Contribution of the Starch, Protein, and Lipid Fractions to the Physical, Thermal, and Structural Properties of Amaranth (*Amaranthus caudatus*) Flour Films

D. TAPIA-BLÁCIDO, A.N. MAURI, F.C. MENEGALLI, P.J.A. SOBRAL, AND M.C. AÑÓN

ABSTRACT: Amaranth protein–lipid (PL) and protein (P) films were elaborated and compared with amaranth flour films in order to determine the contribution of the interactions between the biopolymer (starch and protein) and the lipids to the film properties. The films were made by the casting method, using the same glycerol concentration (0.9 g glycerol/100 g solution). A separation of the lipid fraction in the PL films and a polymorphic transformation of the corresponding fatty acids were observed by differential scanning calorimetry (DSC) and verified by an analysis of the microstructure by scanning electron microscopy (SEM). The flour films showed no separation of the lipid fraction, evidence that the lipids were strongly associated with the proteins and homogenously distributed throughout the starch network, contributing to the good mechanical properties when compared to the PL films and to the excellent barrier properties when compared to both the PL and P films. The protein-protein interactions also contributed to the mechanical properties of the flour films. The presence of proteins and lipids in the flour films had an important effect on film solubility, and also on the color and opacity of the films. This study showed that the flour film properties depended on the interactions formed by their polymers (starches and proteins) and by the lipid, on the distribution of these interactions within the film matrix and on the concentrations of each component in the film.

Keywords: amaranth flour, edible films, mechanical properties, microstructure, protein

Introduction

The use of agricultural biopolymers for the development of edible and/or biodegradable films could be an alternative to increase their applications and create new markets, as well as substituting nondegradable synthetic plastic in pharmaceutical and food applications. Polysaccharides, proteins, and lipids or a combination of these have been used to prepare edible films.

Among the polysaccharides, starch is the most widely used in the elaboration of films, due to its low cost and abundance in nature. Studies on the mechanical and barrier properties of edible films based on starch are abundant in the literature (Mehyar and Han 2004; Mali and others 2005; Vicentini and others 2005). In general, the starch films present good mechanical properties and good oxygen barrier properties, but these films are sensitive to humidity (Forsell and others 2002).

In order to improve the properties of these materials, some authors have elaborated films based on starch and protein mixtures (Arvanitoyannis and others 1997; Jagannath and others 2003; Coughlan and others 2004), and, with the particular objective of improving water vapor permeability, some lipids have also been added to the film formulations (García and others 2000; Shaw and others 2002; Bravin and others 2004; Colla and others 2006).

MS 20060660 Submitted 12/1/2006, Accepted 3/12/2007. *Authors Tapia-Blácido and Menegalli are with Food Engineering Dept., FEA, UNICAMP, CEP 13083-862 – Campinas, SP, Brazil. Authors Mauri and Añón are with Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA). 47 y 116 (1900) La Plata, Argentina. Author Sobral is with Food Engineering Dept., FZEA-USP, P.O. Box 23, CEP 13630-000 – Pirassununga, SP, Brazil. Direct inquiries to author Menegalli (E-mail: fcm@fea.unicamp.br).*

To produce such films, researchers have commonly used extracted and purified biopolymers (such as commercial starches and proteins) and lipids, that are then mixed during film processing. Another interesting alternative is the use of flours prepared from agricultural crops, which are natural complex blends of starch, protein, and lipids (Mariniello and others 2003; Tapia-Blácido and others 2005). Tapia-Blácido and others (2005) produced amaranth flour films with interesting mechanical and water vapor barrier characteristics. According to these authors, these characteristics were a result of the natural interactions occurring between the starch, protein, and lipids during drying of the filmogenic solution.

Amaranth flour is produced from the amaranth grain, which is considered to be a pseudocereal. The amaranth is a dicotyledonous plant cultivated in different countries in South America, Central America, Africa, India, and Asia. *Amaranthus caudatus* species are mainly cultivated in the Andean countries such as Peru and Bolivia.

The main biopolymer present in the amaranth grain (approximately 62%) is starch, which has a polygonal shaped granule that has attracted the attention of many researchers due to its size (1 μm). The protein content (approximately 14%) of the amaranth grain is higher than that of other cereals, thus presenting a balanced composition of essential amino acids and also an important concentration of sulfur amino acids. The main protein fractions present in the amaranth grain are albumins, 11S-globulin, globulin-P, and glutelins (Scilingo and others 2002). The lipid content of the amaranth grain is in the range from 4.8% to 8.1% (Saunders and Becker 1984).

The objective of this work was to study the effect of the starch, protein, and lipid fractions and the interactions of these biopolymers on the mechanical, barrier, structural, and thermal properties of amaranth flour films.

doi: 10.1111/j.1750-3841.2007.00359.x

Materials and Methods

Raw materials and reagents

Amaranth flour, protein-lipid (PL), and protein (P) were obtained from amaranth grains (*Amaranthus caudatus*) grown in Callejón de Huaylas (Huaraz, Peru). The grains were transported to Brazil, cleaned, and stored at 10 °C.

