), who observed amylose values ranging from 16% to 20% in cassava starches. Amylose rich solution presented 100% of amylose and 5.5 mg solids/mL solution.

3.2. Mechanical properties

A food packaging generally requires high stress with deformation according to the intended application. In general, a food packaging must be an undeformable material to provide structural integrity or reinforce food structure, or a deformable film for other applications (Gontard, Guilbert, & Cuq, 1992).

Glycerol content affected significantly mechanical properties of cassava starch films (Tukey test, $p \leq 0.05$). With constant amylose content (15.6 g/100 g starch) and glycerol content ranging from 20 to 45 g/100 g of starch, strain at break and puncture deformation were enhanced and stress at break, Young's modulus and puncture strength decreased (Table 2). Control films without glycerol (run 6 and 7, Table 2) were brittle and had the higher stress at break, Young's modulus and puncture strength and the lower strain at break and puncture deformation values. These effects of glycerol are well-known and its explanation is found in literature (Cuq, Gontard, Cuq, & Guilbert, 1997; Mali et al., 2002; Mali, Grossmann, García, Martino, & Zaritzky, 2004, 2005; Parris, Coffin, Joubran, & Pessen, 1995; Sobral, Menegalli, Hubinger, & Roques, 2001; Sothornvit & Krochta, 2001); when glycerol was incorporated, some structural modifications occurred in starch network, the film matrix became less dense and under stress, movements of polymer chains were facilitated, improving film flexibility.

Amylose content affected significantly mechanical properties of cassava starch films; when glycerol content remained constant (32.5 g/100 g starch) and amylose content increase from 6.3 to 25 g/100 g starch (runs 5, 4 and 3, Table 2), could be observed a significantly increase in stress at break from 2.2 to 7.0 MPa, in Young's modulus from 0.7 to 8.6 MPa and in puncture strength from 7.4 to 10.1; strain at break and puncture deformation decreased with increase of amylose content. Comparing the control samples prepared without glycerol (runs 6 and 7, Table 2), the enrichment with amylose increased stress at break and puncture strength values, but strain at break, Young's modulus and puncture deformation were not affected and

Table 2
Mechanical properties of cassava starch films

Run	Film formulation (g/100 g starch)		Mechanical properties				
	Glycerol	Amylose	Tensile tests			Puncture tests	
			Y_1	Y_2	Y_3	Y_4	Y_5
1	45	15.6	$5.4 \pm 1.08\text{d.e}$	$153.2 \pm 26.73\text{b}$	$0.2 \pm 0.12\text{d}$	$7.9 \pm 0.48\text{e}$	$5.7 \pm 0.66\text{c}$
2	20	15.6	$21.7 \pm 6.08\text{c}$	$5.2 \pm 1.89\text{e}$	$40.5 \pm 6.44\text{b}$	$19.6 \pm 0.61\text{c}$	$1.6 \pm 0.06\text{d}$
3	32.5	25.0	$7.0 \pm 0.72\text{e}$	$132.1 \pm 24.3\text{c}$	$8.6 \pm 2.35\text{c}$	$10.1 \pm 0.55\text{d}$	$6.0 \pm 0.78\text{c}$
4	32.5	15.6	$5.2 \pm 1.07\text{d}$	$104.0 \pm 43.51\text{d}$	$1.8 \pm 0.84\text{d}$	$10.4 \pm 0.70\text{d}$	$6.9 \pm 0.51\text{b}$
5	32.5	6.3	$2.2 \pm 0.48\text{f}$	$263.1 \pm 19.25\text{a}$	$0.7 \pm 0.15\text{d}$	$7.4 \pm 0.34\text{e}$	$9.0 \pm 0.94\text{a}$
6	–	6.3	$52.8 \pm 3.37\text{a}$	$4.5 \pm 1.76\text{e}$	$80.6 \pm 4.86\text{e}$	$32.6 \pm 2.39\text{a}$	$1.74 \pm 0.11\text{d}$
7	–	–	$47.6 \pm 3.3\text{b}$	$4.6 \pm 1.52\text{e}$	$78.9 \pm 5.00\text{e}$	$29.4 \pm 1.94\text{b}$	$1.83 \pm 0.18\text{d}$

