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BIOFILMS BASED ON CANIHUA FLOUR (*Chenopodium Pallidicaule*): DESIGN AND CHARACTERIZATION

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This work aims to (1) produce and characterize the flour obtained from two varieties of canihua, *cupi* and *illpa-inia*, and (2) evaluate the ability of these flours to form biofilms. The flours produced contain proteins, starches, lipids, organic substances containing phenol groups, and high percentages of unsaturated fatty acids. Films produced from the *illpa* variety presented lower water vapor permeability and larger Young's modulus values than the films formed from the *cupi* variety. Both films were yellowish and displayed a high light blocking ability (as compared with polyethylene films), which can be attributed to the presence of phenolic compounds. Furthermore, they showed lesser solubility and water permeability than other polysaccharide films, which may be the result of the higher protein (12%–13.8%) and lipid (11%) contents in canihua flours, as well as the formation of a larger number of S–S bonds. On the other hand, these films presented a single vitreous transition temperature at low temperatures (< 0 °C), crystallization of the A and V_h types, and an additional diffraction peak at $2\theta = 7.5°$, ascribed to the presence of essential fatty acids in canihua flour. Canihua flour can form films with adequate properties and shows promise for potential applications in food packaging, because it acts as a good barrier to incident ultraviolet light.

Keywords: films; biodegradable; canihua; flour.

INTRODUCTION

Over the last decades, several authors have prepared biodegradable films and coatings to explore their properties for food packaging applications. These films can potentially increase the shelf life of many food products, diminish the environmental impact of non-biodegradable packaging, and reduce the traditional dependence on petroleum.[1,2]

Biopolymers originate from natural plant or animal products, such as proteins, polysaccharides, lipids, and resins.[3] The physical and chemical characteristics of the biopolymer will strongly influence the properties of the films and coatings it will form.[4] Starch is one of the most abundant natural polysaccharide raw materials; it is a renewable, inexpensive, and widely available resource. In the last decades, scientists have evaluated the ability of starch to generate films for application in the food packaging area. However, starch can only afford films with satisfactory mechanical properties when it is blended with other materials like lipids, hydrocolloids, fillers, or active compounds.[5-11]

Films consisting of proteins and polysaccharides are designated composite films. Such composites should exhibit improved mechanical and barrier properties as compared with films consisting of polysaccharides alone.[12-14]

Recently, several researchers have employed flour from various sources such as fruit, tubers, grains, and pseudocereals to produce composite films. For example, edible films were prepared from banana, amaranth, rice and achira flours, respectively.[10,15-17] These flours are a combination of proteins, starch, lipids, and fibers, so they constitute an ideal natural blend to fabricate biofilms.[17,18] The features of the resulting flour film will depend on (1) the natural interactions taking place between starch, proteins, and lipids during drying of the filmogenic solution; (2) the distribution of the interactions within the polymeric matrix; (3) the balance between hydrophilic and hydrophobic interactions; and (4) the concentration of each component in the film.[17,18]

The flour obtained from the canihua grain seems to be an interesting alternative to produce biodegradable composite films. Canihua (*Chenopodium pallidicaule*) is an annual pseudocereal belonging to the Chenopodiaceae family; it grows in the Andes of southern Peru and Bolivia at altitudes ranging from 3600 to 4400 m. The canihua grain has high starch content. Carbohydrates and proteins make up 63-66% and 14-18% of its total weight, respectively; these percentages are higher than those found in other cereals and milk protein equivalents.[19] The canihua grain also presents high protein quality—it contains lysine, tryptophan, and isoleucine, not to mention that it is a potential source of dietary fiber (18-25%), iron, calcium, sugars, polyphenols and other antioxidants.[20,21] In addition, the oil of these grains is rich in unsaturated fatty acids and tocopherols.[21,22]

Despite the nutritional value of canihua, its consumption has decreased. Its cultivation is poorly disseminated, and few studies have investigated the nutritional quality of the canihua grain and its use to fabricate new products. This has posed difficulties to the peoples inhabiting the Andean areas of Peru and Bolivia, for whom the cultivation of canihua is an important source of income. Worse still is the fact that some varieties of canihua are in serious danger of extinction.[23] Therefore, the Peruvian government (law nº 29196) and the Food and Agriculture Organization of the United Nations (FAO) have been encouraging the consumption of canihua worldwide, to promote organic production, alleviate poverty, contribute to food security, and conserve the ecosystems and biological diversity of Peru.[24,25]

This work aimed to assess the potential of canihua (*Chenopodium pallidicaule)* flours to form biodegradable films. To this end, we isolated the flours from two little investigated varieties of canihua, *cupi* and *illpa-inia*. We also characterized the mechanical, barrier, optical, structural, and thermal properties of the prepared films.

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MATERIALS AND METHODS

Material

Canihua flours were isolated from canihua grains (*cupi* and *illpa-inia* varieties) supplied by the Agronomical Experimental Station-INIA Puno, Peru. The grains were transported to Brazil, cleaned (small impurities were removed by screening), and stored at 10 ºC. The moisture of the grains canihua *cupi* and *illpa-inia* varieties were 9.62% and 10.56% respectively.

All the reagents were of analytical grade.

Production of canihua flours

The alkaline wet milling method[26] was used to obtain the canihua flour, which was isolated from the canihua grains using a 0.25% sodium hydroxide solution and a steeping time of 24 h, at 5 ºC. The seeds were milled, and the ground mass was passed through 80, 200, and 270 mesh screens. The pH of the suspension obtained during sieving was adjusted to 4.5 with HCl 0.2 mol equi L^{-1}, the suspension was then centrifuged at 4500 × g for 20 min, at 10 ºC, and the supernatant was discarded. The precipitate was neutralized to pH 7.0 with NaOH 0.1 mol equi/L and dried in a forced air convection oven at 37 ºC, for 24 h. After drying, the material was ground in a knife mill (Marconi, model MA340, São Paulo, Brazil), consecutively sieved through screens (80 US mesh), and stored at 5 ºC and 36% RH in a sealed container.