All reagents were of analytic grade. Glycerol, NaOH, and HCl were purchased from Synth (São Paulo, Brazil). All solutions were prepared with deionized water.

Production of amaranth flour, PL, and P

Amaranth flour was obtained using the alkaline wet milling method of Perez and others (1993), modified by Tapia-Blácido (2003). PL was isolated from amaranth grains with a 0.25% sodium hydroxide solution, using a steeping time of 24 h at 5 °C, then milled and filtered through a 270-mesh screen. The filtrate was centrifuged (4000 × *g*) for 20 min at 10 °C and the starch discarded. The supernatant was adjusted to pH 5 with 1N HCl and then centrifuged at (6000 × *g*) for 20 min at 10 °C. The pellet was resuspended in water, neutralized with 0.1N NaOH, and dried by freeze-drying. Finally, the PL was defatted with petroleum ether to obtain only amaranth protein (P).

Chemical analysis

The amaranth flour, PL, and P samples were analyzed following standard AOAC methods (AOAC 1997) for the determinations of moisture, protein, ash, and lipid contents. The amylose content was also determined in the amaranth flour using a colorimetric method (Juliano 1971).

Fatty acid profile of the amaranth oil

The lipids were extracted from the amaranth flour by refluxing the samples in a Soxhlet apparatus with petroleum ether. The fatty acid composition was determined using the methodology of Hartmann and Lago (1973). The lipids were hydrolyzed with KOH in methanol for 5 min at 100 °C. The free fatty acids formed at this stage were then methylated with boron trifluoride (BF_3) in methanol at 100 °C. The fatty acid methyl esters and sterols were then identified by gas chromatography in an Agilent 6850 Series GC System (Agilent, Santa Clara, Calif., U.S.A.) using the following operational conditions: DB-23 AGILENT (50% cyanopropyl)-methylpolysiloxane 60-m long capillary column, with Φ int. 0.25-mm and 0.25-μm film; oven temperature of 195 °C for 20 min, 195 to 215 °C (5 °C/min), 215 °C for 16 min; detector temperature: 280 °C; injector temperature: 250 °C; stripping gas: helium; split: 1:50.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The amaranth flour, PL, and P samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) following Laemmli's method (Laemmli 1970) as modified by Petruccelli and Añón (1994). Runs were carried out with 12% (w/v) polyacrylamide separating gels with a stacking gel of 4% (w/v) in mini-slabs (BioRad Mini Protean II Model). The protein molecular weights were estimated using the Low MW markers (Pharmacia calibration kit) that included phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa). The samples were dissolved in the sample buffer (0.125 M Tris–HCl, pH 6.8, 20% (v/v) glycerol, 1% (w/v) SDS, and 0.05% (w/v) bromophenol blue) and the gels fixed and stained with Coomassie brilliant blue.

Film production

The films were produced by the casting method, which consists of drying a film-forming solution (FFS) previously applied to a support. Amaranth flour films were prepared using the methodology proposed by Tapia-Blácido and others (2005). A 4% w/w suspension of flour in water was homogenized in a mixer for 25 min, and the pH was regulated to 10.7 with NaOH (0.1N) to dissolve the protein. This suspension was then heated at 82 °C for 15 min and finally 0.9 g glycerol/100 g solution added.

In previous tests, it had been observed that the heat treatment of PL and P water suspensions provoked the precipitation of globulin molecules (Scilingo and others 2002) resulting in nonhomogeneous films. So the procedure developed for the elaboration of P and PL films was as follows: a 4% w/w suspension of PL or P in water was homogenized on a magnetic stirrer for 3 h. The pH was adjusted to pH 10.7 with NaOH (0.1N) and stirred for an additional 10 min. The suspensions were then centrifuged at (6000 × *g*) for 20 min at 10 °C to separate the starch residues, heated (82 °C for 15 min) with gentle stirring and finally centrifuged again at (6000 × *g*) for 20 min at 25 °C to eliminate the insoluble protein fractions. The supernatant was then reheated and the glycerol (0.9 g/100 g solution) added.

For each film, 85 ± 3 g of the solution was poured onto acrylic plates (18 × 21 cm) to obtain a constant thickness of 80 ± 5 μm. The films were dried in an oven with air circulation and a controlled temperature and relative humidity system (model MA 415UR, Marconi, Piracicaba, Brazil). The films were dried at 40 °C and 55% RH to a final water content that allowed for easy peeling from the plates. All the films were preconditioned for at least 48 h in desiccators containing a saturated solution of NaBr (58% RH) prior to characterization. The final concentration of glycerol in the films was 22.5-g glycerol/100 g biopolymer. The thickness of the films was measured with a digital micrometer (model FOW72-229-001, Fowler, Newcastle, Calif., U.S.A.). The mean thickness of each film was determined from an average of 20 measurements.