Means at same column with different letters are significantly different ($p \leq 0.05$). Y_1 = stress at break (MPa); Y_2 = strain at break (%); Y_3 = Young's modulus (MPa); Y_4 = puncture strength (N) and Y_5 = puncture deformation (mm).

this probably occurred because a low content of amylose was added. The amylose enrichment originated stronger films and this could be explained because during drying of film-forming solutions, water evaporates, allowing the formation of starch network, and during this stage, the proximity of starch chains induced by higher amylose contents could facilitate the formation of a matrix with more polymer content per area. According to Rindlav-Westling, Stading, Hermansson, and Gatenholm (1998), amylose network structure is very stable, with strong molecular orientation, forming films denser than amylopectin films.

The influence of glycerol and amylose content on starch films could be easily observed when looking at the primary tensile results (Fig. 1). The tensile stress–strain curves show the more brittle character of the film prepared with the lower glycerol content (20 g/100 g starch) and without glycerol (run 2, 6 and 7, Table 2) which was also observed when handling the films, compared to the flexible behavior of the films with higher glycerol/starch ratio (run 1, 3, 4 and 5, Table 2). Myllärinen, Partanem, Sppälä, and Forsell (2002), comparing the stress–strain curves of amylose and amylopectin films, observed that glycerol and starch polymers were strongly interacted, and at low glycerol contents (<20%), this interaction resulted in more brittle structure for both polymer networks.

Lourdin et al. (1995) observed that the addition of glycerol as plasticizer above 20% changed the mechanical behavior of starch films by extending the plasticity zone,

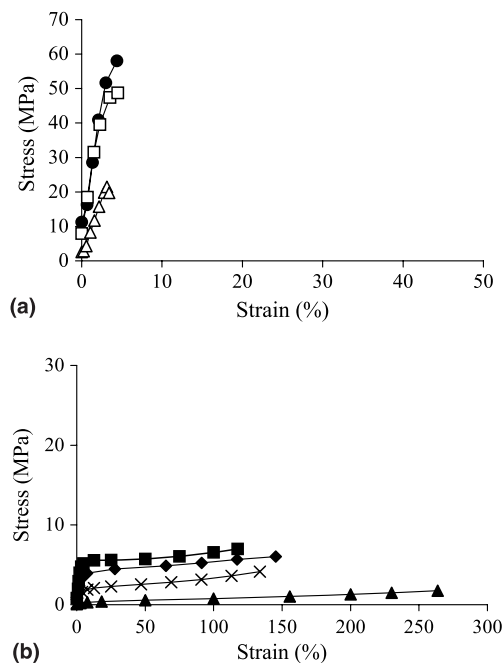


Fig. 1. Stress–strain curves of brittle films (a) and flexible films (b) with different formulations: ● = films without plasticizer, □ = films without plasticizer and without amylose, △ = 20 g glycerol and 15.6 g amylose/100 g starch, ◆ = 45 g glycerol and 15.6 amylose/100 g starch, ▲ = 32.5 g glycerol and 6.3 g amylose/100 g starch, ■ = 32.5 g glycerol and 25.0 g amylose/100 g starch and × = 32.5 g glycerol and 15.6 g amylose/100 g starch.

though this influence was only apparent for amylose contents between 0% and 40%. This fact was observed in this work, when amylose enrichment produced filmogenic solutions with amylose contents ranging between 20.8% (in starches without enrichment) and 36.6% in starches with the high level of amylose enrichment (run 3, Table 2).

As seen in Table 2, stress at break of cassava starch films ranged from 2.2 to 52.8 MPa and strain at break from 4.5% to 263.1%; the lowest stress and the highest strain values were obtained for films formulated with the highest glycerol/amylose ratio (32.5/6.3, run 5, Table 2), likewise, the highest stress and the lowest strain values were obtained for films formulated without glycerol enriched with amylose solution (run 6, Table 2). These values were higher than those of other starch films reported in literature (Mali et al., 2004; Mali, Grossamann, García, Martino, & Zaritzky, 2005), but depending of film formulation, not quite than those of synthetic films, such as HDPE (high density polyethylene) or LDPE (low density polyethylene), which present stress at break of 16 and 26, respectively, and strain at break of 300% and 500%, respectively (Bader & Göritz, 1994; Salame, 1986).

