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Development and characterization of biofilms based on Amaranth flour (*Amaranthus caudatus*)

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Abstract

The aim of the present paper was to study the filmogenic capacity of Amaranth flour. The films were obtained in a casting process using glycerol as plasticizer. The influence of the glycerol content, pH, temperature of heating process and drying temperature and relative humidity on mechanical and barrier properties were evaluated. The effect of these variables was analyzed according a $2^{(5-1)}$ fractional experimental design that allowed the selection of significant factors: glycerol content, pH and process temperature. These then were used in a full factorial design. Solubility and mechanical properties were measured to obtain the optimal processing variables and casting solution formulation. The biofilms presented a yellowish color, moderate opacity, and high flexibility but low tensile strength. Nevertheless they showed less oxygen and water permeability than other protein and polysaccharide films.

Keywords: Amaranth; Starch; Edible films; Physical properties; Solubility

1. Introduction

Proteins and polysaccharides are well known for being good film formers (Gennadios, McHugh, Weller, & Krochta, 1994; Nisperos-Carriedo, 1994; Torres, 1994). Protein films are made of raw materials of high molecular weight such as gelatin, myofibrilar proteins, gluten, whey protein among others. These films are characterized by good mechanical properties, although they are usually quite permeable to water and gases (McHugh & Krochta, 1994). Starch films have also received a lot of interest since they are a very low cost materials, the most abundant and important polysaccharide in nature. Numerous studies have been reported on its film forming capabilities and industrial applications (Otey, Westoff, & Doane, 1980). Starches from different origins, such as potato, corn, wheat, rice, and cassava, both natural and modified, have been utilized, mainly in the manufacture of edible films (Lourdin, Della Valle, & Colonna, 1995; Ollett, Parker, & Smith, 1991; Vicentini, Sobral, & Cereda, 2002). The matrix of starch-based film is normally formed during the drying of a gelatinized dispersion, as hydrogen bonds form between hydroxyl groups (Lourdin et al., 1995). As these interactions are weak, the mechanical properties of starch based films are of poor quality. Various studies have attempted to improve the functional properties of these films, by using mixtures of protein and starch as the raw material for the formation of edible films. Parris, Dickey, Kurantz, Moten, and Craig (1997) verified that the addition of 1% of corn starch to Zein films decreased the water vapor permeability. Whereas blends of gelatin and potato starch showed greater elongation and tensile stress at break in comparison with pure starch films (Arvanitoyannis, Psomiadou, Nakayama, & Yamamoto, 1997). However, the addition of casein was reported to decrease the mechanical resistance of

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corn starch films (Arvanitoyannis, Psomiadou, & Nakayama, 1996). When dealing with cereals, it is not necessary to extract the starch nor the proteins for film production. Rayas and Hernández (1997) prepared edible films from three types of wheat flours, and more recently, Mariniello et al. (2003) used whole soy flour and apple pectin as the raw materials for producing edible films.

It is interesting to investigate other new renewable resources for the production of edible and biodegradable materials. Amaranth is a pseudocereal of rapid growth, with a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown, so many countries have been adapting certain varieties to their soils. The main cultivars used are *Amaranthus hipochondriacus*, *Amaranthus cruentus* and *Amaranthus caudatus*. The grain composition is a function of the cultivars and the environmental conditions of culture (Saunders & Becker, 1984). Amaranth seeds contain a significant proportion of protein (14–17%), fat (5–9%) and starch (62%) which made their flour a promising material for film formation.

The objective of this study was thus to investigate the feasibility of the use of Amaranth flour for the formation of film and find the best formulation of casting solution in terms of the physical properties of the films. The optimal process conditions for film preparation were investigated, as well as the mechanical characterization (puncture and tensile tests), and their solubility, permeability to oxygen and water. Optical properties such as color and opacity were also determined.

2. Materials and methods

2.1. Raw material

Amaranth flour was prepared using the seeds of *Amaranthus caudatus*, grown in Callejón de Huaylas (Perú). Flour was obtained using the modification of the alkaline wet milling method of Perez, Bahnassey, and Breene (1993), proposed by Tapia-Blácido (2003). Glycerol and all chemical used were reagent grade.

2.2. Chemical analysis

The content of water, protein, ashes and lipid of the Amaranth flour samples was analyzed following the standard methods of the AOAC (1997). Amylose content was determined using a colorimetric method (Juliano, 1971) modified by Martínez and Cuevas (1989).

