Relationships among the Composition and Physicochemical Properties of Starches with the Characteristics of Their Films

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The physical, molecular, and functional properties of corn, cassava, and yam starches were related to the film properties of these starches. Corn, cassava, and yam starches contained 25%, 19%, and 30% amylose, respectively. Amylose from yam starch showed the smallest molecular weight among the starches and amylopectin from corn starch the smallest molecular weight. Cassava starch presented a higher amylopectin content, and its gels and films were less strong, more transparent, and more flexible than corn and yam films. Plasticized films of the three starches were more flexible, with a higher strain and lower stress at break when the glycerol content increased. Unplasticized films were brittle and had water vapor permeability values ranging from 6.75×10^{-10} to 8.33×10^{-10} g m^{-1} s^{-1} Pa^{-1}. These values decreased when the glycerol content reached 20 g/100 g of starch because a more compact structure was formed. Then, at a glycerol content of 40 g/100 g of starch, the WVP increased because the film matrixes became less dense.

KEYWORDS: Corn; cassava; yam; mechanical properties

1. INTRODUCTION

Over the past few years, there has been a renewed interest in films made from renewable and natural polymers such as starch (*1*). Several studies have analyzed the properties of starch-based films (*2*−*8*). Edible and/or biodegradable films are not meant to totally replace synthetic packaging films; however, they do have the 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 (*8*).

Starch consists primarily of branched and linear chains of glucose molecules, named amylopectin and amylose, respectively. Amylose is essentially a linear molecule with a few branches, whereas amylopectin is a highly branched molecule. The preponderance of amylose in starches gives stronger films. The branched structure of amylopectin generally leads to films with different mechanical properties, such as decreased tensile stress (*9*).

Starch is present in a semicrystalline form in granules that varies in composition, size, shape, and functionality when obtained from different botanical sources (*10*, *11*). The starches from various botanical sources have unique characteristic functional properties that are influenced by the granular and molecular structures.

Several different starches are available on the market, including corn, cassava, wheat, and rice, among others, and each type has specific properties and, hence, special application (*12*). Other starches, such as yam starch, although not commercially available, have some interesting functional properties. Worldwide, corn represents the major commercial source of starch, while cassava is an important starch source in some parts of the word, such as Brazil, which is the largest cassava-producing country (*13*). Cassava starch is appreciated for its paste clarity, low gelatinization temperature, and good gel stability (*14*). Native yam starch (*Dioscorea alata*) contains about 30% amylose. This is important for film production because amylose is responsible for the film-forming capacity of starches (*8*).

The objective of this work was to relate the physical, molecular, and functional properties of starches from different botanical sources (corn, cassava, and yam) to the properties of cast films obtained from these starches.

2. MATERIALS AND METHODS

2.1. Materials. Corn starch was provided by Refinações de Milho Brasil (Curitiba, PR, Brazil) and cassava starch by Hiraki Industry (São Paulo, SP, Brazil). Yam starch was extracted from fresh tubers of yam (*D. alata*) according to Alves, Grossmann, and Silva (*15*). The amylose

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and amylopectin contents of yam starch were determined simultaneously by the Landers, Gbur, and Sharp (*16*) method; ash, protein, and fat were determined according to the standard AOAC (*17*) methods.

2.2. Starch Granule Morphology. The granule morphology of native starches was studied by scanning electron microscopy. Starch samples were mounted onto aluminum stubs with double-sided stick tape, coated with gold, and then examined in a Philips scanning electron microscope (model XL 30, Holland) at an accelerated potential of 20 kV.