3.3. Water vapor permeability (WVP)

Since a main function of a food packaging is often to avoid or at least to decrease moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product, water vapor permeability should be as low as possible (Gontard et al., 1992).

Films without glycerol were very brittle and could not be analyzed for this property because they broke during the tests (Table 3). WVP values were influenced by glycerol and amylose contents; WVP decreased significantly (Tukey test, $p \leq 0.05$) when lower glycerol content was employed (Table 3). This effect of glycerol was extensively related by other authors (Forsell, Hulleman, Myllärinen, Moates, & Parker, 1999; Forsell, Mikkilä, Moates, & Parker, 1997; García, Martino, & Zaritzky, 2000; Mali et al., 2002, 2004). Similar results have been reported concerning the effect of glycerol on WVP of protein films (Gontard et al., 1992). This behavior could be related to structural

Table 3
Water vapor permeability of cassava starch films

Run	Glycerol (g/100 g starch)	Amylose (g/100 g starch)	WVP ($\times 10^{-9}$ g m ⁻¹ s ⁻¹ Pa ⁻¹)	Water content (g/100 g film)
1	45.0	15.6	4.90 ± 0.10a	24.07a
2	20.0	15.6	2.40 ± 0.20e	7.57e
3	32.5	25.0	4.60 ± 0.10b	11.44d
5	32.5	15.6	3.20 ± 0.2d	14.35c
4	32.5	6.3	3.90 ± 0.10c	18.86b
7	–	6.3	–	11.80d
6	–	–	–	11.30d

Means at same column with different letters are significantly different (Tukey test, $p \leq 0.05$).

modifications of starch network that might become less dense, added to the hydrophilic character of glycerol, which is favorable to adsorption and desorption of water molecules. Moreover, in previous works water sorption isotherms showed that above 43% RH the equilibrium water content of starch films with glycerol was higher than those of non-plasticized films (Mali et al., 2002; Myllärinen et al., 2002).

Cassava starch films exhibited increased WVP values at increased amylose addition (Table 3), what could be related with a higher number of free hydroxyl groups, that might enhanced interactions with water, favoring water vapor transmission through the films. According to Cuq, Gontard, and Guilbert (1998) most free hydrophilic groups in proteins films favor sorption and water vapor transfer, rather than hydrophobic gas transfer like that of CO₂ and O₂; Mali et al. (2004) related similar results for yam starch films.

Water content of cassava starch films ranged from 7.57 to 24.07 g water/100 g film, and was influenced by glycerol and amylose contents. Water content results showed the same trend observed for WVP values, increasing significantly (Tukey test, $p \leq 0.05$) with glycerol increase.

With regard to synthetic polymers, cassava starch films obtained in this work have WVP values higher than those of low-density polyethylene ($0.0036 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$) studied by Shellhammer and Krochta (1997). However, cassava starch film permeabilities were close than those of other biodegradable films such as wheat gluten plasticized with glycerol ($7.00 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$), amylose ($3.80 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$), hydroxypropyl-methylcellulose with plasticizer and oil ($1.90 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$) (Gennadios, Weller, & Gooding, 1994), and slightly higher than those of cellophane ($0.84 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$), (Shellhammer & Krochta, 1997) and methyl cellulose films ($0.500 \times 10^{-10} \text{ g m}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$), (Turhan, Sahbaz, & Güner, 2001).

4. Conclusions

This study showed the possibility of using amylose-rich starch solution extracted from cassava starch without reagents and with temperature of 60 °C for film production.

Mechanical and barrier properties of cassava starch films were influenced by glycerol and amylose contents. The enrichment of filmogenic solutions with amylose solution originates stronger and more permeable films. Glycerol behaved as a typical plasticizer in starch films; with increasing glycerol concentration, water vapor permeability, strain at break and puncture deformation increased, and stress at break, Young's modulus and puncture strength decreased. Depending on formulation, cassava starch films can have stress and strain similar to low-density polyethylene films.

The selection of the optimum film-forming condition depends on the specific use of the film, application techniques and other considerations. For example, if the film

had to be used to protect products from handling, the prime property to optimize would be mechanical. Films with higher amylose and glycerol contents produced flexible films with stress at break of 7.0 MPa and strain at break of 132.1%, interesting to be used in products exposed to consumer handling.

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