2.3. Film preparation

Films were made by the casting method which consists of drying a film-forming solution (FFS) which has been applied on a support. Certain preliminary tests were necessary to obtain clear and easily spread solutions. Both glycerol and sorbitol were tested as possible plasticizers, but the former gave better results. The procedure developed was as follows: a water solution of 4% Amaranth was homogenized in a mixer for 25 min.; after that, the pH was regulated with NaOH (0.1 N) to dissolve the protein. The solution was then heated to processing temperature with gentle stirring. The plasticizer was added at this point, and the casting solution was then maintained at this temperature for 15 min. For each film, 40 ± 0.08 g of solution was poured onto acrylic plates (9.2×17.9 cm), to obtain a constant thickness of 85 ± 5 μm. The films were dried for different periods of time to assure a final moisture content of approximately $22 \pm 1.1\%$ (w.b). This was verified to be the only final water content that would allow easy peeling from the plates.

2.4. Film characterization

The thickness of the films was measured with a digital micrometer Fowler (average of 8 measurements). To carry out the mechanical tests, the film samples were pre-conditioned and tested at 25 °C and 58% relative humidity. A TA.XT2 (SMS) texturometer was used for puncture and tensile tests. The puncture tests followed the methodology of Gontard, Duchez, Cuq, and Guilbert (1994) while tensile tests were performed according to the standard method D882-95 (ASTM, 1995). A permeability test was also performed using a modified E 96-80 ASTM Standard method (ASTM, 1995). Solubility was measured by immersing 2.0 cm diameter film disks, in water at 25 ± 2 °C for a period of 24 h (Gontard, Guilbert, & Cuq, 1992). Oxygen transmission was measured using the ASTM F 1927 method with a Mocon 2/20 Oxtran. The color, represented as difference of color, was determined according Gennadios, Weller, Hanna, and Froning (1996) and opacity, according the HunterLab method (Sobral, 1999), both using a Miniscan XE Colorimeter HunterLab.

2.5. Statistical analysis

For the previous screening of the most relevant variables influencing the quality of the films, a fractional experimental design of $2^{(5-1)}$ trials plus 3 central points, was used. The following independent variables were included: glycerol content (30, 35 and 40 g glycerol/100 g flour), pH (10, 10.5 and 11), temperature of heating process (85, 90 and 95 °C), temperature of drying (30, 40 and 50 °C) and relative humidity (40, 55 and 70%). The dependent variables were force and deformation at break in puncture tests and tensile strength, elongation and Young modulus, in tensile tests. This preliminary analysis facilitated selecting the statistically

Table 1
Results of full factorial design

Test	Cg (X_1)[a]	pH (X_2)	Tp (X_3)	Puncture test		Tensile test			Solubility (%)
				Force (N)	Deformation (%)	Strength (MPa)	Elongation (%)	Young modulus (MPa)	
1	25 (−1)	10.5 (−1)	80 (−1)	3.0	8.5	1.6	77.8	24.3	42.1
2	25 (−1)	10.5 (−1)	90 (+1)	3.0	9.2	1.1	79.7	16.8	47.2
3	25 (−1)	11.5 (+1)	80 (−1)	2.1	14.0	1.6	60.4	37.2	44.8
4	25 (−1)	11.5 (+1)	90 (+1)	2.5	13.3	0.6	107.4	3.1	56.1
5	35 (+1)	10.5 (−1)	80 (−1)	0.8	34.0	0.4	129.4	1.7	53.5
6	35 (+1)	10.5 (−1)	90 (+1)	0.9	30.4	0.4	116.0	0.3	55.0
7	35 (+1)	11.5 (+1)	80 (−1)	1.1	31.8	0.4	152.4	1.4	57.0
8	35 (+1)	11. 5 (+1)	90 (+1)	0.9	32.0	0.7	137.8	2.3	54.0
9	21.59 (−1.68)	11 (0)	85 (0)	4.3	3.4	2.7	11.7	93.8	51.8
10	38.41 (+1.68)	11 (0)	85 (0)	0.5	37.2	0.3	167.8	1.4	61.7
11	30 (0)	10.16 (−1.68)	85 (0)	0.9	28.4	0.8	113.6	4.7	59.2
12	30 (0)	11.84 (+1.68)	85 (0)	1.1	27.8	0.4	110.4	1.5	62.7
13	30 (0)	11 (0)	76.6 (−1.68)	1.2	28.4	0.6	121.0	3.3	53.2
14	30 (0)	11 (0)	93.4 (+1.68)	1.3	23.7	0.6	104.8	3.4	55.3
15 (C)	30 (0)	11 (0)	85 (0)	1.4	23.3	0.7	124.0	2.6	40.7
16 (C)	30 (0)	11 (0)	85 (0)	1.4	23.3	0.7	124.7	2.8	40.6
17 (C)	30 (0)	11 (0)	85 (0)	1.4	21.5	0.7	120.2	2.8	39.8

