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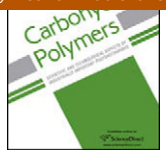

Development and optimization of biodegradable films based on achira flour

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ABSTRACT

The influence of glycerol concentration (C_g), process temperature (T_p), drying temperature (T_s), and relative humidity (RH) on the properties of achira flour films was initially assessed. The optimized process conditions were C_g of 17 g glycerol/100 g flour, T_p of 90 °C, T_s of 44.8 °C, and RH of 36.4%. The films produced under these conditions displayed high mechanical strength (7.0 MPa), low solubility (38.3%), and satisfactory elongation values (14.6%). This study showed that achira flour is a promising source for the development of biodegradable films with good mechanical properties, low water vapor permeability, and solubility compared to films based on other tubers.

1. Introduction

Concentrated efforts toward the development of biodegradable materials with properties that ensure food safety and security while minimizing the environmental impact of their use has been the focus of different studies. To produce such films, researchers have usually employed commercial biopolymers and lipids, which are then mixed during film processing (Arvanitoyannis, 1999; Arvanitoyannis & Biliaderis, 1998, 1999; Arvanitoyannis, Nakayama, & Aiba, 1998; Arvanitoyannis, Psomiadou, & Nakayama, 1996; Psomiadou, Arvanitoyannis, & Yamamoto, 1996). Results have not always been favorable due to the thermodynamic incompatibility of biopolymers, which may also cause phase separation (Grinberg & Tolstoguzov, 1997). To overcome this problem, researchers have turned to natural mixtures of starch, protein, lipids, and fibers, which can be obtained in the form of flour from raw materials of plant origin such as cereals and legumes. The use of natural mixtures (flours) from agricultural crops has also attracted increasing attention because of the several botanical sources available as material for the development of edible films, allied with the possibility of improving their mechanical and barrier properties. The characteristics of the films based on flour are a result of the natural interactions occurring between the starch, protein, and lipids during drying of the casting suspension (Tapia-Blácido, Mauri, Menegalli, Sobral, & Añón, 2007), which can also be influenced by the different variables employed during the production process (Araujo-Farro, Podadera, Sobral, & Menegalli, 2010; Dias, Müller, Larotonda, & Laurindo, 2010; Gontard, Guilbert, & Cuq, 1993; Parris, Dickey, Kurantz, Moten, & Craig, 1997; Tapia-Blácido, Sobral, & Menegalli, 2005a, 2011). The functional properties of the films can also be modified by changing the temperature and relative humidity applied during the drying step (Alcantara et al., 1998; Arvanitoyannis et al., 1998, 1996; Denavi et al., 2009; Tapia-Blácido, Sobral, & Menegalli, 2005b, among others).

Tubers and roots are important sources of flours in tropical regions of Latin America, the Caribbean, Africa, and Asia. Among these sources, achira (*Canna indica* L.) is a widely cultivated plant in different countries of Latin America and Asia, and it has been commercially employed for starch extraction (Leonel, Sarmento, Cereda, & Guerreiro, 2002; Peroni, Rocha, & Franco, 2006). Several studies have reported on some interesting features of the achira starch, such as paste clarity, high viscosity, and high resistance to hydrolysis by α-amylase (Hung & Morita, 2005; Puncha-Arnon, Puttanlek, Rungsardthong, Pathipanawat, & Uttapap, 2007; Santacruz, Ruales, & Eliasson, 2003). The high viscosity and clear paste of the achira starch makes it a potentially useful thickening agent (Puncha-Arnon et al., 2007) and may lead to its application in the production of excellent transparent noodle (Hung & Morita, 2005). Other studies have indicated that the achira starch has high amylose content and high level of phosphorus, as compared to starch obtained from other commercially important tubers, like potato and cassava. These components play an important role in the functional properties of the achira starch (Cisneros, Zevillanos,

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& Cisneros-Zevallos, 2009; Peroni et al., 2006; Piyachomkwan et al., 2002; Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003). Considering these properties, it is surprising that no studies on the production of films from achira flour have been carried out to date. Only the starch obtained from this tuber has been explored in an *in vitro* investigation on the digestibility of edible films (Hernández, Emaldi, & Tovar, 2008). The present study aims to determine the optimal formulation of achira flour film by using response surface methodology and multi-response analysis, in order to obtain films with low solubility, moderate elongation, and high resistance to break.

2. Material and methods

2.1. Raw material

Achira flour was obtained from rhizomes cultivated in humid subtropical climate (Köppen *Cwa*) in the city of Conchal, SP, Brazil (22°19′48″S, 47°10′22″W, 591 m.a.s.l.). Seven-month-old rhizomes were harvested and supplied by Corn Products Brazil. The rhizomes were cleaned and peeled manually, cut into 3-mm slices, and immersed into $K_2S_2O_5$ solution (0.8%, w/v), to prevent oxidation of the slices. Then, the slices were dried at 40 °C for 45 h in a temperature-controlled oven (model MA 415UR, Marconi, Piracicaba, Brazil). Finally, dry-milling and sieving (115/400 mesh) were used to obtain the achira flour with granulometry ≤43.5 μm.

2.2. Chemical analysis

The moisture, crude protein, total dietary fiber, and ash contents of the achira flour were determined using AOAC methods (2005); the starch content was calculated by the method of Diemair (1963). The Bligh and Dyer (1959) method was used for determination of the total lipid content. The amylose content was obtained by following the ISO 6647 method and is expressed as the percentage (%) of total dry starch content. All the analyses were performed in triplicate.

2.3. Preparation and conditioning of the flour films

The films were prepared according to the casting technique. To this end, an aqueous suspension of achira flour (4% w/w) was magnetically stirred for 30 min, at room temperature, and then heated up to the processing temperature (T_p: 70, 75, 80, 85, or 90 °C), while gentle stirring was maintained, in order to avoid the formation of bubbles. When the solution reached the T_p value, glycerol was added as a plasticizer (C_g: 15, 20, 25, 30, or 35 g glycerol/100 g flour). After this the film-forming suspension was kept at the processing temperature for 15 min. Next, 76 ± 0.5 g of the suspension was poured and spread on Teflon plates (18 × 21 cm) and dried at 35, 40, 45, 50, or 55 °C and 30, 40, 50, 60, or 70% RH in an oven (Model MA-415UR, Marconi, Brazil) equipped with a control system for drying temperature (T_s) and relative humidity (RH). Prior to the characterization, the dried films were peeled from the Teflon plates, cut into specific shapes for various tests, and stored at 25 °C and 58% RH for 48 h in desiccators containing a saturated NaBr solution. Film thickness was measured with the aid of a micrometer (model FOW72-229-001, Fowler, Newcastle, CA) and was determined as a random average of 15 measurements made at five different regions of the film.

The values of glycerol content (C_g), process temperature (T_p), drying temperature (T_s), and relative humidity (RH) varied according to four variables-five levels of a Central Composite Design (CCD), as reported in Table 1. Preliminary tests were performed under process conditions at the CCD central point, to determine the ideal moisture content of the films. For this purpose, a film sample was weighed every 15 min, until a condition in which the film was easily removed from the teflon plates without any damage was reached.