Differential scanning calorimetry (DSC)

The thermal properties of the films were determined by differential scanning calorimetry, using a DSC TA 2010 calorimeter controlled by a TA 5000 module (TA Instruments, New Castle, Del., U.S.A.), with a quench cooling accessory. Prior to the determination, the samples were conditioned in desiccators containing silica gel (0% RH) and a saturated solution of NaBr (58% RH), at 25 °C for 3 wk. For the analysis, the samples were heated at 10 °C/min between −150 and 150 °C. The glass transition temperature (T_g) was considered to be the inflexion point of the base line, caused by the discontinuity of the specific heat of the sample. The melting temperature was considered as the maximum peak temperature of the endothermic phenomenon. All these properties were calculated with the help of the software Universal Analysis V1.7F (TA Instruments, New Castle, Del.,U.S.A.) (Sobral and others 2002).

Scanning electron microscopy (SEM)

SEM analyses were performed according to the procedure described by Colla and others (2006). Film samples were maintained in a desiccator with silica gel for 7 d, and film pieces (4 × 4 mm) were then mounted on cylindrical aluminum stubs using a double-sided cupper tape, and coated with gold in a VG Microtech (Cambridge, U.K.) model SC 7620 sputter coater. They were finally observed using a JEOL Model JSM-5800LV scanning electron microscope, at an accelerated voltage of 10 kV.

Mechanical properties

The tensile strength, Young's modulus, and elongation at a break of the films were determined following the procedures outlined in the ASTM methods D882–95 (ASTM 1995), taking an average of 5 determinations in each case. The films were cut into 25.4-mm-wide and 130-mm-long strips using a scalpel, and mounted between the grips of the texture analyzer TA.XT2i (SMS, Surrey, U.K.). The initial grip separation was set at 80 mm and the crosshead speed at 1.0 mm/s. The tensile strength (force/initial cross-sectional area) and elongation at break were determined directly from the stress × strain curves using the software Texture Expert V.1.15 (SMS, Surrey, U.K.), and Young's modulus was calculated as the slope of the initial linear portion of this curve.

Barrier properties of the films

The water vapor permeability (WVP) test was performed using a modified E96-95 ASTM Standard method (ASTM 1995) at 25 ± 2 °C. Film samples were sealed over the circular opening of a permeation cell containing silica gel. The cells were then placed in desiccators containing distilled water. The weight loss of the cells was monitored every 24 h for 7 d.

Oxygen permeability (OP) was determined in duplicate, according to the ASTM D3985-81 (ASTM 1989) method. The oxygen transmission rate was determined in an OX-TRAN 2/20, Mocon Inc. (Minneapolis, Minn., U.S.A.) at 25 ± 1 °C and atmospheric pressure. Oxygen permeability (OP) was calculated, dividing the oxygen transmission rate by the oxygen pressure and multiplying this result by the mean thickness of the sample.

Solubility and moisture content

Solubility was measured by the immersion of 2.0-cm-diameter film disks in water containing sodium azide, at 25 ± 2 °C for a period of 24 h (Gontard and others 1992). The amount of dry matter in the initial and final samples was determined by drying the samples at 105 °C for 24 h. The water content was determined gravimetrically by drying film samples in an oven at 105 °C for 24 h, and calculated according to ASTM D644-94 (ASTM 1994).

Color and opacity

The color, represented as the difference in color (ΔE^*), was determined according to Gennadios and others (1996) and opacity using the HunterLab method (Sobral 1999), both analyses using a colorimeter (HunterLab, model Miniscan XE, Reston, Va., U.S.A.). The difference in color was calculated as

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^*, and Δb^* are the differentials between the color parameter of the samples and of the white standard ($L^* = 94.83$, $a^* = -0.78$, $b^* = 1.44$) used as the film background.

Statistical analysis

Statistical analyses were performed using Statistic 6.0 software (Basic Statistics and Tables). The Tukey test was applied at a 5% significance level to compare means for mechanical, color, opacity, solubility, moisture content, and barrier properties of the films.

Results and Discussion

Raw material composition

Table 1 shows the proximate composition of the amaranth flour and of the protein-lipid (PL) and protein (P) fractions extracted from the amaranth grain by moist grinding. As expected, the more purified materials presented higher protein contents and could be considered as protein concentrates. Nevertheless it was not possible to completely eliminate the starch fraction from the PL and P fractions, which presented starch contents of 17% and 23%, respectively. Although the difference in starch content between these 2 fractions was important, it should be observed that the ratio between the protein and starch contents was practically the same for the 2 fractions (0.36 for PL and 0.32 for P). The starch present in these samples was composed of 7.58% ± 0.40% amylose and 92.42% amylopectin. Considering that it was not possible to completely separate the starch from the PL and P fractions by the extraction method used, a centrifugation step was considered within the methodology for the elaboration of these films, with the objective of separating the residual starch, thus minimizing its effect and allowing for the exclusive evaluation of the contribution of the protein–lipid and protein on the properties of the amaranth flour films.

On the other hand, the lipid content of the amaranth flour was practically the same as that of the grain (Saunders and Becker 1984), indicating minimal losses during flour extraction. It was also shown that the lipid-to-protein ratio was constant, observing similar values in the flour (0.628) and in the PL fraction (0.627), which could suggest a strong association between the lipid and protein phases.