Chemical and thermal properties and color analysis

The ash, protein, and moisture contents were obtained using the standard AOAC methods.[27] The lipid content was calculated by the method of Bligh and Dyer.[28] The onset (T_o), peak (T_p), and final (T_f) gelatinization temperatures as well as the enthalpy (ΔH) were determined with the aid of a differential scanning calorimeter (TA-Instruments, model 2920, Pennsylvania, USA) equipped with a cooling system. The differential scanning calorimetry measurements were performed in triplicate; the results are presented as mean values. Canihua flour samples (varieties *cupi* and *illpa-inia*) were also subjected to color analysis on a colorimeter (UltraScan VIS, HunterLab, Virginia, USA) operating in the reflectance mode, using the CIELAB and illuminant D65 (daylight) classification system.[29] The fatty acid composition of the canihua flour oil was determined via the methodology of Hartmann and Lago.[30]

Film production

The films were produced by the casting method, which consists of drying a film-forming solution (FFS) previously applied to a support. A 4% (w/w d.b.) suspension of canihua flour (variety *cupi* or *illpa-inia*) in water was homogenized in a mixer for 1 h, at room temperature; NaOH 0.1 mol equi L^{-1} was used to adjust the pH to 10.7, to dissolve the protein. The suspension was then heated at 92 ºC for 15 min, under gentle stirring. Next, 20 g of glycerol/100 g of flour was added, and the solution was maintained at this temperature for 15 min. Finally, 70 g of the FFS was poured and spread on Teflon plates (18 × 21 cm) and dried at 45 ºC and 50% relative humidity in an oven (Model MA-415UR, Marconi, Brazil), equipped with a system that controlled the drying temperature and the relative humidity.

Film characterization

Before characterization, the films were conditioned in desiccators containing a saturated NaBr solution under 58% relative humidity, at 25 ºC, for 48 h. Film thickness was measured using a manual micrometer (Fowler, model FOW52-229-001, Pennsylvania, USA), with an accuracy of 0.0001 mm. The mean thickness of each film was determined from an average of 15 random measurements.

Visual aspect

The visual aspect was assessed subjectively; the following parameters were considered: flexibility, ease of handling without risk of rupture, continuity (absence of fissures or cracks after drying), and homogeneity (absence of insoluble particles or opacity zones and uniform coloration).[31]

Moisture content

The moisture content of the film was measured for samples (2 g) collected at the end of the drying process and after conditioning, using the ASTM D644-99 methodology.[32] Three measurements were accomplished for each determination.

Solubility in water

The solubility (S) values of the films were measured the following methodology:[33] initially, three discs (diameter = 2 cm) of each film were stored in a desiccator containing silica gel (0% relative humidity) for 48 h. The samples were weighed, to obtain the initial dry weight (W_i), and immersed in 50 mL of water containing sodium azide (0.02% w/v) at 25 ºC for 24 h, under sporadic agitation. After this period, the solution containing the film discs was filtered, the insoluble matter was dried at 105 ºC for 24 h, and the resulting material was weighed, to determine the final dry weight (W_f). Analyses were carried out in quadruplicate, and the solubility of the films in water (%) was computed according to Equation (1):

$$S = \frac{W_i - W_f}{W_i} \times 100, \tag{1}$$

where W_i and W_f are the initial and the final dry weight of the sample (g), respectively.

Mechanical properties

The mechanical tests were conducted on a texture analyzer TA.XT2i (SMS, Surrey, England). The tensile strength and elongation at break were obtained according to the ASTM D882-02 method.[34] The secant modulus is the slope of the line connecting the origin and a given point on the stress–strain curve; it was calculated by dividing the corresponding stress value by the designated strain (1%) on the stress–strain curve. This calculation was performed using the software Exponent (SMS, 2003). Five measurements were accomplished for each mechanical test.

Water vapor permeability

The water vapor permeability (WVP) was determined by gravimetry, using the standard method E96-95 with modifications.[35] WVP was measured using appropriate capsules and three different ranges of relative humidity gradient (ΔRH = 2–33%, ΔRH = 33–64%, and ΔRH = 64–90%); the system was stored in a desiccator containing different saturated salt solutions of known equilibrium relative humidities (RH, water activities), at 25 ºC.[6] Silica gel was used to ensure minimal moisture condition for the first gradient (~0% RH). Analyses were conducted in triplicate for each relative humidity range. WVP was calculated using Equation (2):

$$WVP = \frac{W\delta}{AP_s(a_{w1} - a_{w2})}, \tag{2}$$

where δ is the mean sample thickness (m), P_s is the saturation pressure of the vapor at the experimental temperature at 25 ºC (Pa), A is the sample permeation area (m^2), a_{w1} and a_{w2} are the water activities in the interior of the desiccator and in the interior of the capsule, respectively, and W = G/t (g of water/h, calculated using the linear regression of mass variation over time under steady-state permeation condition).

Color and opacity

The color, represented as color difference (ΔE*), was determined;[36] the opacity was analyzed by means of the HunterLab method.[37] A colorimeter (HunterLab, model Miniscan XE) was employed in both cases. The difference in color was calculated as:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}\ , \tag{3}$$

where ΔL^*, Δa^*, and Δb^* are the differences between the color parameter of the samples and of the white standard ($L^* = 93.49$, $a^* = -0.77$, $b^* = 1.40$) used as film background.

Light transmittance (Tr)

Film samples were cut into rectangles (1 cm × 3 cm). Light transmittance was measured by transmittance (%) using a UV-visible spectrophotometer (Varian Model Gary 1G, Mulgrave, Australia) operating in the wavelength range of 190–780 nm, with an accuracy of 0.1 nm. Three replicates of each film were tested.

Differential scanning calorimetry (DSC)

The thermal properties of the flours and the films were analyzed on a differential scanning calorimeter (TA-Instruments, model 2920, Pennsylvania, USA) equipped with a cooling system. Prior to the determination, the samples were weighed (8 mg) in aluminum pans and conditioned in hermetically sealed flasks containing a saturated NaBr solution (58% RH) at 25 ºC, for seven days. For the analysis, the sample pans were hermetically sealed and scanned at a heating rate of 10 ºC min^{-1} over a temperature range of -70 to 150 ºC. A sealed, empty aluminum pan was used as reference for all the DSC runs. The glass transition temperature (T_g) was considered to be the inflexion point of the baseline, caused by the discontinuity of the specific heat of the sample. To calculate the melting temperature, the onset (T_o), peak (T_p), and final (T_f) temperatures of the endothermic phenomenon were considered. All these properties were obtained from the thermograms of the samples using the Universal Analysis 3.9A software. The DSC measurements were performed in triplicate, and the results are presented as mean values.