[a]Independent variables values (the values between brackets are the coded variables. Cg: glycerol content (g/100 g flour); Tp: process temperature (°C).

significant variables. With these variables, a full factorial design (2^3 plus star configuration) with three replicates at the central point was performed. The selected variables were glycerol content, pH and process temperature. The statistical design and the coded levels considered are shown in Table 1. An analysis of variance (ANOVA), a multiple comparison test and all statistical analysis were performed using Statistica 5.0 software. The data were fitted to a second order equation (Eq. (1)) as a function of the dependent variables.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where b_n are constant regression coefficients, Y_i dependent variables (mechanical properties and solubility) and X_1, X_2 and X_3 are the coded independent variables (glycerol content, pH and process temperature).

The relationship between dimensionless coded variables (X_1, X_2 and X_3) and real variables (Cg, pH and Tp) was as follows:

$$X_1 = (\mathrm{Cg} - 30) * 1.682/8.41;$$
$$X_2 = (\mathrm{pH} - 11) * 1.682/0.84;$$
$$X_3 = (\mathrm{Tp} - 11) * 1.682/8.4 \quad (2)$$

After optimization, those properties, as well as the color, opacity and permeability for water vapor and oxygen were determined for the optimized films.

3. Results and discussion

The method of flour extraction developed, produced a material containing, on dry basis, 7.97% ± 0.18 water, 2.14% ± 0.03 ashes, 8.93 ± 0.03 lipids, 14.21% ± 0.77 protein, 7.58% ± 0.40 amylose and nearly 67% amylopectin. Consequently this material is thus a complex mixture of a waxy starch with Amaranth protein and fat. All films produced were self-supporting and peelable. They were transparent, with a light yellow coloration and had an average thickness of 85 ± 5 μm.

3.1. Film production and formulation optimization

3.1.1. Fractional experimental design

The previous screening of variables resulting from the fractional experimental design, revealed that glycerol content, pH, temperature of heat treatment and relative humidity were the most relevant variables in relation to the mechanical properties. The effect of glycerol content was significant ($p < 0.05$), for all properties and was the most influencing effect. The second most important influence involved heat treatment and pH was the third. These three variables were thus selected for

the complete fractional design analysis (Tapia-Blácido, 2003). Although variables related to drying were statistically relevant their effects on the responses were weaker so they were fixed in the following statistical study. The drying of casting solutions was conducted at 40 °C and 55% relative humidity.

3.1.2. Full factorial design

The results of the complete experimental design and the value of independent and coded variables are given in Table 1.

For the force at break (puncture test) all effects and interactions were statistically significant ($p < 0.05$) and the following coded model was obtained:

$$F = 1.346 - 0.985X_1 + 0.417X_1^2 - 0.052X_2 - 0.070X_2^2 + 0.027X_3 + 0.017X_3^2 + 0.210X_1X_2 - 0.057X_1X_3 + 0.031X_2X_3 \quad (R^2 = 0.97) \tag{3}$$

The analysis of variance for the model for force at break indicated that the model was satistically significant ($p < 0.05$) with an $R^2 = 0.97$ and the F value was six times higher than the listed value.