Table 2 shows the fatty acid profile of the amaranth flour lipid fraction. The predominant fatty acids found in the flour lipid phase were linoleic (C18:2), oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids, the unsaturated C18 fatty acids representing more than 70% of the total. These results agree with those of Ayorinde and others (1989). According to Yang and Paulson (2000), stearic (4%) followed by palmitic (19%) acids provide the best water vapor barrier characteristics in edible films.

Figure 1 shows the results of the SDS-PAGE analysis of the proteins present in the flour and the PL and P fractions. The electrophoretic profile typical of fraction P showed bands at approximately 54, 56, and 33 kDa, other low molecular weight bands (<20 kDa) and high molecular weight aggregates (>67 kDa). These bands can be attributed to the subunits of the 11S globulins, globulin P, glutelins, and albumins (Martínez and Añón 1996). The flour and

Table 1—Raw material composition

Composition (g/100 g dry solids)	Flour	PL	P
Moisture content	7.97 ± 0.18	2.23 ± 0.1	2.58 ± 0.54
Protein	14.21 ± 0.77	48.87 ± 0.4	73.83 ± 0.74
Fat	8.93 ± 0.03	30.65 ± 0.9	-
Ash	2.14 ± 0.03	2.9 ± 0.02	2.87 ± 0.21
Starch	74.72	17.58	23.30

PL = protein–lipid; P = protein.

Table 2—Fatty acid profiles (%) of lipids extracted from amaranth flour

Fatty acids	(%)
C14:0	0.22 ± 0.004
C16:0	19.08 ± 0.012
C16:1	-
C17:0	0.62 ± 0.007
C18:0	4.10 ± 0.01
C18:1	28.82 ± 0.02
C18:2	44.48 ± 0.004
C18:3	0.89 ± 0.005
C20:0	0.97 ± 0.005
C20:1	0.22 ± 0.001
C22:0	0.35 ± 0.006
C24:0	0.22 ± 0.004

PL fraction showed a polypeptide composition similar to that of fraction P. High molecular weight aggregates were also observed in the PL fraction, some of which failed to enter the separator gel.

Differential scanning calorimetry (DSC)

Figure 2a and 2b shows the DSC traces obtained for the amaranth flour, PL, and P films conditioned at 0% RH and 58% RH, respectively. The traces of the flour films, in contrast to those of the P films, showed 2 glass transition temperatures (T_{g1} and T_{g2}), typical of systems presenting phase separation (Sobral and others 2001, 2002), and whose values varied according to the water content of the film (Table 3). At low water contents (0% RH), the T_{g1} and T_{g2} values were higher than those obtained for films conditioned at 58% RH. This behavior, also observed for the T_g values of the PL and P films conditioned at 0% and 58% RH, was due to the plasticizing effect of water, which provoked a depression in T_g due to an increase in the mobility of these systems. Various other authors working with edible films have also observed this effect (Biliaderis and others 1999; Sobral and others 2001, 2002).

The amaranth PL films conditioned at 58% RH also presented 2 glass transition temperatures (Table 3). The 1st one (T_{g1}), occurring at very low temperatures (−77 °C) and also observed on the DSC traces of the flour (−65 °C) and P films (−81 °C) conditioned at 58% RH, could be related to a glycerol-rich fraction. On the other hand, the 2nd T_g (−47 °C), observed on the DSC traces of PL films conditioned at 58% relative humidity, was associated with another protein rich fraction. The 2nd T_g (−26.3 °C) of the amaranth flour films conditioned at 58% RH also corresponded to those of a protein-rich phase. However, the value for the 2nd T_g observed in the flour films conditioned at 0% relative humidity was above zero (57.5 °C), and should correspond to a starch-rich fraction such as observed in the work of Myllärinen and others (2002). The values determined in the present work for the 1st T_g agree with those verified in highly plasticized films based on fish myofibrillar proteins (≤−50 °C) (Sobral and others 2002).

The endothermic peak observed in the amaranth PL films conditioned at both 0% and 58% RH could be associated with the lipid-rich fraction, possibly related to the polymorphic transformation of fatty acids from the γ- to the α-form, characteristic of oleic acid (−3 °C). Briefly, the difference between the 2 polymorphs can be recognized from the conformational structure of the ω-chain (the hydrocarbon chain between the double bond and the terminal methyl group); the ordered all-*trans* structure in the γ-form compared with the disordered liquid-like conformational structure in the α-form (Inoue and others 2004). These authors verified that a mixture of oleic acid with saturated fatty acids such as lauric, palmitic, and myristic acids produced a decrease in the transformation temperature of the γ- to the α-form, and the peaks were wider than those obtained with pure oleic acid.