2.3. Molecular Profile of Starches. Corn, cassava, and yam starches were fractionated into amylose and amylopectin by an aqueous leaching method (*18*), and then, amylopectin molecules were debranched. The debranching of amypolectin was studied according to the Jane and Chen (*19*) method. Fractionated amylopectin from each starch (50 mg) was dispersed in 1 mL of dimethyl sulfoxide (DMSO), precipitated with methanol, filtered, and redispersed in distilled water. The mixtures were incubated for 1 h at 96 °C and then dissolved in a buffered solution (pH 5.0) with 15 mg of pullulanase (Novozymes Latin America Limited, Araucária, PR) for 90 min at 57.5 °C. Placing the mixtures in a boiling water bath for 10 min inactivated the enzyme. The digests were centrifuged to remove the precipitated enzyme, and the debranched amylopectin was precipitated with methanol and collected.

The molecular profiles of native and fractionated starches were determined according to the Bradbury and Bello (*20*) method by high-performance size exclusion chromatography (HPSEC) and were made to compare qualitatively the amylose and amylopectin apparent molecular weights. To prepare the samples for injection, native and fractionated starches were treated to facilitate their solubility: native starches were pregelatinized with an autoclave (121 °C for 20 min), and amylose and amylopectin samples (10 mg) were treated with 2 mL of DMSO (90% w/v), heated (100 °C for 1 h), and incubated at 60 °C until the analysis. All solutions were filtered with a 0.45 μm nylon filter.

Then the samples (100 μL) were injected into a high-performance size exclusion chromatograph (Shimadzu LC 10AD), which contains a sample injector (Shimadzu SIL 10A), a Bio-Silect 125-5 (300 × 7.5 mm) column and a one-guard column (75 × 7.5 mm) from Bio-Rad Laboratories (Richmond, CA), and a differential refractometer (Shimadzu RID 10A). The mobile phase was 30% DMSO in water, and the columns and the detector were maintained at 30 and 40 °C, respectively. Amylose (type III, Sigma) and amylopectin resulting from fractionation were used as standards and injected separately at the same conditions. The areas of the detector response peaks for the specimens were normalized to facilitate comparison among starches. The retention time was used as a parameter to compare molecular weights: the larger molecules thus can leave the column first with lower retention times, and the smaller molecules, which diffuse in gel beads and are delayed in their passage down the column, with higher retention times, in the order of their sizes. Preliminary tests were made, and the eluted materials were separated in fractions according to their retention times: amylopectin molecules with the largest hydrodynamic volume and apparent molecular weight eluted first with retention times of 9.0–10.5 min from the HPSEC columns, and amylose molecules eluted with retention times of 11–13.5 min. All samples were analyzed in duplicate.

2.4. Light Transmittance of Starch Pastes. The percent transmittance (%T) of starch solutions was determined by the method proposed by Craig, Maningat, Seib, and Hoseney (*21*). The starch solutions (1% w/w in water) were heated in a boiling water bath and stirred for 30 min. After the solutions were cooled to room temperature, the %T at 650 nm was measured against a water blank with a Cintra 20 spectrophotometer (Cintra, Australia). All samples were analyzed in triplicate.

2.5. Starch Gel Retrogradation. Gel retrogradation was studied using the method of Biliaderis (*22*) with some modifications. Starch pastes (6% w/v) were prepared in a Brabender viscograph (Pt 100, Germany). The samples were heated from 30 to 95 °C (3 °C/min) and maintained for 10 min at 95 °C. The paste from each starch type was poured into cylindrical plastic tubs (30 mm in diameter and 45 mm in height). These were sealed with a plastic film and stored at 4 °C. After

1 and 7 days the water released was removed and the percent of weight decrease expressed as retrogradation. All samples were analyzed in triplicate.