X-ray diffraction

Film samples were cut into small pieces (2 cm × 2 cm) and dehydrated in a desiccator containing silica gel (~0% RH) for three weeks. X- ray diffraction analysis was performed using an X-ray diffractometer (Siemens, model D5005, Baden-Württemberg, Germany) operated at a voltage of 40 kV and a current of 30 mA; the target was Cu. The diffraction data of the samples were collected over angles ranging from 5 to 70º (2θ), at a scanning rate of 1.2º min^{-1}. The crystallinity index (%) of the films was quantitatively estimated as the ratio of crystalline area to the total area of the diffractogram,[38] using the Origin 8.0 software (OriginLab Corporation, Massachusetts, USA). Therefore, a smooth curve connecting the peak baselines is plotted on the diffractograms. The area above the smooth curve (upper diffraction peak area) corresponds to the crystalline portion, while the lower area between the smooth curve and a linear baseline connecting the lower points of the diffraction pattern corresponds to the amorphous section. The crystallinity index (Ic) was calculated using Equation (4)

$$I_C = \frac{A_c}{(A_c + A_a)}\ , \tag{4}$$

where: I_C refers to the degree of crystallinity; A_c refers to the crystallized area on the X-ray diffractogram; A_a refers to the amorphous area on the X-ray diffractogram

Scanning electron microscopy (SEM)

To evaluate the microstructure, the surface and cross-section of the films were analyzed by SEM. The film samples were cut into small pieces (2 cm × 2 cm) and dehydrated in a desiccator containing silica gel (~0% RH) for three weeks. After this period, the dried samples were fractured with the help of tweezers, to obtain small fragments. Samples of these fragments were fixed on aluminum stubs by means of a double-sided tape and were then coated with a layer of gold (Sputter Coater Polaron, model SC7620), to improve conductivity. The coated samples were viewed under a scanning electron microscope (LEO, model LEO 440i, Cambridge, England) operating at an acceleration voltage of 15 kV.

Statistical analysis

An analysis of variance (ANOVA) and a Tukey test of multiple comparisons with a significance level of 5% were run using the Statistic 7.0 software for comparison of the differences between the means of the properties of the canihua flour films.

RESULTS AND DISCUSSION

Raw material characterization

The flours isolated from canihua grains of the varieties *cupi* and *illpa-inia* did not differ significantly in terms of the lipid, ash, or starch content; however, they were statistically different with respect to the moisture and protein contents (Table 1). The canihua flour from variety *illpa-inia* presented higher protein content.

DSC furnished the temperature range in which gelatinization occurred (T_0, T_p and T_f). The canihua flour from variety *illpa-inia* presented lower gelatinization temperatures and enthalpy (T_0, T_p, T_f, and ΔH). The gelatinization temperatures were close to values reported by Steffolani *et al.*[39] in the case of canihua starch. The canihua flours obtained here displayed larger lipid but smaller protein and ash contents as compared with the whole flour and grains of canihua of the *cupi* variety reported by others researchers.[40,23] The color parameters and ΔE values of the canihua flour varieties investigated in this work differed significantly—the variety *illpa-inia* afforded lower b*, L*, ΔE values and was less yellowish. Rosell *et al.*[40] described larger a* and b* but smaller L* values for the whole canihua flour, which displayed a brownish color.

Table 1. Chemical composition, thermal properties, and optical properties of the canihua flour

Component	Canihua flour (*Cupi*)	Canihua flour (*Illpa-inia*)
Moisture content (%)	9.6 ± 0.1^{b}	8.3 ± 0.1^{a}
Protein (% d. b.)	12.3 ± 0.3^{a}	$13.8 \pm 0,2^{b}$
Fat (% d. b.)	11.0 ± 0.8^{a}	10.7 ± 0.2^{a}
Ash (% d. b.)	1.6 ± 0.02^{a}	1.55 ± 0.01^{a}
Starch (% d. b.)	$74.6 \pm 0.4^{a*}$	$73.9 \pm 0.04^{a*}$
T_0 (ºC)	58.9 ± 1.2^{a}	58.3 ± 0.3^{a}
T_p (ºC)	64.8 ± 0.3^{b}	62.8 ± 0.2^{a}
T_f (ºC)	72.9 ± 2.0^{b}	70.9 ± 0.2^{a}
ΔH	4.8 ± 0.6^{b}	3.5 ± 0.3^{a}
a^*	1.7 ± 0.1^{a}	1.2 ± 0.2^{a}
b^*	11.1 ± 0.3^{b}	8.9 ± 0.4^{a}
L^*	76.9 ± 0.5^{b}	73.9 ± 0.1^{a}
ΔE	9.1 ± 0.3^{b}	6.3 ± 0.4^{a}

Different letter superscripts in the same line indicate a statistically significant difference ($p < 0.05$).

Table 2 summarizes the fatty acids profile of the oils extracted from the canihua flours of the varieties *cupi* and *illpa-inia*. The extracted oils presented higher percentage of linoleic acid (C18:2), followed by oleic (C18:1), palmitic (C16:0), linolenic (C18:3), and stearic (C18:0) acids; 75 and 25% of the fatty acids were unsaturated and saturated, respectively. Repo-Carrasco *et al.*[22] reported lower values of linoleic and oleic acids and higher values of palmitic acid for the oil extracted from canihua grains. According to the Tukey test ($p < 0.05$), the oil extracted from the canihua flour of the variety *illpa-inia* contained slightly larger percentage of linolenic acid (C18:3, 5.69%) as compared with the oil extracted from the canihua flour of the variety *cupi*.