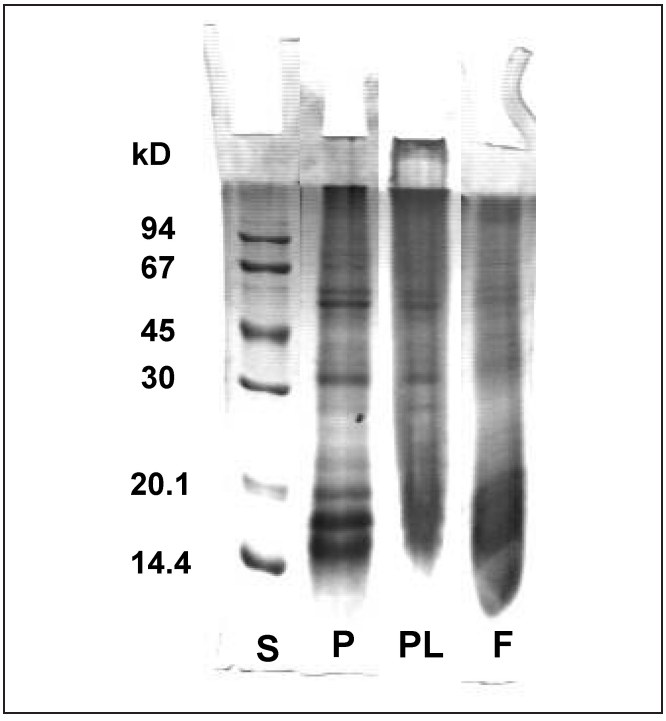

Figure 1—SDS-PAGE of the raw materials. Lane S = standard molecular weight proteins; lane P = protein fraction; lane PL = protein–lipid fraction; and lane F = amaranth flour.

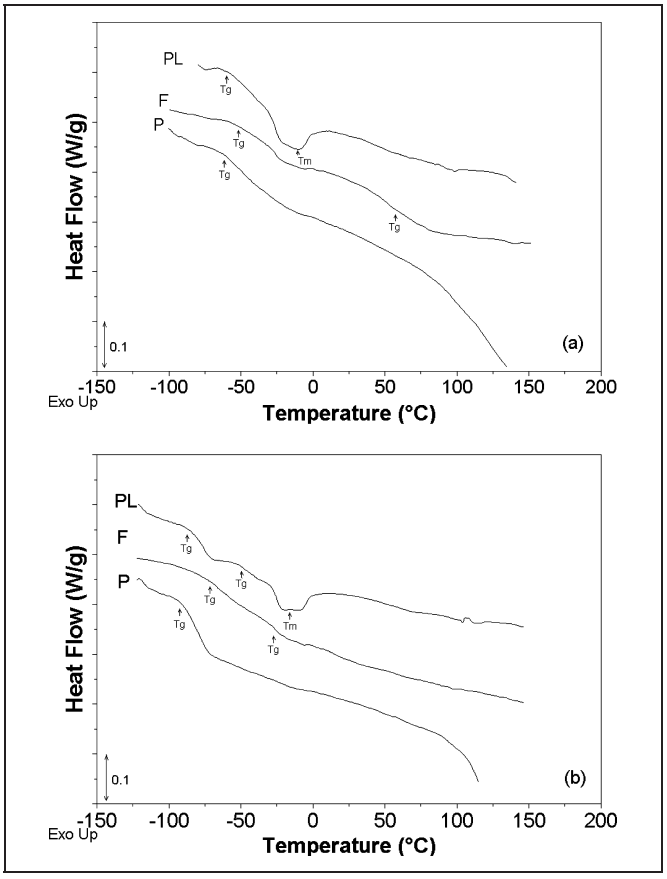

Figure 2—DSC curves of amaranth films: (F) flour, (PL) protein-lipid, and (P) protein, conditioned at (a) 0% RH and (b) 58% RH and 25 °C for 3 wk

Table 3—Glass transition temperatures (T_g) and melting points (T_m) of flour, PL, and P amaranth films, obtained by DSC

Film	T_{g1}	T_{g2}	T_m
0% RH			
Flour	−25.9 ± 0.8	57.5 ± 2.4	–
PL	−47.5 ± 0.6	–	−11.0 ± 1.3
P	−50.8 ± 2.1	–	–
58% RH			
Flour	−64.8 ± 2.5	−26.3 ± 1.1	–
PL	−77.3 ± 1.8	−47.0 ± 0.02	−20.3 ± 0.2
P	−81.2 ± 2.9	–	–

PL = protein-lipid; P = protein.

This peak was present, but was less intense, in the flour films, either because the lipid concentration in the flour was lower or due to protein–lipid interactions in these films. Thus, this result indicated a structural difference in the lipid phase of the flour films as compared to the PL films.

The lipid fraction of the PL and flour films showed an expressive oleic acid content (Table 2), justifying the presence of these low-temperature endothermic peaks, which are related to the polymorphic transformation of this fatty acid.

Microstructure of the films

As can be seen in Figure 3, the flour films presented a dense surface (Figure 3–1a) similar to that of the P films (Figure 3–3a), although the latter showed a smoother surface than the flour films. However, the surface and the cross-sectional microstructures of the PL films (Figure 3–2a and 3–2b) showed a clear separation of the lipid phase from the protein matrix, with the presence of different sized lipid droplets, not homogenously distributed in the protein matrix, confirming the behavior observed in the respective DSC trace (Figure 2).