Puncture deformation was more significantly affected by the glycerol content ($p < 0.05$). The temperature (Tp) and pH only had a weaker influence although statistically significant. The model for the coded variables is as follows:

$$D = 23.50 + 10.26X_1 - 1.72X_1^2 + 1.04X_2^2 - 0.83X_3 - 1.27X_1X_2 \quad (R^2 = 0.96) \tag{4}$$

The response surfaces for puncture force and deformation can be seen in Figs. 1 and 2 as a function of pH and glycerol content. The strong influence of the glycerol content which is due to its plasticizing effect is seen in decrease of the force and in the increase in the deformation. Myllärinen, Buleon, Lahtinen, and Forssell (2002a) report that amylopectin films are more sensitive to glycerol content than amylose films. Since mechanical properties are not as affected by Tp and pH, the influence of these variables is better seen when plotted for fixed glycerol concentrations. Figs. 3 and 4 show puncture force and relative deformation at break at a fixed value of Cg of 21.59%, as a function of pH and temperature of processing. Puncture and deformation are not affected by temperature and pH has a little effect. Lower values of deformation and higher forces were obtained at lower pHs. The following Eqs. (5)–(8) represent the

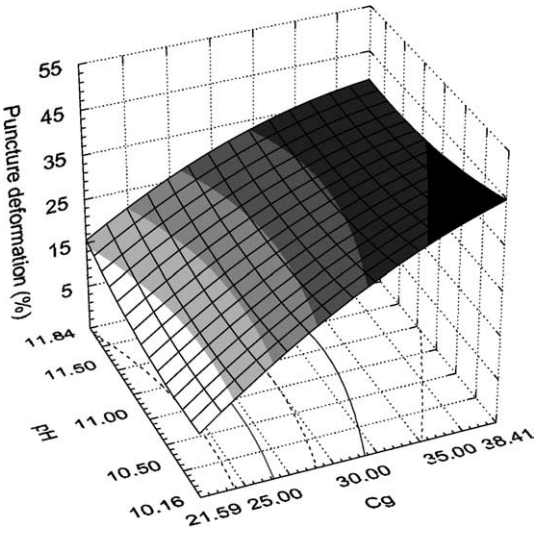

Fig. 2. Response surface of deformation at break—puncture test—as function of glycerol content (Cg) and pH. Tp = 85 °C.

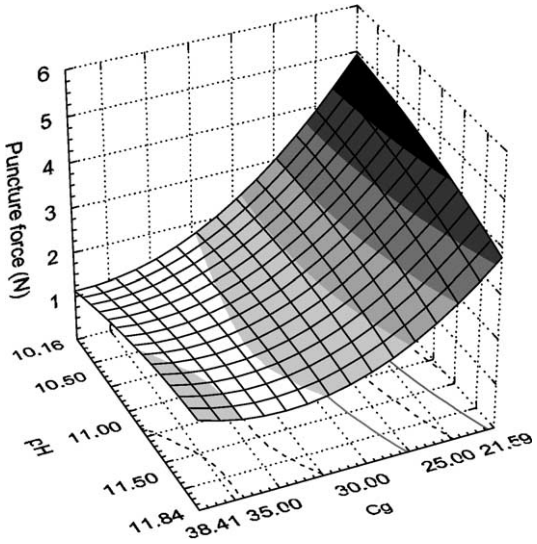

Fig. 1. Response surface of force at break—puncture test—as function of glycerol content (Cg) and pH. Tp = 85 °C.

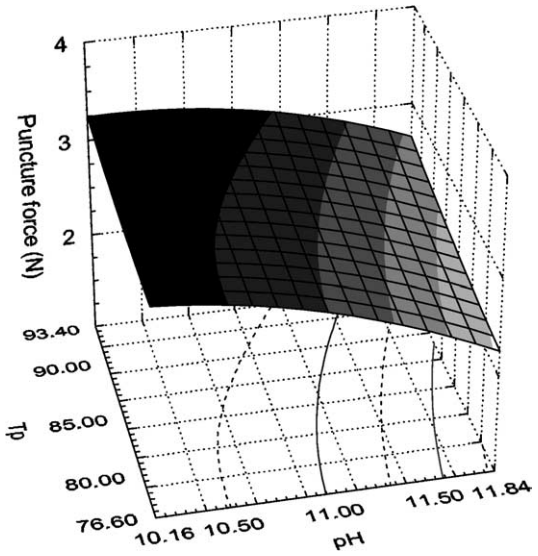

Fig. 3. Response surface of force at break—puncture test—as function of pH and process temperature (Cg = 21.59%).