2.6. Starch Film Preparation and Characterization. *2.6.1. Film Preparation.* Starch films were prepared by casting, employing corn, cassava, and yam starches (3 g of starch/100 g of filmogenic solution), with different glycerol concentrations (0, 20, and 40 g/100 g of starch). For each experiment, the quantity of filmogenic solution poured onto the plate was calculated to obtain a constant weight of dried matter of approximately 9.00 mg/cm², resulting in films with 0.08 ± 0.01 mm thickness, measured with a Mitutoyo micrometer (São Paulo, Brazil) with an accuracy of ±1 μm. The starch suspensions were dried (40 °C) in a ventilated oven, model TE-394-3 (Tecnal, Piracicaba, SP, Brazil) to constant weight (about 20 h) to evaporate the solvent. The result was translucent films, which can be easily removed from the plate. The films were equilibrated at 20 °C and a relative humidity (RH) of 64%, for 48 h, before being tested.

2.6.2. Film Characterization. 2.6.2.1. Opacity. The opacity of the films was determined using a BSI standard procedure (*23*) modified by Gontard, Guilbert, and Cuq (*24*). Film samples were cut to 1 × 3 cm and placed on the internal side of a spectrophotometer cell (GBC Cintra 20, Vitoria, Australia) to record the absorbance spectrum between 400 and 800 nm. The film opacity was defined as the area under the curve and expressed as absorbance units × nanometers (AU·nm). All tests were conducted in duplicate.

2.6.2.2. Mechanical Properties. The tensile properties were studied using a TA.TX2i Stable Micro Systems texture analyzer (Surrey, England) in accordance with ASTM D-882-91 (*25*). Five sample strips (25.4 × 100 mm) of each formulation were cut and clamped between pneumatic grips. Force (N) and deformation (mm) were recorded during extension at 50 mm·min⁻¹ and with an initial distance between the grips of 50 mm. The parameters determined were the stress at break (MPa) and strain at break. The stress at break and strain at break are referred to in the literature as tensile strength and elongation, respectively.

2.6.2.3. Water Vapor Permeability (WVP). WVP tests were conducted using ASTM (*26*) method E96 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 desiccator. The RH inside the cell was always lower than that outside, and water vapor transport was determined from the weight gain of the permeation cell. After steady-state conditions were reached (about 2 h), the weight measurements were made every 2 h, 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 transfer area (m²). After the permeation tests, the film thickness was measured and WVP (g s⁻¹ m⁻¹ Pa⁻¹) was calculated as WVP = $[(WVTR)/[S(R_1 - R_2)D]]$, where S is the saturation vapor pressure of water (Pa) at the test temperature (25 °C), R_1 the RH in the desiccator, R_2 the RH in the permeation cell, and D the film thickness (m). Under these conditions, the driving force $[S(R_1 - R_2)]$ was 1753.55 Pa. All tests were conducted in duplicate.

2.7. Statistical Analysis. Analysis of variance (ANOVA) and the Tukey mean comparison test ($p \leq 0.05$) were performed employing Statistica software (Statsoft, Tulsa, OK).

3. RESULTS AND DISCUSSION

3.1. Morphological Granular Characterization of Starches. Corn, cassava, and yam starches differed in shape and size (**Figure 1**). In corn starch, the granules presented a polyhedral irregular shape with an average granule diameter of 5–20 μm; these were according to other authors (*27*), who reported the average size of individual corn starch granules in ranges of 1–7 μm for small and 15–20 μm for large granules. Cassava starch granules were elliptical and truncated in shape with diameters
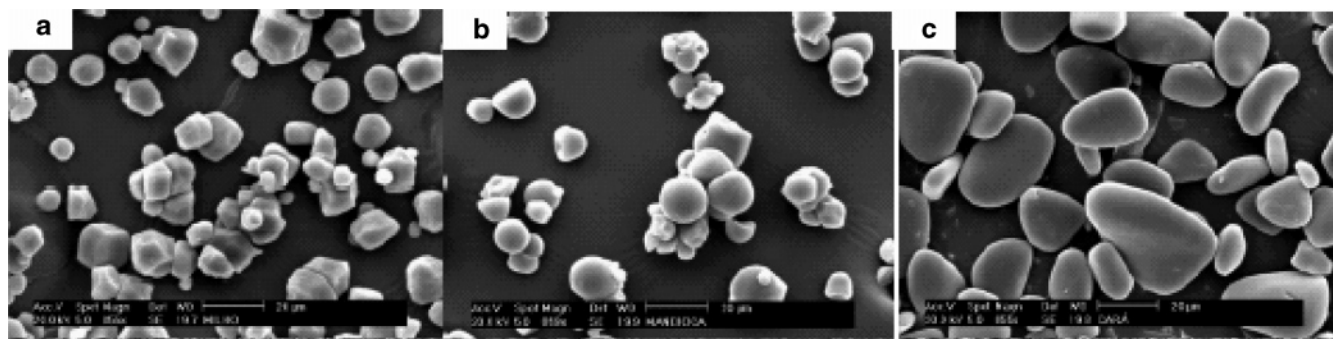
Figure 1. SEM micrographs of corn (**a**), cassava (**b**), and yam (**c**) starch granules. Magnification: 20 μm between marks.