2.4. 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 (ASTM, 2002). The secant modulus is the slope of the line connecting the origin and a given point on the stress–strain curve, and 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.

2.5. Solubility in water and moisture content

The solubility in water was calculated as the percentage of dry matter of the solubilized film after immersion for 24 h in water at 25 ± 2 °C (Gontard, Guilbert, & Cuq, 1992). Film discs (diameter = 2 cm) were cut, weighed, immersed into 50 mL distilled water, and slowly and periodically agitated. The amount of dry matter in the initial and final samples was determined by drying the samples at 105 °C for 24 h.

The moisture content of the film was measured in samples (2 g) collected at the end of the drying process and after conditioning, by using the ASTM D644-99 methodology (ASTM, 1999). Three measurements were accomplished for each determination.

2.6. Color and opacity

The color, represented as color difference (ΔE^*), was determined according to Gennadios, Weller, Handa, and Froning (1996), and the opacity was analyzed by means of the HunterLab method (Sobral, 1999). A colorimeter (HunterLab, model Miniscan XE) was employed in both cases. The difference in color was calculated as:

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

where ΔL^*, Δa^*, and Δb^* are the differentials 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.

2.7. Scanning electron microscopy (SEM)

SEM analyses were carried out as described by Tapia-Blácido et al. (2007). Film samples were maintained in a desiccator with silica gel for 7 d. Film pieces (4 mm × 4 mm) were then mounted on cylindrical aluminum stubs using a double-sided cupper tape, and coated with gold in a VG Microtech (Cambridge, UK) model SC 7620 sputter coater. The film specimens were finally observed under a JEOL Model JSM-5800LV scanning electron microscope, at an accelerated voltage of 10 kV.

2.8. Water vapor permeability

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, and the cells were then placed in desiccators containing distilled water. The weight loss of the cells was monitored every 1 h, for 9 h.

Table 1
Central composite design matrix with values of the factors and responses to moisture content, mechanical properties, solubility, color, and opacity.

Run	Factors				Responses						
	C_g [b]	T_p (°C)	T_s (°C)	RH (%)	Moisture[c] (%)	Tensile Strength (MPa)	Elongation (%)	Secant modulus (MPa)	Solubility (%)	ΔE^*	Opacity (%)
	X_1 [a]	X_2 [a]	X_3 [a]	X_4 [a]	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
1	20(−1)	75(−1)	40(−1)	40(−1)	19.9 ± 0.8	3.2 ± 0.7	7.7 ± 2.5	170.3 ± 6.4	47.0 ± 2.8	19.2 ± 0.9	26.4 ± 1.6
2	30(1)	75(−1)	40(−1)	40(−1)	17.7 ± 0.5	2.2 ± 0.2	13.0 ± 0.9	58.4 ± 3.4	40.9 ± 2.1	17.4 ± 0.2	21.5 ± 0.3
3	20(−1)	85(1)	40(−1)	40(−1)	18.5 ± 0.7	4.5 ± 0.5	13.2 ± 1.6	154.8 ± 5.7	30.9 ± 1.8	18.3 ± 0.1	20.6 ± 0.1
4	30(1)	85(1)	40(−1)	40(−1)	18.1 ± 0.8	2.5 ± 0.1	16.3 ± 0.2	62.3 ± 0.9	43.8 ± 1.7	17.2 ± 0.2	20.8 ± 0.4
5	20(−1)	75(−1)	50(1)	40(−1)	17.1 ± 1.1	2.2 ± 0.2	7.5 ± 1.5	92.2 ± 8.1	41.9 ± 1.2	21.6 ± 0.1	36.0 ± 1.4
6	30(1)	75(−1)	50(1)	40(−1)	18.9 ± 0.8	2.8 ± 0.6	10.9 ± 0.5	87.1 ± 22.2	46.0 ± 2.0	22.9 ± 0.5	34.1 ± 1.4
7	20(−1)	85(1)	50(1)	40(−1)	17.7 ± 0.2	4.8 ± 0.4	16.7 ± 1.8	131.3 ± 11.4	48.3 ± 1.2	14.4 ± 0.3	17.5 ± 0.5
8	30(1)	85(1)	50(1)	40(−1)	19.2 ± 0.9	2.5 ± 0.4	16.7 ± 0.6	56.8 ± 7.5	48.2 ± 1.4	17.7 ± 0.4	20.8 ± 0.3
9	20(−1)	75(−1)	40(−1)	60(1)	17.8 ± 1.0	3.0 ± 0.01	10.4 ± 0.8	105.0 ± 2.8	47.1 ± 1.3	22.0 ± 1.3	37.8 ± 0.7
10	30(1)	75(−1)	40(−1)	60(1)	19.1 ± 1.0	1.6 ± 0.3	12.5 ± 0.2	41.6 ± 7.1	53.4 ± 2.0	22.6 ± 0.5	26.1 ± 0.7
11	20(−1)	85(1)	40(−1)	60(1)	17.3 ± 1.0	4.6 ± 0.4	16.7 ± 1.5	117.0 ± 16.0	45.1 ± 0.8	17.1 ± 0.2	21.2 ± 0.2
12	30(1)	85(1)	40(−1)	60(1)	18.8 ± 1.2	2.5 ± 0.3	15.4 ± 0.9	58.6 ± 6.7	47.0 ± 2.3	17.8 ± 1.6	18.6 ± 0.4
13	20(−1)	75(−1)	50(1)	60(1)	17.4 ± 0.3	4.0 ± 0.3	9.3 ± 1.0	147.3 ± 21.4	47.7 ± 2.1	23.0 ± 0.3	33.2 ± 1.3
14	30(1)	75(−1)	50(1)	60(1)	18.0 ± 0.9	2.5 ± 0.1	10.6 ± 0.6	66.1 ± 2.3	47.0 ± 1.7	19.9 ± 0.7	19.5 ± 0.6
15	20(−1)	85(1)	50(1)	60(1)	19.1 ± 0.3	5.2 ± 0.4	14.5 ± 1.2	154.8 ± 5.0	40.3 ± 0.8	16.4 ± 0.9	19.6 ± 0.4
16	30(1)	85(1)	50(1)	60(1)	20.1 ± 0.2	2.5 ± 0.2	14.6 ± 0.7	57.0 ± 2.3	43.1 ± 0.8	17.4 ± 0.9	17.8 ± 1.4
17	15($-\alpha$)	80(0)	45(0)	50(0)	17.5 ± 0.2	6.9 ± 0.7	14.2 ± 2.3	235.8 ± 7.8	55.9 ± 1.9	16.5 ± 0.3	19.9 ± 0.6
18	35(α)	80(0)	45(0)	50(0)	20.5 ± 0.4	1.9 ± 0.5	16.0 ± 1.4	34.7 ± 4.4	45.7 ± 2.3	20.8 ± 0.6	21.6 ± 0.7
19	25(0)	70($-\alpha$)	45(0)	50(0)	19.6 ± 0.6	1.5 ± 0.1	11.9 ± 1.1	46.4 ± 1.4	55.6 ± 1.8	21.9 ± 0.5	44.1 ± 0.7
20	25(0)	90(α)	45(0)	50(0)	17.4 ± 0.1	4.5 ± 0.3	14.5 ± 1.2	129.1 ± 7.0	40.1 ± 1.6	16.0 ± 0.9	17.6 ± 0.3
21	25(0)	80(0)	35($-\alpha$)	50(0)	20.3 ± 0.5	3.1 ± 0.5	15.4 ± 1.0	82.1 ± 8.3	44.5 ± 1.2	16.7 ± 0.9	21.0 ± 0.5
22	25(0)	80(0)	55(α)	50(0)	18.0 ± 0.7	3.3 ± 0.2	14.7 ± 0.8	88.3 ± 4.8	40.9 ± 3.0	17.8 ± 1.0	20.7 ± 0.2
23	25(0)	80(0)	45(0)	30($-\alpha$)	20.3 ± 0.8	3.6 ± 0.2	15.0 ± 0.5	94.9 ± 5.6	32.4 ± 2.2	18.2 ± 1.1	21.9 ± 0.8
24	25(0)	80(0)	45(0)	70(α)	18.5 ± 0.7	3.1 ± 0.3	13.6 ± 0.5	75.5 ± 5.7	53.5 ± 1.7	19.0 ± 0.6	20.5 ± 0.4
25	25(0)	80(0)	45(0)	50(0)	19.6 ± 0.3	3.0 ± 0.4	15.9 ± 0.7	74.3 ± 6.5	46.4 ± 1.7	15.9 ± 0.5	20.7 ± 0.2
26	25(0)	80(0)	45(0)	50(0)	19.8 ± 0.5	3.0 ± 0.1	15.3 ± 1.3	73.5 ± 2.0	44.3 ± 0.6	15.3 ± 0.6	19.6 ± 0.5
27	25(0)	80(0)	45(0)	50(0)	19.1 ± 0.4	3.1 ± 0.2	14.7 ± 1.7	77.2 ± 2.0	43.8 ± 1.0	16.1 ± 0.5	20.6 ± 0.3
28	25(0)	80(0)	45(0)	50(0)	19.3 ± 0.3	3.3 ± 0.7	15.1 ± 1.2	80.4 ± 5.4	44.2 ± 0.6	15.9 ± 0.3	19.7 ± 0.5