Table 2. Fatty acid profiles of lipids extracted from the flours of canihua seeds

Fatty acid (%)	Canihua flour (*Cupi*)	Canihua flour (*Illpa-inia*)
C14:0	0.169 ± 0.01^{a}	0.164 ± 0.00^{a}
C15:0	0.06 ± 0.00^{a}	0.07 ± 0.01^{a}
C16:0	14.6 ± 0.5^{a}	14.0 ± 0.1^{a}
C16:1	0.31 ± 0.00^{a}	0.29 ± 0.12^{a}
C17:0	0.078 ± 0.005^{a}	0.079 ± 0.017^{a}
C17:1	0.078 ± 0.022^{a}	0.074 ± 0.013^{a}
C18:0	1.54 ± 0.17^{a}	1.40 ± 0.00^{a}
C18:1	$25.9 \pm 0.4^{a,b}$	24.8 ± 0.1^{a}
C18:2	46.9 ± 1.0^{a}	48.5 ± 0.1^{a}
C18:3	5.3 ± 0.1^{a}	5.7 ± 0.0^{b}
C20:0	$0.7 \pm 0.01^{a,b}$	0.8 ± 0.041^{b}
C20:1	$1.03 \pm 0.02^{a,b}$	$1.03 \pm 0.00^{a,b}$
C22:0	0.65 ± 0.02^{a}	0.64 ± 0.02^{a}
C22:1	0.78 ± 0.03^{a}	0.78 ± 0.01^{a}
C24:0	0.26 ± 0.02^{a}	0.21 ± 0.05^{a}

Different letter superscripts in the same line indicate a statistically significant difference ($p < 0.05$).

Film characterization

Visual appearance and optical properties

Figure 1 reveals that the canihua films obtained from the flour of canihua grains of the variety *cupi* and *illpa-inia* (FCC and FCII, respectively) displayed homogeneous and continuous surface, without fissures or insoluble particles. Both FCC and FCII exhibited good handling and flexibility properties, which enabled their easy manipulation during the characterization procedures, with no risk of rupture. The biofilms were yellowish and had good visual aspect.

Table 3 lists the color and opacity parameters measured for FCC and FCII. Both FCC and FCII exhibited similar color difference values (ΔE*), which were higher than those reported for the films prepared from banana, achira, or amaranth flour.[10,17,26] The proteins present in the biofilm flours could affect the ΔE* of the films.[26] The b* values attested that FCC and FCII had intense yellow color, but FCII was lighter than FCC. Indeed, the b* parameter was lower in the former case ($b^* = 24.10 \pm 0.39$ and 31.94 ± 1.5 for FCII and FCC, respectively). Other researchers[41] described similar b* values for films prepared from soybean protein ($b^* = 32.54$). Proteins and pigments like carotenoids and flavonoids could account for the yellow color of the canihua flour films.[10,42] Repo-Carrasco-Valencia *et al.*[21,22] reported that flavonoids and tocopherols exist in canihua oil and grains.

The a* parameter was positive for both FCII and FCC, indicating typical red shades. The opacity of these films was similar: 51.4 ± 1.2% and 56.7 ± 0.6% for FCC and FCII, respectively. These values were much higher than reported for amaranth flour films.[18] The large opacity of the films could stem from the high contents of proteins, lipids, fibers, and phenolic compounds present in the flours.[10]

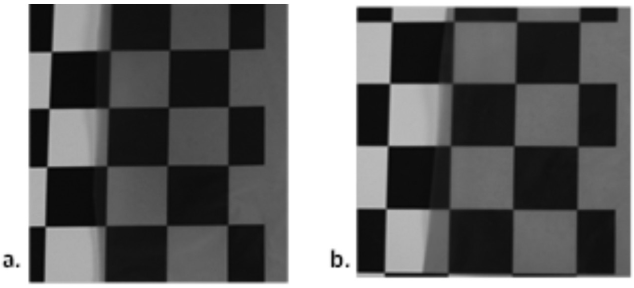

Figure 1. *Visual appearance of canihua films of the varieties (a) cupi and (b) illpa-inia*

Table 3. Optical properties of canihua flour films (*Chenopodium pallidicaule*)

	FCC	FCII
a^*	2.8 ± 0.4^{a}	2.7 ± 0.2^{a}
b^*	31.9 ± 1.5^{b}	24.1 ± 0.4^{a}
L^*	61.9 ± 1.1^{a}	61.7 ± 0.7^{a}
ΔE	44.9 ± 1.4^{a}	39.3 ± 0.8^{a}
Opacity (%)	51.4 ± 1.2^{a}	$56.7 \pm 0{\cdot}6^{a}$

FCC= Films prepared from the canihua flour of the variety *Cupi*, FCII= Films prepared from the canihua flour of the variety *Illpa-Inia*. Different letter superscripts in the same line indicate a statistically significant difference ($p < 0.05$).

Mechanical properties of canihua flour films

Table 4 depicts the tensile strength, elongation at break, and Young modulus values for FCC and FCII. Tensile strength corresponds to the resilience of the material to support traction stresses and depends largely on the intensity and distribution of the intra- and intermolecular interactions within the polymeric matrix.[43] According to Table 4, FCII and FCC had similar tensile strength values. However, FCII had lower elongation and higher Young modulus values than FCC. The high tensile strength and Young modulus as well as the low elongation values detected for the biofilms evaluated in this work may have resulted from protein denaturation during the process of alkaline extraction of the canihua flour. This process may have elicited reticulated intra- and intermolecular bonds involving protein S-S bonds,[44,45] to give more rigid films. These films should present weaker interactions between polymers and the lipids or antioxidants present in the polymeric matrix, but stronger intra- and intermolecular interactions between the polymers. On the other hand, the larger Young modulus values registered for FCII could arise from the higher protein content in the original flour (Table 1), as reported by other authors.[10]

Table 4. Properties of the canihua flour films

Property	FCC	FCII
Moisture content (%)	14.8 ± 0.3^{a}	13.8 ± 0.5^{a}
Solubility in water (%)	35.8 ± 1.6^{a}	33.9 ± 0.9^{a}
Tensile strength (MPa)	6.3 ± 0.2^{a}	6.0 ± 0.7^{a}
Elongation at break (%)	23.6 ± 2.4^{a}	14.9 ± 1.6^{b}
Young's modulus (MPa)	379.7 ± 33.2^{a}	456.3 ± 56.7^{b}

FCC= Films prepared from the canihua flour of the variety *Cupi*, FCII= Films prepared from the canihua flour of the variety *Illpa-Inia*. Different letter superscripts in the same line indicate a statistically significant difference ($p < 0.05$).