Table 1. Chemical Composition of Corn, Cassava, and Yam Starches (Dry Basis)[a]

component	corn starch	cassava starch	yam starch
ash (%)	0.06 ± 0.005 c	0.22 ± 0.01 a	0.17 ± 0.01 b
fat (%)	0.29 ± 0.01 a	0.28 ± 0.01 a	0.27 ± 0.01 a
protein (%)	0.27 ± 0.01 a	0.11 ± 0.01 c	0.20 ± 0.01 b
carbohydrates[b] (%)	99.38 ± 5 a	99.39 ± 5 a	99.56 ± 5 a
amylose (%)	25 ± 2 b	19 ± 2 c	30 ± 2 a
amylopectin (%)	75 ± 5 b	81 ± 5 a	70 ± 5 b

[a] Numbers after "±" are the standard deviation of the analysis, and means in the same row with different letters are significantly different (Tukey test, $p \leq 0.05$).
[b] Calculated by difference.

ranging from 7 to 14 μm. Yam starch had granules with diameters ranging from 12 to 37 μm. Corn and cassava starch granules, unlike those of yam, showed a tendency to exist in clusters of individual granules, as described by Hoover (*28*).

3.2. Chemical Composition of Starches. Corn, cassava, and yam starches presented a carbohydrate content of up to 99% (**Table 1**). The amylose and amylopectin contents are shown in **Table 1**; corn starch showed 25% amylose and cassava 19%. These data agree with those of other authors (*28, 29*), who observed values of 25% for corn and values ranging from 16% to 20% in cassava starches. Yam starch showed the highest amylose level (30%), higher than the values obtained by some authors (*30−32*) and similar to those reported by other*s* (*7, 15*). The differences could be explained by the different plantation conditions of yam tubers and by the methods used in this analysis. The amylose content is relevant for the film-forming capacity of the starch, although in plasticized films the final characteristics are strongly influenced by amylopectin and plasticizer interaction.

3.3. Molecular Profile of Starches. In the HPSEC profile of native starches (**Figure 2**), two fractions were observed. The first fraction was related to corn, cassava, and yam amylopectin, a macromolecule with a high molecular weight and thus with a minor retention time (9.0−10.5 min). The second fraction, with peaks appreciably smaller, was attributed to the amylose fractions (retention time from 11.0 to 14.0 min). Amylopectin peaks were similar for corn, cassava, and yam starch profiles, although cassava had a more intense peak, agreeing with its higher amylopectin content.