[a] Independent variables (the values in parentheses are the coded variable).
[b] Glycerol content (g/100 g of flour).
[c] Moisture content of film after conditioning.
T_p, process temperature; T_s, drying temperature; RH, relative humidity; ΔE, total color difference.

2.9. Experimental design and statistical analysis

The surface response methodology was used to study the effect of glycerol concentration (C_g), process temperature (T_p), drying temperature (T_s), and relative humidity of drying (RH) on the moisture content, mechanical properties, solubility in water, total difference in color, and opacity of the films. The C_g and T_p levels were defined according to a 2^4 full-factorial central composite design (star configuration) (Table 1). An analysis of variance (ANOVA), a multiple comparison test, and all statistical analyses were performed using the Statistica v.7.0 software (StatSoft, Inc., USA). The data were fitted to a second order equation (Eq. (1)) as a function of the independent variables:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} + \beta_{44} X_{42} + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4, \quad (2)$$

where β_i are constant regression coefficients, Y_i are responses (moisture content, mechanical properties, optical properties, and solubility), and X_i are the coded process variables (factors).

2.10. Optimization of the process conditions and experimental validation

The optimization of the process conditions for the production of achira flour films was accomplished using a multi-response method called desirability (Derringer & Suich, 1980). This method involves the transformation of each response Y_i into an individual desirability d_i (Eqs. (3) and (4)). The desirability scale ranged from 0 to 1. If the response fell outside an acceptable region, it was set to $d_i = 0$; if the response was fully desirable (at its goal or target), it was set to $d_i = 1$.

$$d_i = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}}, \quad (3)$$

where $Y_{\min}$ is the response minimum value and $Y_{\max}$ is the response maximum value. In the case of solubility, Eq. (3) had to be redesigned, so that the minimum values for these responses could be obtained (Eq. (4)).

$$d_i = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \quad (4)$$

The individual desirability functions from the considered responses were then combined, to obtain the overall desirability (D), defined as the geometric average of the individual desirability (Eq. (5)). D was later maximized using the Statistica 7.0 software® (Statsoft, USA).

$$D = (d_1^{n_1}, d_2^{n_2}, \ldots, d_k^{n_k})^{1/k}, \quad (5)$$

where k is the number of considered responses and n_i *is* the weight of each response. Thus, since $0 \le D \le 1$, a high D value shows that all d_{ks} are toward the target value, which is considered as the optimal solution of the system. Finally, achira flour films were prepared in the optimal conditions, in order to validate the optimized process conditions obtained by the multiresponse analysis. The validation experiments were performed in duplicate, and the resulting films were characterized with respect to their mechanical properties, solubility, microstructure, and water vapor permeability.

3. Results and discussion

3.1. Chemical composition of the achira flour

The achira flour contains 4.6 ± 0.08 g/100 g moisture, 5.3 ± 0.04 g/100 g ash, 1.2 ± 0.2 g/100 g lipids, 4.5 ± 0.07 g/100 g protein, 17.3 ± 1.6 g/100 g total dietary fiber, and 71.7 ± 0.8 g/100 g starch (among which 30.7 ± 0.1 g/100 g of dry starch was amylose). The results are expressed on dry basis.

3.2. Full experimental design

The results of the 2^4 full-factorial central composite design (star configuration) are summarized in Table 1. Data regarding the dependent variables (moisture content, tensile strength, elongation at break, secant modulus, solubility, total difference in color, and opacity) were fitted to a second-order equation (Eq. (2)) as a function of the independent variables. The statistical analysis of these data was performed at the 95% ($P < 0.05$) and 90% ($P < 0.1$) confidence levels, and the regression coefficients of the obtained second-order models are listed in Table 2.

The models achieved for tensile strength (Y_2), elongation at break (Y_3), secant modulus (Y_4), difference in color (Y_6), and opacity (Y_7) had a good R^2 value (between 0.77 and 0.90). Analysis of variance (ANOVA) for these models indicated that they were statistically significant ($P < 0.05$, F ratio value $> F$ critical value). However, the coded models for moisture content (Y_1) and solubility (Y_5) had a low determination coefficient (R^2 of 0.54 and 0.61 respectively), suggesting that data variation was not adequately explained for the model, and that a higher-order model would be required (Gontard et al., 1992). On the other hand, SSE/SST ratio values (pure error sum of squares/total sum of squares) were low for all the studied dependent variables (Table 2), evidencing that the process variables were controlled during the experiments, and that the experimental errors were minimal.