The flour films also presented a dense cross section, although slightly less dense than that of the P films (Figure 3–1b and 3–3b), due to the presence of a starch network (starch–starch interactions) in the film matrix. The structure of the flour film appears to be strongly stabilized by the interactions of the biopolymers and of the lipid that make up the film matrix, and consequently no separation of the lipid phase was observed. The existence of amylose–lipid complexes was

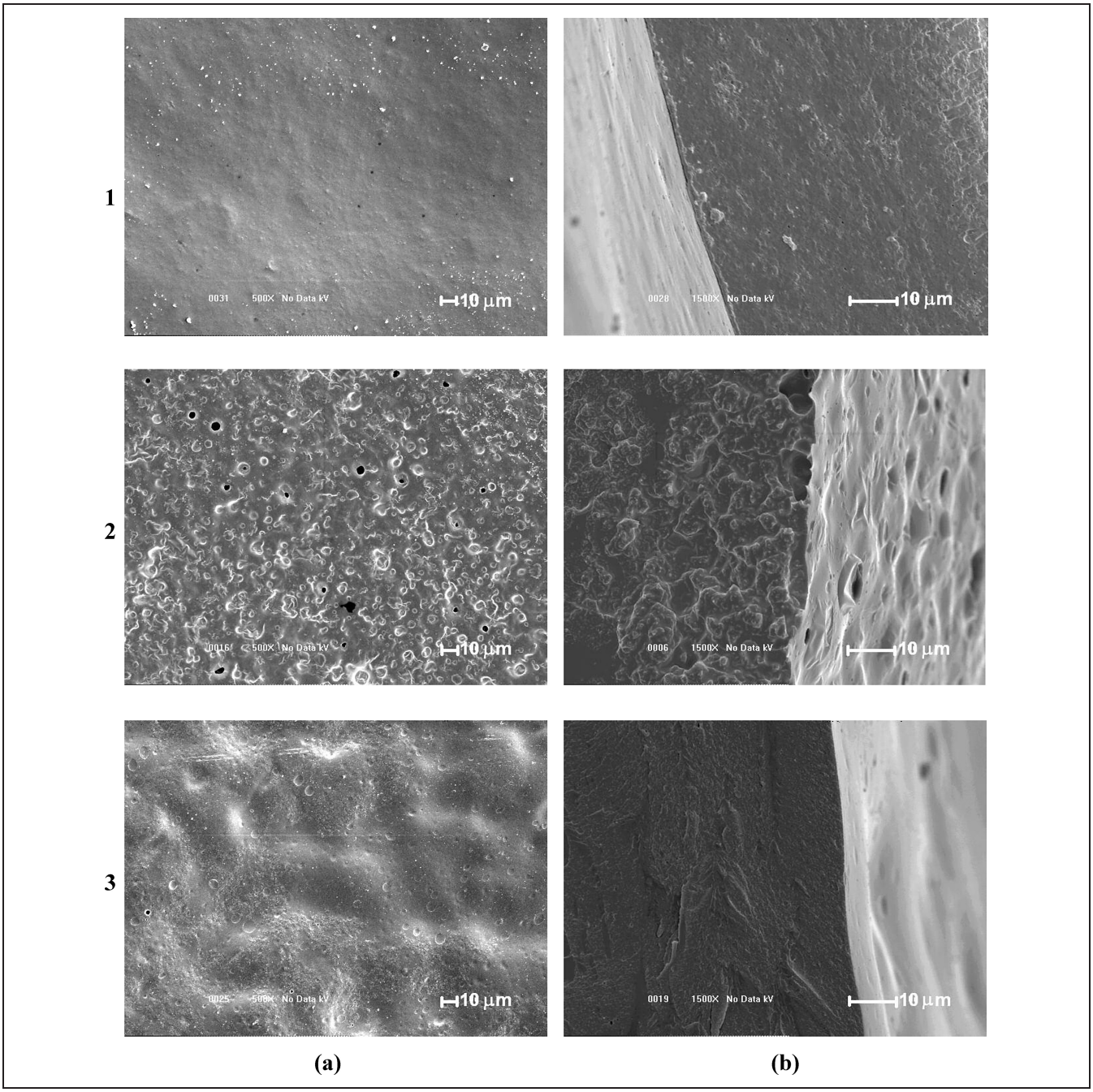

Figure 3—SEM micrographs of amaranth films: (a) surfaces at × 500 magnification and (b) cross sections at × 1500 magnification. The numbers 1, 2, and 3 represent the flour, PL (protein–lipid), and P (protein) films, respectively.

not shown, since there was no sign of fusion peaks of these complexes in the flour DSC curves. The stabilization of the lipid and protein phases found emulsified in the starch phase could be due to a greater thermodynamic compatibility of these phases in their native state in the starch phase. The protein–lipid and protein–protein interactions forming the film matrix, together with the starch–starch interactions and starch–protein, allowed the amaranth flour films to present good water vapor barrier properties as compared to the P and PL films, and also better mechanical properties than the PL films (Table 4), as shown below.