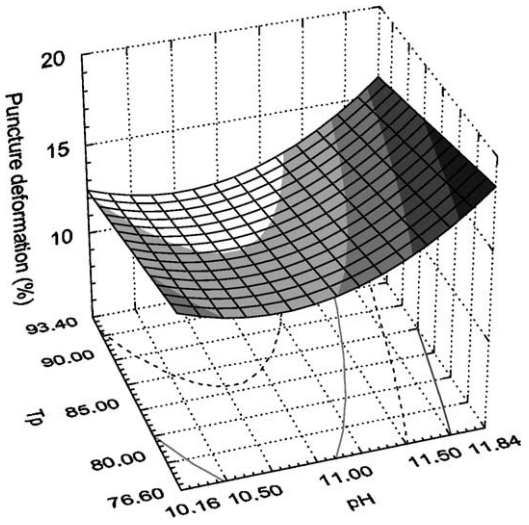

Fig. 4. Response surface of deformation at break—puncture test—as function of pH and process temperature (Cg = 21.59%).

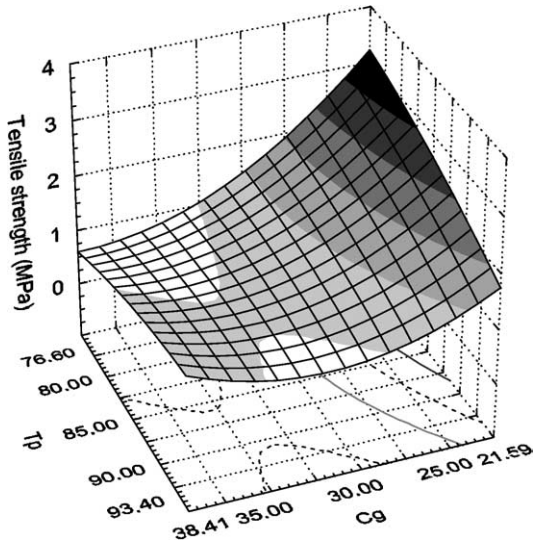

Fig. 5. Response surface of tensile strength as function of glycerol content and Tp, (pH = 11).

surface responses for tensile tests: strength (T), elongation (E) and Young Modulus and solubility determination. All data were fitted by Statistic 5.0 software and analysis of variance (ANOVA) of data show that all models were predictive ($p < 0.05$), with F values greater than the critical values.

$$T = 0.673 - 0.517X_1 + 0.272X_1^2 - 0.069X_2 - 0.036X_2^2 - 0.094X_3 - 0.038X_3^2 + 0.105X_1X_2 + 0.205X_1X_3 - 0.040X_2X_3 \quad (R^2 = 0.90) \tag{5}$$

$$E = 117.07 + 34.62X_1 - 9.58X_1^2 + 3.63X_2 - 3.02X_2^2 - 2.70X_3^2 + 4.32X_1X_2 - 9.60X_1X_3 + 5.48X_2X_3 \quad (R^2 = 0.90) \tag{6}$$

$$\text{Young modulus} = 3.26 - 16.92X_1 + 14.07X_1^2 - 0.32X_2 - 1.67X_2^2 - 3.06X_3 - 1.57X_3^2 + 0.32X_1X_2 + 5.12X_1X_3 - 3.03X_2X_3 \quad (R^2 = 0.83) \tag{7}$$

$$\text{Solubility} = 40.82 + 3.36X_1 + 4.20X_1^2 + 1.46X_2 + 5.70X_2^2 + 1.36X_3 + 3.32X_3^2 - 1.14X_1X_2 - 2.22X_1X_3 \quad (R^2 = 0.80) \tag{8}$$

The response surfaces generated using Eqs. (5)–(8) are given in Figs. 5–7. Tensile test results verified the same trends as the puncture tests: high influence of glycerol content on response variables with pH and temperature contributing less. The analysis of these figures shows that a higher tensile strength for lower glycerol content and lower process temperatures, corresponding with a

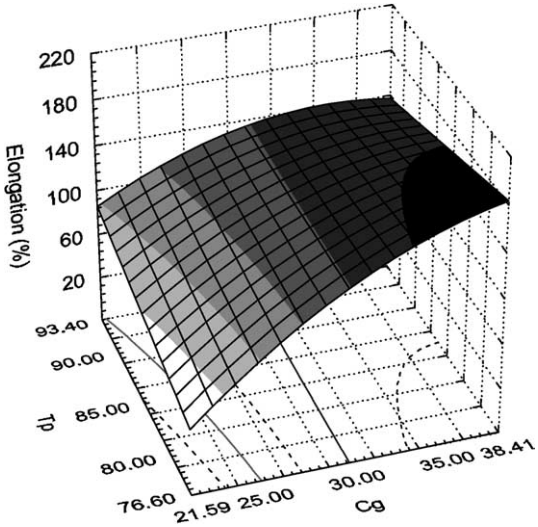

Fig. 6. Response surface of tensile elongation as function of glycerol content and Tp, (pH = 11).

reduced elongation at break. Consideration of stress and elongation suggested that the optimum values were obtained at temperatures ranging from 78 to 85 °C and at pH between 10.5 to 11.5. The highest values for the Young modulus were found in the same variables range.