Solubility in water (S) and moisture content

The solubility values are shown at Table 4. The Tukey test revealed that FCC and FCII had not significantly different moisture and S values. Both canihua films were less soluble than other flour films, such as those prepared from amaranth15 and achira17 flours (S = 42.2 ± 1.8 and 38.3 ± 1.9, respectively). The lower solubility of certain flour films was attributed to the presence of lipids and proteins homogeneously distributed throughout the starch matrix, which should improve hydrophobicity and reduce solubility.[15] On the other hand, the lower solubility and moisture of both FCC and FCII could stem from protein denaturation during the alkaline extraction procedure, as mentioned previously. This should prompt stronger protein-protein (reticulated) interactions and the establishment of hydrophobic bonds (Van der Waals forces) as well as the formation of a larger number of S-S bonds.[44] The overall result should be lower film solubility. Other authors[45] verified a similar behavior for films consisting of proteins extracted from amaranth flour using NaOH.

Water vapor permeability (WVP)

We determined WVP at three gradients of relative humidity (RH), low (2-33%), medium (33-65%), and high (65-90%), at 25 ºC. This methodology is interesting to determine the kind of food for which this packing material is useful.[6] As expected, Figure 2 evidences that the WVP of the canihua films FCC and FCII rose with increasing RH, due to the hydrophilic nature of the biopolymers. The WVP of FCC and FCII ranged from 0.25×10^{-10} to 1.73×10^{-10} g m^{-1} s^{-1} Pa^{-1} and from 0.27×10^{-10} to 1.18×10^{-10} g m^{-1} s^{-1} Pa^{-1}, respectively. In the medium (33-64%) and high (64-95%) range of RH, the film FCII displayed lower WVP values as compared with the film FCC, which might have resulted from the higher protein content in the canihua flour of the variety *illpa-inia* (13.8% d.b.). The proteins present in this flour may be homogeneously distributed along the film, increasing its hydrophobicity and decreasing its affinity for water and hence the WVP.[15] The presence of larger hydrophobic sites in the structure of the film FCII should make diffusion of water molecules through the biofilm difficult. The higher hydrophobicity of the film FCII also affected the elongation values: this film furnished lower elongation data (see Table 4).

Light transmittance (tr)

Light barrier measures ultraviolet and visible light transmission; it is expressed as a percentage of light transmission within a wavelength range.[46]

Figure 3 illustrates the Tr percentage in the films FCC and FCII within the wavelength of 190 to 780 nm as compared with polyethylene (PE) films; air transmittance was the reference.

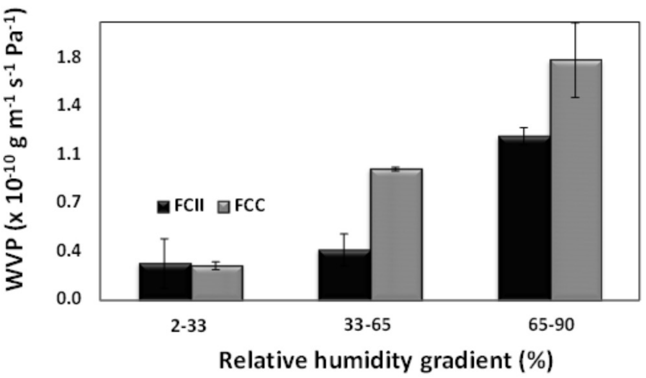

Figure 2. Water vapor permeability of canihua films of the varieties (FCC) cupi and (FCII) illpa-inia

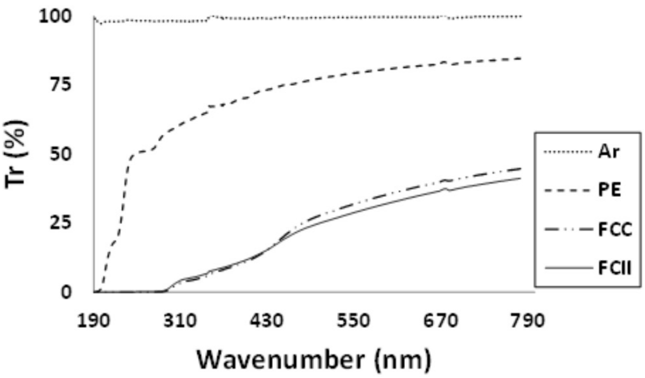

Figure 3. Light transmittance (Tr) of canihua films of the varieties (FCC) cupi and (FCII) illpa-inia and light transmittance of air (Ar) as compared with light transmission of a polyethylene film (PE)

The films FCC and FCII had significantly lower Tr values than the PE film with similar thickness. At a wavelength of 300 nm, the films PE, FCC, and FCII afforded Tr values of 59, 1.9, and 2.8%, respectively. The low Tr registered for FCC and FCII as compared with PE could be due to the phenolic compounds present in canihua, which account for the yellowish color of these films.[21,23] Organic substances containing phenol groups bearing intramolecular hydrogen bonds absorb UV light to a certain extent.[47] In this way, they dissipate UV radiation in the form of thermal energy by means of photophysical reactions. Other authors also observed that the addition of phenolic compounds or antioxidants diminished the UV light transmittance of chitosan films.[48]

Differential scanning calorimetry (DSC)

Figure 4 presents the DSC thermograms of the films FCC and FCII. Both films displayed a single vitreous transition temperature at low temperature (< 0 ºC), followed by an endothermic fusion peak. FCII had higher T_g than FCC— -30.5 ± 0.7 ºC and -32.4 ± 0.4 ºC, respectively—as attested by the Tukey test ($p < 0.05$). This T_g value is related to the fraction enriched with plasticizer, as reported by other researchers for starch and protein films.[8,15,49] Indeed, several authors have described that the addition of plasticizers, like water, glycerol, or fatty acids, tends to reduce the T_g.[7,8,50-52] For water, T_g is -134 ºC; in the case of glycerol, T_g ranges from -50 to -80 ºC.[52,53] Hence, the negative T_g values obtained for both FCC and FCII may be related to the plasticizer and moisture content.