Mechanical properties of amaranth films

Table 4 shows the results of the tensile tests for the mechanical properties of the flour, PL, and P films. No significant differences ($P > 0.05$) were observed between the values for tensile strength, elongation, and Young's modulus of the amaranth flour and P films. However, the flour and P films were stronger and more deformable than the PL films. These results confirmed that the high concentration of lipids in the PL films hindered protein–protein interactions, provoking a decrease in Young's modulus and the tensile strength, while these interactions must have occurred more easily in the P films. In addition, the high lipid concentration in the PL films caused segregation of the lipid phase, possibly due to a thermodynamic incompatibility between the phases at the concentrations encountered in these films, or a destabilization of the protein–lipid complex due to the actual extraction process. Such segregation of the lipid phase from the protein phase in the PL films, as observed by DSC (Figure 2) and by the analysis of the microstructure (Figure 3), would provoke an early rupture of the film during elongation. In studies carried out with artificial emulsions, it was also reported that an increase in the lipid concentration did not contribute to film elongation. Shaw and others (2002) observed that an increase in the soy oil concentration in WPI films (in oil:protein ratios from 0.0 to 0.2) increased film elongation, but that oil:protein ratios of 0.3 and 0.4 decreased film elongation.

On the other hand, the lipid present in the flour films did not affect the mechanical properties of the films, as in the case of the PL films. To the contrary, the lipid contributed to a more effective plasticization, increasing film elongation, since the flour films would have been more brittle due to the high starch content. According to Tapia-Blácido (2003), the amaranth starch films prepared by the same procedure used here for the flour films were more rigid, with values for tensile strength of 3 MPa and for elongation of 18.9%. The presence of proteins and lipids in the flour evidently interfered with the formation of the starch network, conferring greater plasticity on the entire network. In addition, the strong association of the lipid phase with the protein phase and the homogenous distribution of these interactions within the starch network conferred interesting mechanical properties on the amaranth flour films when compared to pectin–soy flour films (Mariniello and others 2003). Thus it was confirmed that the mechanical properties of amaranth flour films were not only a consequence of protein–protein interactions, but also of protein–lipid, starch–starch, and starch–protein interactions, in a naturally mixed system.

Barrier properties of amaranth films

As shown in Table 4, the values for WVP and oxygen permeability (OP) of the amaranth flour, PL, and P films presented significant differences ($P < 0.05$). The amaranth flour films showed lower values for water vapor and oxygen permeability than the P and PL films.

The low water vapor permeability of the flour films was a result of the strong association of the lipids with the proteins and the homogenous distribution of these interactions within the film matrix, formed mainly of starch, creating more hydrophobic continuous zones that impeded the diffusion of water vapor.

The amaranth flour films were also less permeable to oxygen than PL and P films ($P < 0.05$) (Table 4). Contrary to that observed with water vapor permeability, the P films were less permeable to oxygen than the PL films. This result suggests that the good oxygen barrier properties of the amaranth flour films were due to the dense matrix, which expressed the strong interaction of the biopolymers (starch and protein) and of the lipids present in the film, resulting in a system with a small free volume, consequently inhibiting the diffusion process. The presence of lipids could also have influenced the low oxygen permeability of amaranth flour films, since it decreased the hydrophilic characteristics of the film.

Colla and others (2006) observed that the addition of 10% stearic acid in the elaboration of films from amaranth flour of the species *Amaranthus cruentus* improved their oxygen barrier properties (4.8 $cm^3\ \mu m\ m^{-2}\ d^{-1}$ kPa). Although the PL films had greater lipid contents than the flour films, they were more oxygen-permeable than the flour films. The high lipid content of the PL films can explain their poor oxygen barrier capacity, since this produces a heterogeneous distribution, failing to form a continuous lipid phase within the matrix, which would facilitate permeation of oxygen molecules through the film. Ayranci and Tunc (2003) also observed that increasing the stearic acid content of the film (15 g/100 g of methyl cellulose) enhanced the oxygen permeability, and the authors attributed this increase to the formation of holes in the crystal structure of edible films as the stearic acid content increased.

Table 4—Properties of amaranth flour, protein-lipid (PL), and protein (P) films

Properties	Flour	PL	P
Tensile strength (MPa)	1.45 ± 0.04^{a}	1.2 ± 0.1^{b}	1.3 ± 0.1^{a}
Elongation at break (%)	83.7 ± 5.1^{a}	39.0 ± 1.1^{b}	85.7 ± 1.1^{a}
Young's Modulus (MPa)	21.5 ± 1.4^{a}	14.8 ± 0.8^{b}	19.4 ± 2.2^{a}
WVP (g mm h^{-1} m^{-2} kPa)	0.3 ± 0.08^{c}	0.4 ± 0.02^{b}	0.7 ± 0.02^{a}
OP ($cm^3\ \mu m\ m^{-2}\ d^{-1}$ kPa)	5.6 ± 3.7^{c}	33.7 ± 7.4^{a}	18.3 ± 6.6^{b}
Moisture content (%)	18.3 ± 2.2^{b}	17.0 ± 0.6^{b}	23.9 ± 0.5^{a}
Solubility (%)	$42.2 \pm 1.8^{a,b}$	39.9 ± 2.5^{b}	48.9 ± 2.8^{a}
Optical properties			
a^*	-1.2 ± 0.01^{b}	0.4 ± 0.1^{a}	-1.5 ± 0.1^{c}
b^*	8.1 ± 0.5^{c}	18.9 ± 0.2^{a}	13.8 ± 1.7^{b}
L^*	90.0 ± 0.3^{a}	78.3 ± 1.0^{c}	86.9 ± 1.3^{b}
ΔE^*	8.9 ± 0.6^{c}	24.1 ± 1.2^{a}	14.9 ± 1.9^{b}
Opacity	15.2 ± 0.9^{b}	20.3 ± 0.1^{a}	13.5 ± 0.3^{c}