In the HPSEC profile of corn, cassava, and yam amyloses (**Figure 3**), two fractions were observed: the first one (retention time of 9.0−10.5 min) was characteristic of contamination with high molecular weight molecules; the second (retention time of 11.0−14.0 min) corresponded to the amylose molecules. Cassava amylose eluted before corn and yam amyloses, indicating its higher molecular weight, while yam amylose had a smaller molecular weight. These results agree with those of other
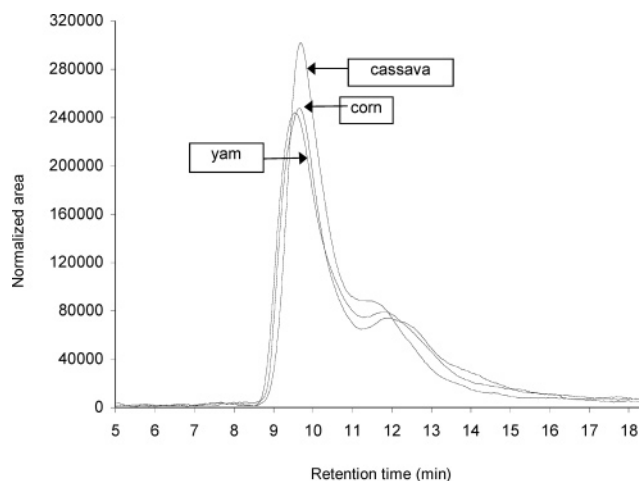

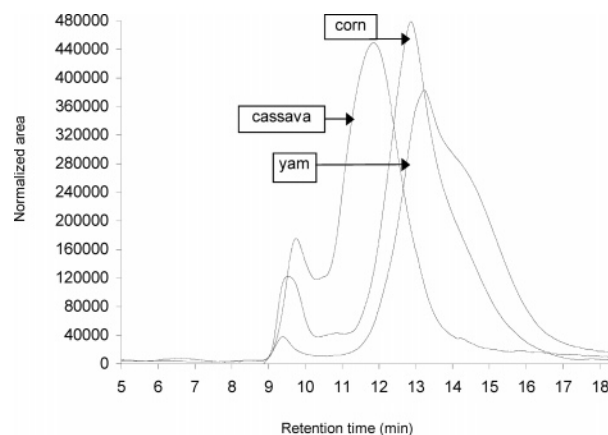**Figure 2.** Molecular profile of native corn, cassava, and yam starches.

Figure 3. Molecular profile of corn, cassava, and yam amyloses.

authors (*18*), who stressed that cassava amylose had a higher molecular weight than corn amylose.

In **Figure 4**, the fractions eluted from 9.0 to 10.5 min were considered to be amylopectin. Corn, cassava, and yam starches showed different intensities of amylopectin peaks, probably because of contamination with amylose (peaks with retention times between 11 and 13.5 min). It was observed that the retention times for the three samples were slightly different, indicative of differences in their molecular weights. Yam amylopectin eluted first, followed by corn and cassava, indicating yam amylopectin had the largest molecular weight.

In the HPSEC profile of debranched amylopectin (**Figure 5**), an amylopectin characteristic peak (retention time of 9.0−10.5 min) was observed, but linear molecules that came from debranched amylopectin, with a smaller molecular weight, also eluted, with retention times higher than 15 min. According to
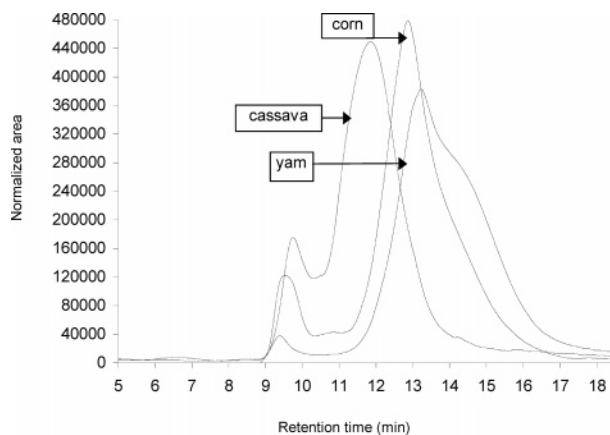
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Figure 4. Molecular profile of corn, cassava, and yam amylopectins.
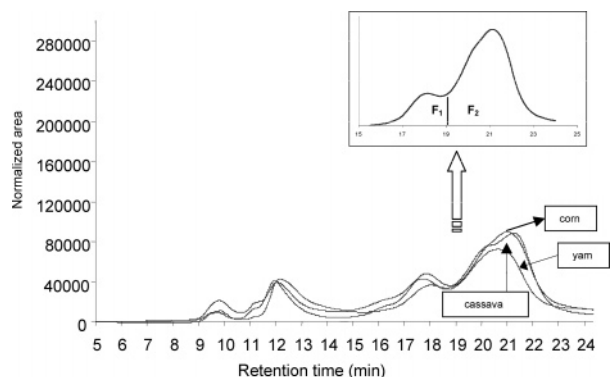