3.3. Moisture content

All the films were obtained with a moisture content of $18.2 \pm 0.7\%$ at the end of the drying process, which is within the range of optimum moisture determined in preliminary experiments ($18.6 \pm 0.6\%$) and ensured that the films were easy to peel off the plates. This shows that the employed methodology was effective in controlling the final moisture content of the films in the different process conditions. After conditioning, there was a very low variation in the moisture content ($18.8 \pm 1.0\%$) (Table 1). This result could be attributed to the presence of fibers in the flour, which restrain water sensitivity and favor an almost constant equilibrium water content in the films. Consequently, the final moisture content was not adequately correlated with the independent variables (C_g, T_p, T_s, and RH), demonstrating that this property does not depend on the studied process conditions. Psomiadou et al. (1996) have reported that the binding of water could depend on the density and on the regulatority of the packing of the polysaccharide chains.

3.4. Mechanical properties

For tensile strength, elongation at break, and secant modulus, the linear and quadratic parameters of plasticizer concentration and process temperature were statistically significant ($P < 0.05$ and $P < 0.10$), so they were considered in the second-order model (Table 2). A rise in C_g from 20 to 30% produced a decrease in resistance and an increase in the flexibility of the achira flour film. Indeed, glycerol has commonly been incorporated into the formulation of films to interact with the polymer chains, thereby reducing the intermolecular forces and enhancing the molecular mobility as well as the flexibility of the films. These effects have been studied by different researchers (Alves, Mali, Beleia, & Grossmann, 2007; Arvanitoyannis & Biliaderis, 1998; Arvanitoyannis et al., 1998; Gontard et al., 1993; Psomiadou et al., 1996; Sobral, Garcia, Habitante, & Monterrey-Quintero, 2004; Tapia-Blácido et al., 2005a,

Table 2
Regression coefficients and analysis of variance for responses variables.

Coefficients	Moisture content (%) Y_1	Tensile strength (MPa) Y_2	Elongation at break (%) Y_3	Secant modulus (MPa) Y_4	Solubility in water (%) Y_5	ΔE^* Y_6	Opacity (%) Y_7
β_o	19.450*	3.083*	15.258*	76.335*	44.683*	15.798*	20.163*
Linear							
β_1	0.462*	−0.939*	0.737*	−41.118*	0.025	0.403*	−1.228*
β_2	−0.062	0.557*	1.981*	7.931*	−2.305*	−1.838*	−5.443*
β_3	−0.179**	0.114*	−0.243**	1.540**	0.014	0.165	0.203
β_4	−0.129	0.012	−0.039	−4.357*	2.745*	0.378*	−0.283
Quadratic							
β_{11}	−0.211*	0.270*	−0.3**	14.598*	1.336*	0.828*	0.318
β_{22}	−0.336*	−0.073**	−0.769*	2.713*	0.581**	0.897*	2.853*
β_{33}	−0.173*	−0.020	−0.305**	2.082*	−0.700**	0.476*	0.350
β_{44}	−0.111	0.010	−0.499*	2.077*	−0.647**	0.813*	0.435*
Interactions							
β_{12}	0.131	−0.361*	−0.634*	−3.849*	0.874**	0.430*	1.953*
β_{13}	0.293*	0.019	−0.275	4.224*	−0.549	0.250	0.311
β_{14}	0.231**	−0.176*	−0.606*	−1.043	−0.036	−0.153	−1.659*
β_{23}	0.406*	−0.032	0.388**	−0.627	1.183*	−0.663*	−1.035*
β_{24}	0.193**	−0.012	−0.326**	1.888**	−0.933**	−0.340*	−0.062
β_{34}	0.181	0.158*	−0.468*	11.344*	−2.269*	−0.447*	−2.036*
R^2	0.54	0.90	0.77	0.89	0.61	0.81	0.88
F ratio value[a]	1.11	20.86	4.27	9.87	2.65	6.35	17.96
F critical value[b]	2.55	2.02	2.02	2.02	2.00	2.46	2.48
SSE/SST	0.010	0.001	0.004	0.001	0.005	0.002	0.001

* Significant at 5% level.
** Significant at 10% level.
[a] Ratio between the mean square of regression and the mean square of residuals.
[b] Tabulated for Fisher test using the significance level and degrees of freedom.

among others). Meanwhile, a 10 °C increase in T_p (from 75 to 85 °C) prompted a slight elevation in tensile strength and elongation at break. The effect of T_p on the mechanical properties of the achira flour film is associated with the gelatinization process. During gelatinization, amylose is leached to the aqueous phase between the granules and the approximation between the polymer chains, which is induced by the high amylose content. This enables the formation of a denser polymer matrix with greater mechanical strength (Alves et al., 2007). High process temperature (T_p) may also favor denaturation of the proteins present in the flour and increase the number of interactions between the different biopolymers (Denavi et al., 2009). Some authors have reported that the high temperature of the prepared films should be understood in terms of a closely packed state where extensive intermolecular bonding occurs, which inhibits further orientation and better alignment of the protein and starch chains (Arvanitoyannis et al., 1998).

Table 2 shows that the linear and quadratic parameters of the drying temperature (T_s) had a significant effect on the elongation at break and secant modulus ($P < 0.05$), while for the tensile strength, the T_s quadratic parameter was not statistically significant ($P > 0.10$). On the other hand, the RH quadratic effect was significant at a 5% level for the elongation at break and secant modulus, while for the tensile strength this was not significant ($P > 0.10$). The interaction parameter (T_s and RH) was statistically significant for all the mechanical properties. At intermediate drying condition (T_s = 45 °C and RH = 50%), a higher secant modulus value was obtained (as shown in Table 1, run 17). As achira flour films have high amylose content (nearly 17.4%), it is possible that under this drying condition the retrogradation of amylose chains was favored.

In Fig. 1(a) it can be observed that the films had maximum tensile strength values when lower C_g values (15%) and high T_p values (80–90 °C) were used. It was seen that intermediate drying conditions such as high T_s (50–55 °C) and also high RH (≥50%) provided tougher achira flour films, as demonstrated in Fig. 1(b). Similar process conditions also corresponded to high elongation at break, and it was noted that higher values of elongation were achieved at high T_p (85–90 °C), regardless of C_g. It is unusual to find a range of process variables that maximize tensile strength and elongation simultaneously. Only at lower T_p (<80 °C) is elongation dependent on C_g (Fig. 1(c)). In relation to the effect of T_s and RH on the elongation at break, Fig. 1(d) gives evidence that a wide range of drying conditions can be used for the attainment of highly flexible films. It is possible to use T_s = 37 °C and RH = 62%, which corresponds to a slow drying rate; or T_s = 45 °C and RH = 42%, corresponding to an intermediate value drying rate, when mean values of C_g and T_p are considered. Analysis of the figures discussed above showed that low C_g (≤25%), high T_p (≥85 °C), intermediate T_s value (45 °C), and a wide range of RH values (50–60%) favor greater mechanical resistance. High tensile strength values and high elongation at break values were obtained in these process conditions. High T_p promoted complete gelatinization of the starch present in the achira flour, due to amylose leaching, which culminated in increased interactions between the components of the polymeric matrix (glycerol, amylose, amylopectin, fibers, protein, lipids, and water). Moreover, the fibers may have facilitated the compatibility between the starch and the glycerol, due to the presence of cellulose (Psomiadou et al., 1996). Finally, the film forming at intermediate drying condition (45 °C and 40% RH) favored re-arrangement of the polymeric matrix, yielding a more homogeneous structure, as reflected in the better mechanical performance of the achira flour films with simultaneous attainment of high tensile strength and elongation.