We did not detect T_g at high temperatures, which indicated good incorporation of starch, proteins, and lipids into the polymeric matrix, to give stable films without phase separation, but with good mechanical resistance and low elongation at room temperature.

On the other hand, the thermograms of the films FCC and FCII evidenced an endothermic peak at low temperatures (< 0 ºC), which was higher for FCC as compared with FCII (-7.3 ± 0.5 °C and -11.5 °C, respectively). This peak could correspond to the polymorphic transformation of fatty acids from the γ to the α form, which is typical of oleic acid (-3 °C), as reported for amaranth protein-lipid films with melting temperature at -20 °C.[15] Recently, was described a similar behavior for starch films containing glycerol at a starch/glycerol ratio of 1:0.25 as well as palmitic, stearic, or oleic acid, stored at different RH values.[8] These authors[8] verified an endothermic peak for the starch-glycerol films upon addition of the fatty acids. The films containing oleic acid displayed an endothermic fusion peak with T_o = -12 °C, T_p = 6.8 °C, and T_f = 30.0 °C at 53% RH. The films containing palmitic or stearic acid also presented an endothermic fusion peak at temperatures higher than 46 °C.

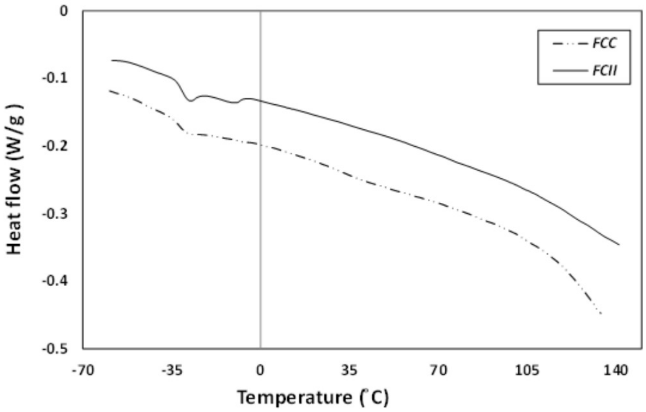

Figure 4. *DSC thermograms of canihua films of the varieties (FCC) cupi and (FCII) illpa-inia*

X-ray diffraction (XRD)

It is important to investigate the crystallinity of biodegradable films, because these studies explain other film properties. In fact, most physical, mechanical, and thermodynamic features of semicrystalline polymers depend on the crystallinity index and morphology of the crystalline regions. The higher the film crystallinity, the better its density, rigidity, mechanical resistance, melting temperature (T_m), and vitreous transition temperature (T_g), but the lower its elongation values.[54] Figure 5 brings the XRD patterns of the films FCC and FCII.

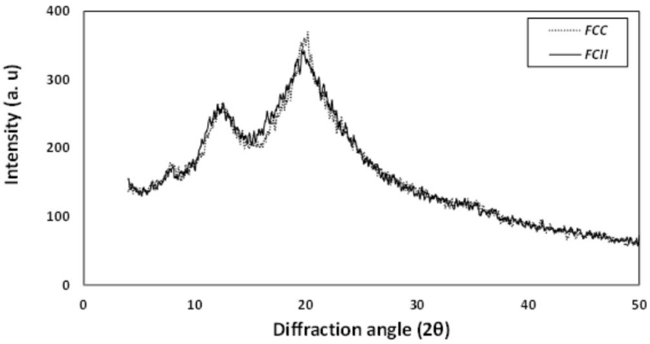

Figure 5. *X-ray diffraction patterns of the canihua films of the varieties (FCC) cupi and (FCII) illpa-inia*

Both FCC and FCII displayed semicrystalline features (amorphous zones and crystalline polymorphs), with diffraction peak at 2θ = 12°, typical of crystalline polymorph type-A and characteristic of cereals.[55,56] These results agreed with data reported by other researchers,[55] who described similar diffraction for the native corn starch. According to these authors, cereals starch present crystallinity with densely packed double helices. The canihua films FCC and FCII also exhibited crystallinity of the V_h type, with intensity peaking at 2θ close to 12 and 19.7°. V_h crystallinity stems from the complexation of amylose with compounds like fatty acids, emulsifiers, surfactants, or glycerol after starch gelatinization.[8,57] This type of crystallinity can originate from rapid amylose recrystallization or retrogradation after starch gelatinization; i.e., during cooling of the filmogenic solution used to prepare the film. Starch initially goes from an amorphous to a crystalline state where the starch molecules rearrange into a more organized structure. This process probably entraps lipids (fatty acids or glycerol) and other non-polar organic components present in the polymeric system, to generate simple conjugations, helices, and crystalline structures or starch-lipid complexes.[8,10,52,58,59] Recently, other authors reported similar 2θ values for crystallinity of the V_h type: 12 and 19°, in the case of banana flour and starch,[10] and 20° for corn starch-glycerol-fatty acids films.[8]

Apart from the crystallinity peaks mentioned previously, FCC and FCII also presented a diffraction peak at 2θ = 7.5°, characteristic of oleic acid crystals. In spite of oleic acid being liquid at the storage temperature, there was a partial crystallization of oleic acid induced in the starch-lipid interface during storage of the films.[58] Recently, other authors[8] also reported a similar behavior for oleic acid in starch-glycerol films, with 2θ peaking at 7.1°. This behavior might have resulted from an ordered oleic acid arrangement in the lipid-starch interface, where organization into a crystalline structure limited molecular mobility.[8,58] The crystallinity of starch-based films depends on various factors such as amylose/amylopectin ratio, starch source, filmogenic solution drying, film storage conditions (temperature and RH), and plasticizer content.[10,60] Crystallization is favored by groups that promote strong secondary intermolecular bonds (like polar groups) or hydrogen bonds.[54] The crystallinity indices of FCC and FCII were 14.3 and 13.8%, respectively. These values were higher than reported for banana flour films.[10]

Scanning electron microscopy (SEM)

Figure 6 depicts the SEM micrographs of the films FCC and FCII, which presented a dense structure, typical of films containing proteins in the polymeric matrix. Other authors also observed a dense structure for amaranth[18] and soybean[61] protein films, respectively.