Figure 5. Molecular profile of debranched amylopectin of corn, cassava, and yam starches.

Hoover (*28*), amylopectin molecules contain several distributions of chains (A, B, and C), which differ in chain length. The A chains (unbranched) are linked to B chains, the B chains carry one or more A and/or B chains, and C chains have the reducing end group of the molecule. The molar ratio of short to long chains is influenced by the starch source.

Specifically observing the small molecular weight fractions of amylopectin (retention times higher than 15 min), two subfractions, F_1 and F_2 (**Figure 5**), could be identified; these subfractions were reported by Hizukuri (*33*, *34*) and were labeled as F_1 (corresponding to the B long chains of amylopectin that eluted first) and F_2 (corresponding to the A and B short chains of amylopectin). The F_2/F_1 ratio may be used as a measure of the average chain length of amylopectin chains, with a higher ratio indicating a higher quantity of short chains in amylopectin (*33*–*35*).

In this work, F_2/F_1 ratios were calculated from normalized peaks area and were 5.32, 2.70, and 2.30 for corn, cassava, and yam starches, respectively. These data suggest structural differences in the amylopectin components of the three starches; among all starches, probably corn had the highest degree of short chains in its amylopectin molecule. Hizuruki (*33*, *34*) reported that cereals contain more short chains and fewer long chains than amylopectins from tubers.

3.4. Light Transmittance of Starch Pastes. The %*T* values of corn, cassava, and yam starch pastes were 7.0 ± 0.5%, 55.5 ± 3.5%, and 5.7 ± 0.7%, respectively. Cassava starch showed the highest %*T*, which was significantly different (Tukey test, $p \leq 0.05$) from those of corn and yam starches. These results agree with data from other authors (*36*, *37*) and could be related to the amylose content of these starches. Cassava starch presented the lowest amylose content, and this probably

conferred it high paste clarity. According to Swinkels (*38*), the higher the amylose content in starches, the lower the %*T*.

Another factor that probably contributed to the higher %*T* of cassava starches was the molecular profile of its amylose chains (**Figure 3**). Amylose from cassava had the highest molecular weight among the starches and consequently was more difficult to align and did not interact as well, leading to a more translucent paste, while yam amylose had the smallest molecular weight, which favored molecular interaction among short chains and contributed to its lower %*T*. According to Cock (*39*), paste clarity is a consequence of molecular interactions (hydrogen bonds) between linear chains of starch.

3.5. Starch Gel Retrogradation. The weight decreases of corn, cassava, and yam starch gels after 24 h of storage at 4 °C were 1.7 ± 0.2%, 1.8 ± 0.2%, and 41.2 ± 4.0%, respectively. Yam starch gel had the highest syneresis (retrogradation), with values of weight decrease up to 39% higher than those of corn and cassava starches, which were not different. This excessive retrogradation of yam starch could be attributed to its higher amylose content, which had a lower molecular weight than corn and cassava amyloses (**Figure 3**).