The solubility results are shown in Fig. 8. According to these results, there is a minimum value of solubility as a function of variables (pH: 10.5–11.5, Cg: 22–35 and Tg: 76–85). The heat treatment of Amaranth flour led to the starch gelatinization and some denaturation of protein. The temperature of gelatinization for Amaranth starch (*Amaranthus Cruentus* ranges from 67 °C up to 75 °C (Pampa, 2003). It can thus be assumed that after the heat treatment of the film forming solution (FFS) (85–95

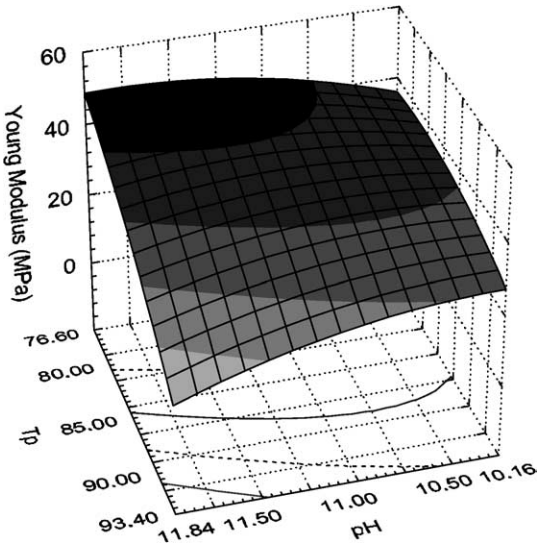

Fig. 7. Response surface of Young Modulus as function of pH and Tp at a fixed glycerol content of 21.59%.

°C/15 min) all starch was gelatinized. However, this heat treatment affects the same variety of starch in different ways. Pampa (2003) did not verify changes in the viscoelasticity of a gel of Amaranth starch during the heating of the gelatinized samples up to 90 °C, although, Pereira (2003), did find a continuous increase in gel consistency in the same variety. It is however well known that heat treatment tends to cause a loss in gel consistency for the most starches. But Vicentini, Sobral, & Cereda (2003) studying the effect of gelatinization temperature (70 and 80 °C) on the mechanical properties of cassava starch-based films prepared from FFS with 2% and 3% starch, found no significant effect of the heat treatments on the properties independent of the starch concentration in FFS.

In relation to the protein fraction, the situation is more complex, since these proteins consist of various fractions with different denaturation temperatures (Td): albumin-1 (Td = 64 °C), albumin-2 (Td = 94 °C), two globulin fractions (Td∼75 and Td = 94 °C) and two glutelin fractions (Td = 70 and 96 °C) (Martínez & Añón, 1996). Moreover, it is known that heating modifies the three-dimensional structure of globular proteins, thus causing exposition of the SH groups, and the consequent production of S-S bonds between adjacent protein chains. This also promotes the exposition of hydrophobic groups which may led to hydrophobic interactions during drying (Perez-Gago & Krochta, 2001). When studying the effect of thermal treatment on the mechanical properties of films based on whey protein isolate, Perez-Gago & Krochta (2001) observed that the increase in temperature of thermal treatment from 70 to 80 °C/20 min increased the tensile stress of these films, although more intense treatments at 90 and 100 °C/20 min caused a reduction. A similar behavior was observed by Iwata, Ishizaki, Handa, & Tanaka (2000) when working about sarcoplasmatic proteins-based films, at temperatures between 55 and 90 °C with a maximum tensile strength obtained at 70 °C.