3.5. Solubility in water

The T_p and RH linear parameters, as well as the C_g, T_p, T_s, and RH quadratic parameters had a significant effect on the solubility in water variable ($P < 0.05$ and $P < 0.10$) (Table 2). An elevation in T_p resulted in less soluble achira flour films, while more soluble films were obtained when the RH values were increased. Thus, it was possible to observe that films produced at the highest drying

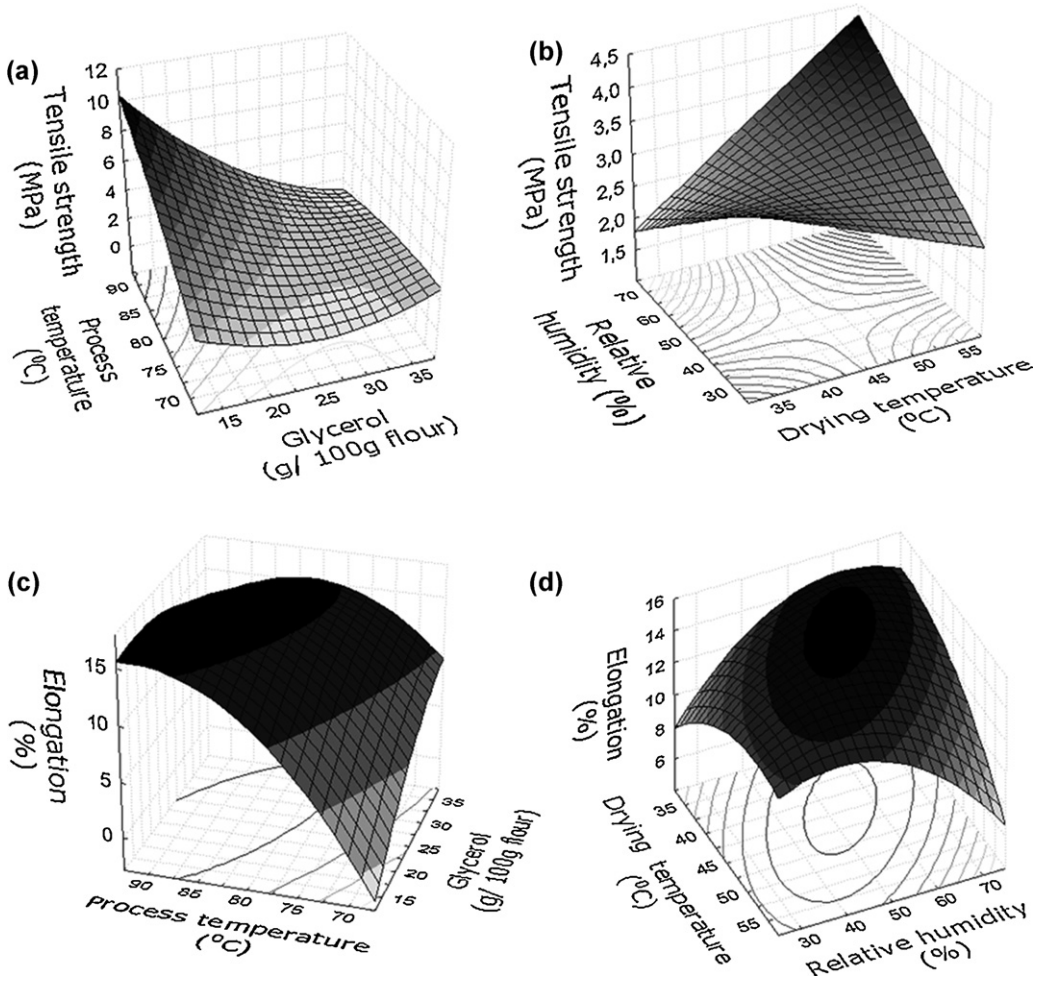

Fig. 1. Response surfaces for the tensile strength (MPa) as a function of (a) the glycerol concentrations (g/100 g of flour) and process temperature, (b) the drying conditions; response surfaces for the elongation at break (%) as a function of (c) the glycerol concentrations (g/100 g of flour) and process temperature and (d) the drying conditions.

rate (severe air-drying conditions at T_s = 45 °C and RH = 30%) were less soluble (run 23), and those produced at the lowest drying rate (T_s = 45 °C and RH = 70%) were more soluble (run 24). This suggests that lower relative humidity favored re-association of the amylose molecules into crystalline segments (retrogradation), allowing for the formation of a more compact network with lower solubility. Meanwhile, a higher relative humidity favored the occurrence of a larger number of interactions between the polymers and glycerol present in the achira flour with the water molecules, so a more hydrophilic network was achieved.

3.6. Color difference and opacity

According to the analysis of variance (ANOVA), the second-order model was statistically significant for the color difference between the films (P < 0.05) (Table 2). In Fig. 2(a), it can be noted that high T_p values (above 80 °C) and high T_s values (between 45 and 50 °C) led to films with lighter color (lower ΔE^*) when C_g and RH were fixed at 25 and 50%, respectively (Table 1). Fig. 2(b) demonstrated that low glycerol concentration (15%) can furnish achira flour films with low opacity levels, provided that the process temperature is maintained above 80 °C. It was also observed that the opacity of the achira flour films decreased when C_g was augmented and a lower process temperature was employed. This effect of C_g has also been reported by Paschoalick, Garcia, Sobral, and Habitante (2003) and Sobral et al. (2004), who assigned it to the physical characteristics of the plasticizer (colorless and transparent) and its dilution effect after its addition to the film solution. If one decides to use high plasticizer concentrations (20–30%) to improve the other properties of these films, it would be necessary to employ high relative humidity (70%) during drying, in order to achieve low opacity levels in achira flour films (Fig. 2(c)). However, the choice of glycerol concentration generally depends on other properties such as the elongation and barrier properties required for these films. Moreover, minimization of the optical properties may be significantly important only in situations where the packaged product has to be visible.