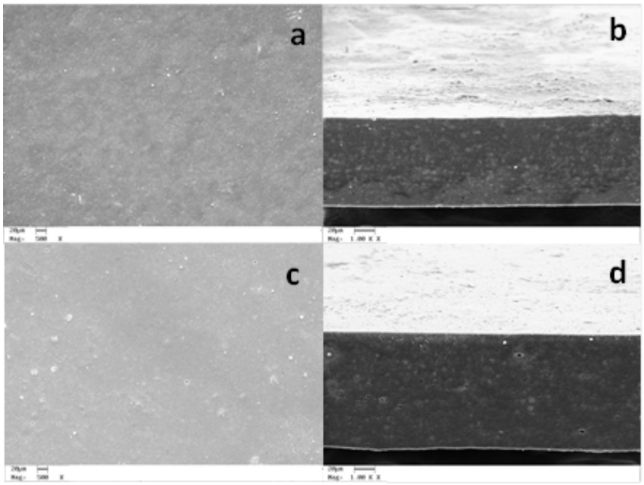

Figure 6. *Scanning electron microscopy of canihua flour films.* ***a.*** *Surface of the film FCC,* ***b.*** *Cross-sections of the film FCC,* ***c.*** *Surface of the film FCII* ***d.*** *Cross-sections of the film FCII*

The canihua films displayed dense cross-section, because the lipids and the other biopolymers present in the polymeric matrix interacted well;[18] there was no lipid phase separation. This behavior of the polymeric matrix may be related to the single T_g value verified for the canihua films.

CONCLUSIONS

The canihua flour can form films with adequate properties and constitutes a promising alternative source of biofilms. The films present excellent handling features, good functional characteristics, high flexibility, and moderate mechanical resistance. Both films investigated here display a single vitreous transition temperature (T_g) at low temperatures (< 0 ºC), related to the fraction enriched with plasticizer. Also, they present X-ray diffraction pattern of the V_h type. The evaluated biofilms have promising application in food packaging, because they act as good barrier to the incident ultraviolet light.