[a]Average ± standard deviation. Different letters (a to c) denote significant difference ($P < 0.05$) between averages obtained by Tukey's test.
[b]Films were conditioned at 25 °C and 58% of relative humidity for 48 h.
[c]Thickness of amaranth flour film: 0.083 ± 0.05 mm.
[d]Thickness of PL and P amaranth films: 0.079 ± 0.03 mm.

These observations can be verified by an analysis of the microstructure of the films presented in Figure 3.

Film solubility and moisture content

The flour and PL films showed no significant differences ($P > 0.05$) between their moisture contents, showing smaller values than the P films (Table 4). The presence of lipid in the flour and PL films increased their hydrophobicity, decreasing water affinity as compared to the P films. Shaw and others (2002) also observed this behavior.

The solubility of the amaranth flour films showed no significant difference ($P > 0.05$) when compared to that of the PL and P films (Table 4). The similarity in solubility of the flour films with those of the PL and P films was also the result of the presence of lipid and protein homogenously distributed throughout the film, interacting with the starch and decreasing its solubility. In addition, some more hydrophobic proteins were found in the flour films, which were not found in the P and PL films, since they were eliminated during the centrifugation step. The presence of these proteins could contribute to an increase in hydrophobicity in certain regions of the flour films.

On the other hand, the solubility of the P films was significantly greater than that of the PL films (Table 4), due to the fact that these films contained no lipid, being formed solely by protein–protein interactions and presenting more exposed hydrophilic groups to react with the water than the PL films.

Independent of their solubility values, the PL and P films did not lose their integrity when immersed in water, but the flour films partially disintegrated due to the presence of starch. However, flour films are less soluble than amaranth starch films, which present solubility values of 62.5% (Tapia-Blácido 2003).

Color and opacity

Table 4 shows the values for the color parameters (a^*, b^*, and L) and for the opacity of the amaranth flour, PL, and P films. The difference in color (ΔE^*) was greatest ($P < 0.05$) for the amaranth PL films, followed by the protein (P) and flour films. This same behavior was also observed for the color parameters a^* and b^* (Table 4). The lower value for ΔE^* and the higher value for L^* of the amaranth flour films could be explained by the presence of starch in these films, thus being explained by its composition.

Table 4 also shows that the protein-based films were more yellow (greater value for b^*) than the flour films. This result indicated that the yellowish color of the flour films would be related to the presence of proteins in their composition, since the amaranth starch films showed b^* values of about 2.0 (Tapia-Blácido 2003).

The flour, PL, and P films showed more color than films based on egg albumin ($\Delta E^* = 1.7$ to 2.3; Gennadios and others 1996) and pigskin gelatin ($\Delta E^* < 3$; Sobral 1999), but presented a color comparable to that of soybean protein films ($\Delta E^* = 8.5$ to 11.6; Kunte and others 1997).

The values for opacity of the flour, PL, and P films presented significant differences ($P < 0.05$). The greater lipid concentration in the PL films associated with phase separation produced an increase in film opacity when compared to the flour and P films. Various other authors have also noted that opacity depended on the lipid concentration of the film matrix (Gontard and others 1994; Shaw and others 2002). This result suggested that the opacity of the flour films was related to the presence of lipid in the biopolymeric matrix. The films produced in this work were more opaque than those made from pigskin gelatin (opacity < 0.5), which were extremely transparent (Sobral 1999).

Conclusions

This study demonstrated that the interactions between the biopolymers (starch and protein) and the lipids formed in the amaranth flour film matrix contributed to the mechanical properties and to the water vapor and oxygen permeability of these films. The flour films showed a strong association between the lipids and the proteins, and these phases were homogenously distributed throughout the starch phase. Stabilization of these phases allowed for the nonobservance of polymorphism transition of the fatty acids (oleic acid) present in the lipid phase, contrary to that observed in PL films. In addition, the nonseparation of the lipid phase of the flour film matrix also contributed to good plasticization and to the excellent barrier properties of the amaranth flour films. Thus the flour film properties were the result of interactions between their components (starch, protein, and lipid) as from the natural state in which they existed in the flour, and the concentration of polymers (starch and protein) and lipid in the film matrix.

Acknowledgments

The authors wish to thank The São Paulo Research Support Foundation (FAPESP), the Natl. Research Council (CONICET) and the Natl. Agency of Scientific and Technological Support (SECyT, PICT 09-12085 and 09-12242) of Argentina, and the CYTED project XI.20, for their financial support.

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