3.7. Optimization of the process variables and experimental validation

The desirability function (D) was calculated for use in a multiresponse optimization of the properties tensile strength, elongation, and solubility. The maximum, minimum, and average values of these variables experimentally obtained in the CCD (Table 1) were considered for evaluation of the D function. It was found that this function has maximum values ($D \cong 1$) under conditions of low C_g (16.68 g glycerol/100 g flour), high T_p (90 °C), intermediate T_s (44.82 °C), and low RH (36.36%). Achira flour films with the best properties, *i.e.*, tensile strength = 5.90 MPa, elongation = 17.39%, and solubility = 40.6%, were obtained in these conditions. This methodology enabled establishment of the range of independent variables that simultaneously optimized all the considered responses. The

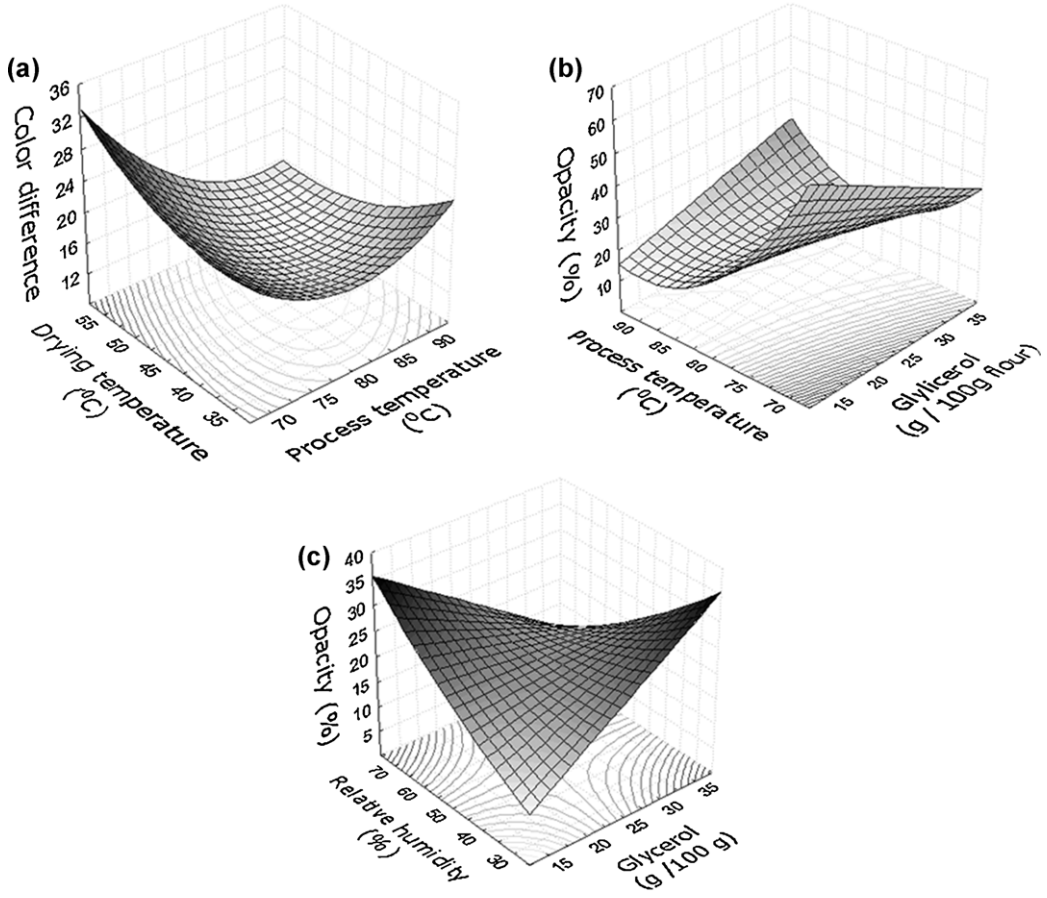

Fig. 2. Response surfaces for the total color difference as a function of (a) the process temperature and drying temperature; response surfaces for opacity as a function of (b) the glycerol concentration (g/100 g of flour) and process temperature and (c) the glycerol concentration (g/100 g of flour) and relative humidity.

conditions were experimentally validated, and the results are presented in Table 3. Based on the relative deviation values (%RD) achieved for each response variable, it can be deduced that the methodology employed for the optimization of the process conditions was satisfactory, and that the surface responses obtained by the full experimental design were suitably validated. The experimental value determined for the tensile strength was higher than the value predicted by the desirability function, which means that there was an increase in the mechanical strength of these films. Improvement of this property is associated with the lower elongation values obtained experimentally, as compared to the predicted value. In other words, the responses achieved for these properties indicated that, under the optimized process conditions, achira flour films had higher mechanical strength, as compared to the films prepared under the several process conditions studied for CCD (Table 1). It is worth noting that the solubility value (38.3%) was also better than the predicted value (40.6%) as a result of the optimization.

Table 3
Results of experimental validation of the optimal conditions for the development of achira flour films.

Responses	Predicted value (optimal)	Experimental value[a]	RD (%)[b]
Tensile strength (MPa)	5.9	7.0 ± 0.3	15.7
Elongation (%)	17.3	14.6 ± 0.3	−18.5
Solubility (%)	40.6	38.3 ± 0.3	−6.0

[a] Values obtained at optimum conditions (17% of C_g, 90 °C of T_p, 44.8 °C of T_s and 36.4% of RH).
[b] Relative deviation = [(experimental value − predicted value)/experimental value] × 100.

3.8. Microstructure of the achira flour films

Micrographs of the film surface and cross-section (Fig. 3(a and b)) revealed that the achira flour films had a cohesive microstructure. It was also possible to notice that fibers were soaked and well incorporated into the starch matrix, and that there was no formation of cracks across the film thickness. Fig. 3(b) also evidenced a laminar structure, suggesting that fibers were naturally oriented. The microstructural features described for these films showed that the optimization of the process conditions was satisfactory, and that a good degree of incorporation of the components of the flour into the polymeric matrix was attained.

3.9. Properties of the achira flour films as compared to other films

Bearing in mind that the optimized formulation of the achira flour film consists of a mixture of 42% (g/g dry film) amylopectin, 18.7% amylose, 1.0% lipids, 3.8% protein, 14.7% fiber, and 15.1% glycerol, the properties of these films were compared to those of films based on other sources, as depicted in Table 4. Whereas the tensile strength is a property that is commonly used to evaluate the resistance of films, the achira flour films displayed high resistance (7.0 MPa) as compared to films made from amaranth flour of *caudatus* and *cruentus* species (1.5–5.4 MPa) (Tapia-Blácido et al., 2007, 2011). Additionally, the achira flour films presented tensile strength values similar to those of the cassava starch films

Table 4
Comparison of the achira flour film properties with those of other films.