After 7 days of storage, corn, cassava, and yam starch gels had 11.5 ± 0.1%, 1.7 ± 0.2%, and 45.5 ± 4.5% weight decreases; only corn starch gel had a significant decreasing weight during storage from 1 to 7 days (Tukey test, $p \leq 0.05$). According to Karim, Norziah, and Seow (*40*), in short-term storage, the development of gel structure occurs via amylose crystallization and, in long-term storage, via amylopectin reordering, which is a much slower process. This affirmation also could explain the corn starch behavior. Probably the higher degree of short chains in its amylopectin molecule (F_2/F_1 ratio, **Figure 5**) favored the retrogradation increase in the 7 days compared to 24 h of storage. Besides, yam and cassava starches had lesser amouts of short-chain amylopectins, and this certainly interfered with the amylopectin reordering process.

3.6. Visual Appearance and Opacity of the Films. All plasticized formulations gave easily handled, homogeneous, and transparent films without any insoluble particles, except cassava starch film formulated with 40 g of glycerol/100 g of starch, which presented a higher adhesiveness and more handling difficulties. Films formulated without glycerol became brittle and difficult to manipulate. The glycerol level did not affect the opacity of the films.

Opacity is a property of prime importance if a film is to be used as a food coating or as food packaging (*24*). Low relative opacity values indicate a transparent film. The film opacity was influenced by the starch source (**Figure 6**): cassava starch films showed the lowest opacity, which was significantly different (Tukey test, $p \leq 0.05$) from those of corn and yam starches. These results agree with paste clarity data, and could be related to the amylose content of these starches. Cassava starch had
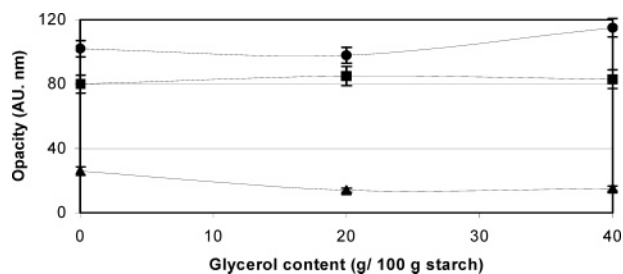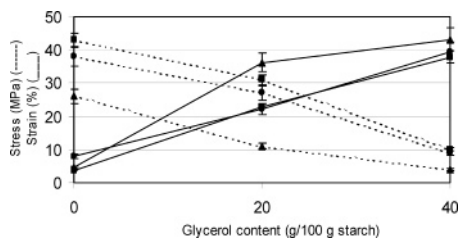

Figure 6. Opacity of starch films: (●) corn starch films, (▲) cassava starch films, and (■) yam starch films. Vertical bars represent standard error values.

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Figure 7. Stress and strain at break of starch films: (●) corn starch films, (▲) cassava starch films, and (■) yam starch films. Vertical bars represent standard error values.

the lowest amylose content, and this probably conferred higher paste clarity and lower film opacity. Corn starch films, although having a lower amylose content, had an opacity slightly higher than those of yam starch films, and this behavior could be justified by differences between the molecular profiles of corn starch amylopectins. Corn amylopectin probably presented a low molecular weight with more short chains (F_2/F_1 ratio, **Figure 5**). This certainly favored the reordering of amylopectin chains to form a more compact and opaque matrix in corn starch films. According to Wang and White (*41*), recrystallization of amylopectin is slower than that of amylose and the presence of numerous and short branches in amylopectin creates an increased ability of amylopectin chains to interact by hydrogen bonds, forming an ordered structure.

3.7. Mechanical Properties. The stress and strain at break in starch films were affected by the glycerol content (**Figure 7**). As seen in **Figure 7**, stress decreased and strain increased significantly with an increase of glycerol in all starch films (Tukey test, $p \leq 0.05$). When a plasticizer, such as glycerol, was incorporated into a starch network, direct interactions and the proximity between starch chains were reduced. Thus, under tensile forces, movements of starch chains were facilitated on plasticized films, decreasing the glass transition temperature of these materials and improving their flexibility (*5−7*).