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REFERENCES

1. Cutter, C. N.; Sumner S.S. *Protein-based Films and Coatings*; Gennadios A., ed.; CRC Press: Boca Raton, 2002, cap. 18.
2. Briassoulis, D. J.; *J. Polym. Environ*. **2006**, *14*, 289.
3. Jones, O.; Mcclements, D.; *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 374.
4. Sothornvit, R.; Krochta, J. M.; *J. Agric. Food Chem*. **2000**, *48*, 6298.
5. Lu, Y. S.; Weng, L. H.; Cao, X. D.; *Macromol. Biosci*. **2005**, *5*, 1101.
6. Muller, C. M. O.; Laurindo, J. B.; Yamashita, F.; *Food Hydrocolloids* **2009**, *23*, 1328.
7. Enrione, J.; Osorio, F.; Pedreschi, F.; Hill, S.; *Food Bioprocess Technol*. **2010**, *3*, 791.
8. Jiménez, A.; Fabra, M. J.; Talens, P.; Chiralt, A.; *Food Hydrocolloids* **2013**, *30*, 408.
9. Bonilla, J.; Talón, E.; Atarés, L.; Vargas, M.; Chiralt A.; *J. Food Eng*. **2013**, *118*, 271.
10. Pelissari, F. M.; Andrade-Mahecha, M. M.; Sobral, P. J. A.; Menegalli, F. C.; *Food Hydrocolloids* **2013**, *30*, 681.
11. Farias, M. G.; Fakhouri, F. M.; Piler de Carvalho, C. W.; Ascher, J. L. R.; *Quim. Nova* **2012**, *35*, 546.
12. Mokrejs, P.; Langmaier, F.; Janacova, D.; Mladek, M.; Kolomaznik, K.; Vasek, V. J.; *Therm. Anal. Calorim*. **2009**, *98*, 299.
13. Kaushik, A.; Singh, M.; Verma, G.; *Carbohydr. Polym*. **2010**, *82*, 337.
14. Vicentino, S. L.; Floriano, P. A.; Dragunski, D. C.; Caetano, J.; *Quim. Nova* **2011**, *34*, 1309.
15. Tapia-Blácido, D. R.; Mauri, A. N.; Menegalli, F. C.; Sobral, P. J. A.; Añon, M. C.; *J. Food Sci*. **2007**, *72*, 293.
16. Dias, A. B.; Müller, C. M. O.; Larotonda, F. D. S.; Laurindo, J. B.; *J. Cereal Sci*. **2010**, *51*, 213.
17. Andrade Mahecha, M. M.; Tapia-Blácido D. R.; Menegalli, F. C.; *Carbohydr. Polym*. **2012**, *88*, 449.
18. Tapia-Blácido, D. R.; Sobral, P. J. A.; Menegalli, F. C.; *J. Food Eng.* **2005**, *67*, 215.
19. White, P.; Alvistur, E.; Dias, C.; Vinas, E.; White, H.; Collazos, C.; J. *Agric. Food Chem.* **1955**, *6*, 531.
20. Peñarrieta, J. M.; Alvarado, J. A.; Åkesson, B.; Bergenståhl, B.; *Mol. Nutr. Food Res*. **2008**, *52*, 708.
21. Repo-Carrasco-Valencia, R.; Hellström, J. K.; Pihlava, J. M.; Mattila, P. H.; *Food Chem.* **2010**, *120*, 128.
22. Repo-Carrasco, R.; Espinoza, C.; Jacobsen, S.; *Food Res. Int*. **2003**, *19*, 179.
23. Repo-Carrasco-Valencia, R.; *Plant Foods Hum. Nutr*. **2009**, *64*, 94.
24. Gonzales L.; Estrada A.; Ley de promoción de la producción orgánica o ecológica, ley N° 29196, Congreso da república del Perú, 29 de enero de 2008.
25. http://www.rlc.fao.org/es/agricultura/produ/cdrom/contenido/libro07/Cap3_3.htm#2, acessada em Junho 2011.
26. Tapia-Blácido, D. R.; *Dissertação de mestrado*, Universidade Estadual de Campinas, Brasil, 2003.
27. AOAC, Official Methods of Analysis, 18th ed., Association of Official Analytical Chemists, Washington, USA, 2005.
28. Bligh, E. G.; Dyer, W. J.; Can.; *J. Physiol. Biochem*. **1959**, *37*, 911.
29. HunterLab, Universal Software Version 3.8, User's Manual. Hunter Associates Laboratory, Reston, VA, USA, 2001.
30. Hartmann, L.; Lago, R.; *Lab. Pract.* **1973**, *22*, 475.
31. Carvalho, R. A.; *Tese de Doutorado*, Universidade Estadual de Campinas, 2002.
32. ASTM. Designation D 644-99: Standard test method for moisture content of paper and paperboard by oven drying. In Annual Book of ASTM Standards. Philadelphia: American Society for Testing and Materials. 1999.
33. Gontard, N.; Guilbert, S.; Cuq, J. L.; *J. Food Sci.* **1992**, *57*, 190.
34. ASTM. Designation D 882-02: Standard test method for tensile properties of thin plastic sheeting. In Annual Book of ASTM Standards. Philadelphia: American Society for Testing and Materials. 2002.
35. ASTM. Designation E 96-95: Standard method for water vapor transmission of materials. In Annual Book of ASTM Standards. Philadelphia: American Society for Testing and Materials. 1995.
36. Gennadios, A.; Weller, C. L.; Handa, M. A.; Froning, G. W.; *J. Food Sci.* **1996**, *61*, 585.
37. Sobral, P. J. A.; *Ciência Engenharia*, **1999**, *8*, 60.
38. Nara, S.; Komiya, T.; *Starch/Stärke* **1983**, *35*, 407.
39. Steffolani, M. E.; Leon, A. E.; Perez, G. T.; *Starch - Stärke* **2013**, 1.
40. Rosell, C. M.; Cortez, G.; Repo-Carrasco, R.; *Cereal Chem.* **2009**, *86*, 386.
41. Soliman, E. A.; Tawfik, M. S.; Sayed, H. E.; Moharam, Y. G.; *Am. J. Food Technol*. **2007**, *2*, 462.
42. Rayas, L. M.; Hernandez, R. J.; Ng, P. K.W.; *J. Food Sci.* **1997**, *62*, 1.
43. Bilbao-Sainz, C.; Avena-Bustillos, R. J.; Wood, D.; Williams, T. G.; McHugh, T. H. ; *J. Agric. Food Chem*. **2010**, *58*, 3753.
44. Valenzuela, C.; Abugoch, L.; Tapia, C. ; Gamboa, A.; *Int. J. Food Sci. Technol*. **2013**, *48*, 843.
45. Condés, M. C.; Añón, M. C.; Mauri, A. N.; *J. Food Eng.* **2013**, *119,* 573.
46. Sarantópoulos, C. I. G. L.; Oliveira, L. M.; Padua, M.; Coltro, L.; Alves, R. M.; Garcia, E. E. C.; *Embalagens Plásticas Flexíveis - Principais Polímeros e Avaliação de Propriedades*, CETEA/ITAL: Campinas, 2002.
47. Sakai, K.; Takahashi, S.; Kobayashi, A.; Akutagawa, T.; Nakamura, T.; Dosen, M.; Kato, M.; Nagashima, U.; *Dalton Trans*. **2010**, *39*, 1989.
48. Wang, L.; Dong, Y.; Men, H.; Tong, J.; Zhou, J.; *Food Hydrocolloids* **2013**, *32*, 35.
49. Araujo Farro, P. C.; *Tese de Doutorado*, Universidade Estadual de Campinas, Brasil, 2008.
50. García, M. A.; Martino, M. N.; Zaritzky, N. E.; *J. Food Sci*. **2000**, *65*, 941.
51. Moore, G. R. P.; Martellia, S. M.; Gandolfoa, C.; Sobral, P. J. A.; Laurindo, J. B.; *Food Hydrocolloids* **2006**, *20*, 975.
52. Jiménez, A.; Fabra, M. J.; Talens, P.; Chiralt, A.; *Food Bioprocess Technol*. **2012**a, *5*, 2058.
53. Bergo, P. V. A.; Carvalho, R. A.; Sobral, P. J. A.; dos Santos, R. M. C.; da Silva, F. B. R.; Prison, J. M.; *Packag. Technol. Sci.* **2008**, *21*, 85.
54. Canevarolo Jr., S.; *Ciência dos Polimeros*, 2ª ed., Artiiber Editora Ltda.: São Paulo, 2006.
55. Pushpadass, H. A.; Marx, D. B.; Wehling, R. L.; Hanna, M. A.; *Cereal Chem*. **2009**, *86*, 44.

56. Carvalho, A. J. F.; *Monomers, polymers and composites from renewable resources*, Belgacem, M. N.; Gandini, A., eds.; Elsevier: Amsterdam, 2008, cap. 15.
57. Famá, L.; Rojas, A.; Goyanes, S.; Gerschenson, L.; *LWT--Food Sci. Technol.* **2005**, *38*, 631.
58. Jiménez, A.; Fabra, M. J.; Talens, P.; Chiralt, A.; *Food Hydrocolloids* **2012b**, *26*, 302.
59. Vázquez, A.; Álvarez, V. A.; *Biodegradable polymer blends and composites from renewable resources*; Yu, L., ed.; Wiley: New York, 2009, p. 241-286.
60. Van Soest, J. J. G.; Vliegenthart, J. F. G.; *Trends Biotechnol*. **1997**, *15*, 208.
61. Denavi, G.; Tapia-Blácido, D. R.; Añón, M. C.; Sobral, P. J. A.; Mauri, A. N.; Menegalli, F. C.; *J. Food Eng*. **2009**, *90*, 341.