Material – film	Material concentration (g/100 g suspension)	Thickness (mm)	Plasticizer (g/100 g raw material)	Tensile Strength (MPa)	Elongation (%)	Young modulus (MPa)	WVP (10^{-10} g m^{-1} s^{-1} Pa^{-1})	Solubility (%)	Reference
Amarant flour (*Amaranthus caudatus*) without fibers	4.0	0.080 ± 0.005	22.5	1.5 ± 0.05	83.7 ± 5.1	21.5 ± 1.4	0.8 ± 0.2[b]	42.2 ± 1.8	Tapia-Blácido et al. (2007)
Amarant flour (*A. cruentus*) without fibers	4.0	0.080 ± 0.08	20.0	2.1 ± 0.3	51.9 ± 3.6	252.0 ± 12.4	3.8 ± 0.2[b]	41.9 ± 2.4	Tapia-Blácido et al. (2011)
Rice flour without fibers	5.0	0.10 ± 0.04	20.0	10.3 ± 1.0	2.7 ± 0.5	560.7 ± 64.3	1.1 ± 0.1[c]	–	Dias et al. (2010)
Rice flour with cellulose fibers (0.3 g/g dry flour)	5.0	0.14 ± 0.03	20.0	20.6 ± 0.8	4.0 ± 0.7	1001.3 ± 194.4	0.8 ± 0.02[c]	–	Dias (2008)
Cassava starch with celulose fibers (0.1 g/g dry starch) and Guar gum (0.01 g/g dry starch)	3.0	0.11–0.12	30.0	8.0	22.0	130.0	2.3 ± 0.2[d]	–	Müller and Laurindo (2009)
Cassava starch	4.0	0.10	20.0	21.7 ± 6.1	5.2 ± 1.9	40.5 ± 6.4	24.0 ± 2.0[e]	–	Alves et al. (2007)
Potato peel	3.0	–	30.0	9.5 ± 1.2	5.3 ± 2.0	366.4 ± 9.2	8.3 ± 1.5[b]	41.2 ± 7.2	Kang and Min (2010)
Achira-flour	4.0	0.084 ± 0.002	17.0	7.0 ± 0.3	14.6 ± 0.3	231.7 ± 19.9[a]	5.3 ± 0.2[b]	38.3 ± 1.9	This work

[a] Calculated as secant modulus.
[b] WVP at relative humidity gradient (ΔRH) = 0–100%.
[c] WVP at relative humidity gradient (ΔRH) = 2–75%.
[d] WVP at relative humidity gradient (ΔRH) = 64–90%.
[e] WVP at relative humidity gradient (ΔRH) = 0–75%.

Fig. 3. SEM micrographs (×1000 magnification) of achira flour films: (a) surface and (b) cross-section.

prepared with 10% cellulose fibers (Müller & Laurindo, 2009), but the achira flour films were considerably thinner. Taking the presence of fibers in the achira flour films into account, the elongation of these films (14.6%) was higher as compared to that of films based on cassava starch without cellulose fibers (Alves et al., 2007), rice flour with and without cellulose fibers (Dias, 2008; Dias et al., 2010), and potato peel (Kang & Min, 2010). Regarding water vapor permeability, the films based on materials obtained from tubers and roots such as cassava, potato, and achira were more permeable than blends based on hydroxypropyl starch/gelatin (Arvanitoyannis et al., 1998), methyl cellulose, and soluble starch (Arvanitoyannis & Biliaderis, 1999) as well as edible films prepared from cereals like rice (Dias et al., 2010), corn starch, and microcrystalline cellulose (Psomiadou et al., 1996) and pseudocereals like amaranth (Tapia-Blácido et al., 2007). However, among the films based on tubers and roots without cellulose fibers, the achira flour film exhibited the lowest water vapor permeability value. It is known that the influence of fibers on the properties of films depends considerably on the fiber–matrix interface, because only good fiber-matrix adhesion allows for the fibers to act as reinforcement agents, thereby increasing the stiffness of the films. The extent of the adhesion between the fiber and the matrix can be affected by the length and diameter of the fibers as well as their orientation and distribution in the polymeric matrix (Wollerdorfer & Bader, 1998). Comparing the solubility values of the films based on amaranth flour, potato peel, and achira flour, it can be realized that all these films have similar solubility, but the achira flour film tends to be the least soluble. In the latter case, the fibers remaining in the achira flour must have been more easily incorporated into the matrix, suggesting that there was a good degree of fiber-matrix interactions, since these fibers are spontaneously distributed in the achira flour. These properties may be improved

with the chemical treatment of these fibers for the attainment of nanosized fibers.

4. Conclusion

This study has shown that the achira flour is an alternative source for the preparation of biodegradable films, whose properties can be modified by controlling the process conditions. A high glycerol content contributes to increased flexibility and reduced opacity of the films; high process temperature favors production of a more resistant polymeric structure with lighter color and lower opacity, as well as solubility in water. A higher drying temperature favors the preparation of films with enhanced resistance and stiffness, whereas lower relative humidity significantly increases the stiffness and reduces the solubility. The optimal conditions for the production of an achira flour film with good mechanical properties and low solubility are C_g = 17%, T_p = 90 °C, T_s = 44.8 °C, and RH = 36.4%. The achira flour films developed in this work present lower water vapor permeability and lower solubility as compared to films prepared from other tubers such as cassava starch and potato peel. These features are associated with fibers that are soaked and well incorporated into the starch matrix, thus yielding a cohesive microstructure. On the other hand, the contribution of fibers to the characteristics of the achira flour film was also important. Reduction in the size of these fibers should enhance their uptake by the film matrix, so the production and utilization of nanofibers from achira fibers as reinforcement material should be further studied in the future.

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References

Alcantara, C. R., Rumsey, T. R., & Krochta, J. M. (1998). Drying rate effect on the properties of whey proteína films. *Journal of Foods Process Engineering*, *21*, 387–405.

Alves, V., Mali, S., Beleia, A., & Grossmann, M. V. (2007). Effect of glycerol and amylose enrichment on cassava starch film properties. *Journal of Food Engineering*, *78*, 941–946.

AOAC. (2005). *Association of Official Analytical Chemist: Official methods of analysis* (18th ed.). Washington, DC.

Araujo-Farro, P. C., Podadera, G., Sobral, P. J. A., & Menegalli, F. C. (2010). Development of films based on quinoa (*Chenopodium quinoa*, Willdenow) starch. *Carbohydrate Polymers*, *81*, 839–848.

Arvanitoyannis, I. (1999). Totally-and-partially biodegradable polymer blends based on natural and synthetic macromolecules: preparation and physical properties and potential as food packaging materials. *Journal of Macromolecular Science – Reviews in Macromolecular Chemistry & Physics*, *C39*(2), 205–271.

Arvanitoyannis, I., & Biliaderis, C. (1998). Physical properties of polyol-plasticized edible films made from sodium caseinate and soluble starch blends. *Food Chemistry*, *62*(3), 3333–3342.

Arvanitoyannis, I., & Biliaderis, C. (1999). Physical properties of polyol-plasticized edible blends made of methyl cellulose and soluble starch. *Carbohydrate Polymers*, *38*, 47–58.

Arvanitoyannis, I., Nakayama, A., & Aiba, S. (1998). Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydrate Polymers*, *36*(2/3), 105–119.

Arvanitoyannis, I., Psomiadou, E., & Nakayama, A. (1996). Edible films made from sodium caseinate, starches, sugars or glycerol: Part 1. *Carbohydrate Polymers*, *31*, 179–192.