Among the films, those from cassava starch were weaker and more flexible with all glycerol contents. Increases in glycerol content resulted in a higher decrease and increase in stress and strain at break, respectively (**Figure 7**). This probably occurred because of the lower amylose content of cassava starch. The linear amylose and the branched amylopectin exhibited different behaviors with regard to gelation, development of crystallinity, and film-forming capacity. When the starch granules were heated in water, they started to swell, ruptured, and collapsed, releasing amylose and amylopectin. Branched amylopectin chains, in solution, had little tendency to interact, and consequently, amylopectin gels and films were weak, cohesive, and flexible. Linear chains of amylose, in solution, had a high tendency to interact by hydrogen bonds, and consequently, amylose gels and films were stiffer and stronger than amylopectin gels and films (*42, 43*).

Though yam starch had a higher amylose content than corn starch, corn and yam films did not differ significantly (Tukey test, $p \leq 0.05$) in their stress at break (**Figure 7**); probably the higher degree of short chains in corn starch amylopectin favored the interaction of these chains, contributing to the formation of the film matrix.

3.8. Water Vapor Permeability. Since a main function of 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 (*24*).

WVP in all films showed the same trend with increasing glycerol content (**Figure 8**); the films formulated without
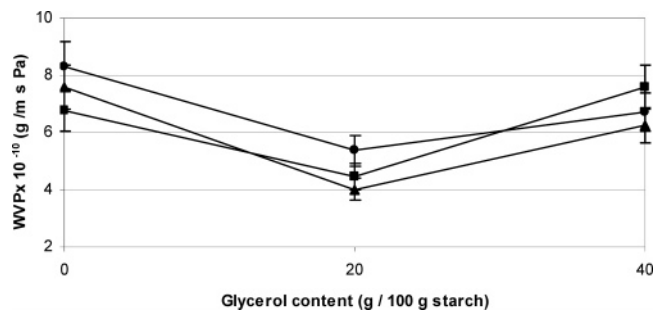

Figure 8. Water vapor permeability of starch films: (●) corn starch films, (▲) cassava starch films, and (■) yam starch films. Vertical bars represent standard error values.

glycerol showed WVP values ranging from 6.75×10^{-10} to 8.33×10^{-10} g m^{-1} s^{-1} Pa^{-1}; these values decreased when the glycerol content reached 20 g/100 g of starch, and then, at a glycerol content of 40 g/100 g of starch, the WVP increased (**Figure 8**). This behavior could be related to structural modifications of the starch network that occurred when glycerol was added. Initially, unplasticized starches formed brittle films with pores or cracks, which facilitated water vapor permeation (*6*). Then, when glycerol was added (20 g of glycerol/100 g of starch), the formation of pores or cracks was avoided and a more compact structure was formed, probably resulting in lower WVP values. Increasing the glycerol content to 40 g/100 g of starch increased the WVP values. The film matrix became less dense, and this, added to the hydrophilic character of glycerol, was favorable to adsorption and desorption of water molecules (*7, 8, 44, 45*).

3.9. Conclusions. Corn, cassava, and yam starch films showed different film properties, which could be related to the molecular, physical, and functional properties of these starches. The amylose content influenced the opacity and strength of starch gels and films; cassava starch had the lowest amylose content, and its gels and films were less strong, more transparent, and flexible than those of corn and yam. The molecular profile of starch amylopectins influenced the film properties; although yam had a higher amylose content than corn starch, corn films were more opaque and corn and yam films did not differ in their stresses at break, probably because corn amylopectin had a lower molecular weight and higher degree of short chains than yam, favoring the reordering of amylopectin chains to form a compact, opaque, and strong matrix in corn starch films. As expected, an increase in glycerol improved the flexibility and water vapor permeability of all starch films. Differences among the molecular profiles of starches did not affect the water vapor permeability of the films. The understanding of the relationships between the molecular profiles of starches and their film properties could be useful in selecting the starch source according to the film application.

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