ASTM. (1995). 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.

ASTM. (1999). 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.

ASTM. (2002). 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.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

Cisneros, F. H., Zevillanos, R., & Cisneros-Zevallos, L. (2009). Characterization of starch from two ecotypes of Andean achira roots (*Canna edulis*). *Journal of Agricultural and Food Chemistry*, *57*, 7363–7368.

Denavi, G., Tapia-Blácido, D. R., Añón, M. C., Sobral, P. J. A., Mauri, A. N., & Menegalli, F. C. (2009). Effects of drying conditions on some physical properties of soy protein films. *Journal of Food Engineering*, *90*, 341–349.

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology*, *12*(4), 214–219.

Dias, A. B. (2008). *Development and characterization of biodegradable films based on rice starch and rice flour* (Magister thesis; p. 103). Federal University of Santa Catarina, Brazil.

Dias, A. B., Müller, C. M. O., Larotonda, F. D. S., & Laurindo, J. B. (2010). Biodegradable films based on rice starch and rice flour. *Journal of Cereal Science*, *51*, 213–219.

Diemair, W. (1963). *Laboratoriumsbuch fur Lebensmittelchemiker, 8 aufl.* Drisden: Verlag 426 Von Theodor Steinkopff.

Gennadios, A., Weller, C. L., Handa, M. A., & Froning, G. W. (1996). Mechanical properties of egg albumen films. *Journal of Food Science*, *61*(3), 585–589.

Gontard, N., Guilbert, S., & Cuq, J. L. (1992). Edible wheat gluten films: Influence of the main process variables on film properties using response surface methodology. *Journal of Food Science*, *57*(1), 190–195.

Gontard, N., Guilbert, S., & Cuq, J. L. (1993). Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, *58*(1), 206–211.

Grinberg, V., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solution. *Food Hydrocolloids*, *11*, 145–158.

Hernández, O., Emaldi, U., & Tovar, J. (2008). In vitro digestibility of edible films from various starch sources. *Carbohydrate Polymers*, *71*, 648–655.

Hung, P. V., & Morita, N. (2005). Physicochemical properties and enzymatic digestibility of starch from edible canna (*Canna edulis*) grown in Vietnam. *Carbohydrate Polymers*, (61), 314–321.

ISO. (1987). *No. 6647: Norme internationale: Riz-détermination de la teneur em amylose*. Geneva, Switzerland: International Organization for Standardization.

Kang, H. J., & Min, S. C. (2010). Potato peel-based biopolymer film development using high-pressure homogenization, irradiation, and ultrasound. *LWT – Food Science and Technology*, *43*, 903–909.

Leonel, M., Sarmento, S., Cereda, M. P., & Guerreiro, L. (2002). Extração e Caracterização do amido de Biri (*Canna edulis*). *Brazilian Journal of Food Technology*, *5*, 27–32.

Müller, C. M. O., & Laurindo, J. B. (2009). Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. *Food Hydrocolloids*, *23*, 1328–1333.

Parris, N., Dickey, L. C., Kurantz, M. J., Moten, R. O., & Craig, J. C. (1997). Water vapor permeability and solubility of zein/starch hydrophilic films prepared from dry Milled Corn Extract. *Journal of Food Engineering*, *32*, 199–207.

Paschoalick, T. M., Garcia, F. T., Sobral, P. J. A., & Habitante, A. M. Q. B. (2003). Characterization of some functional properties of edible films based on muscle proteins of Nile Tilapia. *Food Hydrocolloids*, *17*, 419–427.

Peroni, F. G. H., Rocha, T. S., & Franco, C. M. L. (2006). Some structural and physicochemical characteristics of tuber and roots starches. *Food Science Technology International*, *12*(6), 505–513.

Piyachomkwan, K., Chotineeranat, S., Kijkhunasatian, C., Tonwitowat, R., Prammanee, S., Oates, C. G., et al. (2002). Edible canna (*Canna edulis*) as a complementary starch source to cassava for the starch industry. *Industrial Crops and Products*, *16*, 11–21.

Psomiadou, E., Arvanitoyannis, I., & Yamamoto, N. (1996). Edible films made from natural resources; microcrystalline cellulose (MCC), methylcellulose (MC) and corn starch and polyols: Part 2. *Carbohydrate Polymers*, *31*, 193–204.

Puncha-Arnon, S., Puttanlek, C., Rungsardthong, V., Pathipanawat, W., & Uttapap, D. (2007). Changes in physicochemical properties and morphology of canna starches during rhizomal development. *Carbohydrate Polymers*, *70*, 206–217.

Santacruz, S., Ruales, J., & Eliasson, A. C. (2003). Three under-utilised sources of starch from the Andean region in Ecuador. Part II. Rheological characterisation. *Carbohydrate Polymers*, *51*, 85–92.

Sobral, P. J. A. (1999). Propriedades funcionais de biofilmes de gelatina em função da espessura. *Ciência Engenharia*, *8*(1), 60–67.

Sobral, P. J. A., Garcia, F. T., Habitante, A. M. Q. B., & Monterrey-Quintero, E. S. (2004). Propriedades de filmes comestíveis produzidos com diferentes concentrações de plastificantes e de proteínas do músculo de tilapia-do-nilo. *Pesquisa Agropecuaria Brasileira*, *39*(3), 255–262.

Tapia-Blácido, D., Mauri, A. N., Menegalli, F. C., Sobral, P. J. A., & Añón, M. C. (2007). Contribution of the starch, protein, and lipid fractions to the physical, thermal, and structural properties of amaranth (*Amaranthus caudatus*) flour films. *Journal of Food Science*, *72*(5), 293–300.

Tapia-Blácido, D., Sobral, P. J. A., & Menegalli, F. C. (2005a). Development and characterization of biofilms based on Amaranth flour (*Amaranthus caudatus*). *Journal of Food Engineering*, *67*, 215–223.

Tapia-Blácido, D., Sobral, P. J. A., & Menegalli, F. C. (2005b). Effects of drying temperature and relative humidity on the mechanical properties of Amaranth flour films plasticized with glycerol. *Brazilian Journal of Chemical Engineering*, *22*(2), 249–256.

Tapia-Blácido, D. R., Sobral, P. J. A., & Menegalli, F. C. (2011). Optimization of amaranth flour films plasticized with glycerol and sorbitol by multi-response analysis. *LWT – Food Science and Technology*, *44*(8), 1731–1734.

Thitipraphunkul, K., Uttapap, D., Piyachomkwan, K., & Takeda, Y. (2003). A comparative study of edible canna (*Canna edulis*) starch from different cultivars. Part I. Chemical composition and physicochemical properties. *Carbohydrate Polymers*, *53*, 317–324.

Wollerdorfer, M., & Bader, H. (1998). Influence of natural fibers on the mechanical properties of biodegradable polymers. *Industrial Crops and Products*, *8*, 105–112.