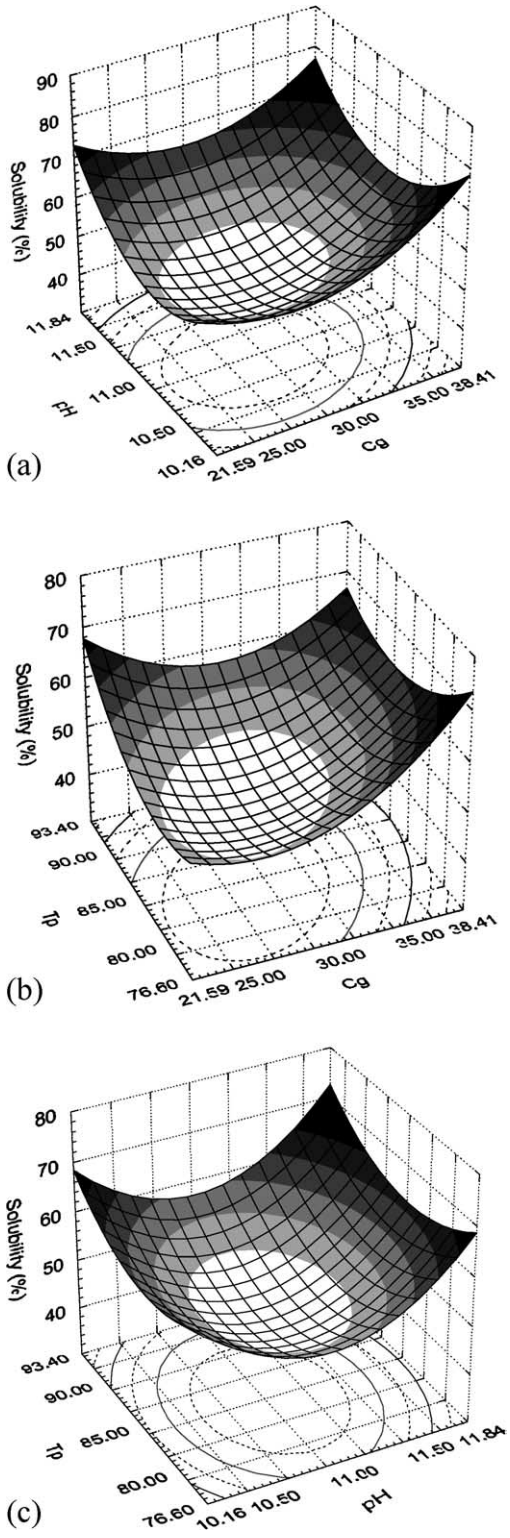

Fig. 8. Responses surfaces of solubility as function of (a) Cg and pH (Tp = 85 °C), (b) Cg and Tp (pH = 11), (c) pH and Tp (Cg = 30%).

Rheological studies of gels of protein isolate obtained from *Amaranthus Hypochondriacus* showed that gel viscoelastic properties improved with an increase of processing temperature (70–95 °C), time and protein concentration. Gels obtained at high temperatures presented a more elastic and ordered structure, which suggest a reinforcement of the hydrophobic interaction (Avanza, Puppo, & Añon, 2003). Therefore, more resistant films should be obtainable either by the leaching of amylose or swelling of amylopectin during starch gelatinization or by the restructuring of the protein with heat treatment.

Amaranth protein is very difficult to be dissolved completely in aqueous solvents (Martínez & Añón, 1996). The pH range chosen was design to achieve maximum dissolution though certain conformation changes may be verified in high alkaline mediums.

Since the integrity of the film is one of the most important aspects, taking the solubility and the mechanical test results into consideration, the optimal casting formulation was considered to be a glycerol content of 22.5%, a pH of 10.7 and process temperature of 82 °C.

Table 2
Properties of Amaranth flour film made with the optimized formulation

Properties[a]	Value
Solubility	42.25 ± 1.82%
Mechanical properties	
Puncture test:	
Force at break	2.34 ± 0.09N
Elongation at break	15.62 ± 0.65%
Tensile test:	
Stress at break	1.45 ± 0.04 MPa
Elongation at break	83.74 ± 5.11%
Young Modulus	215.0 ± 1.4 Mpa
Barrier properties	
Water permeability	0.0093 ± 0.0008 $g\,mm\,h^{-1}\,m^{-2}$ kPa
Oxygen permeability	5.63 $cm^3\,\mu m\,m^{-2}\,d^{-1}$ kPa
Thickness	0.083 ± 0.005 mm
$a^*(a_o^* = -0.8)$	−1.16 ± 0.01
$b^*(b_o^* = 1.44)$	8.09 ± 0.49
$L^*(L_o^* = 94.83)$	89.97 ± 0.28
ΔE^*	8.89 ± 0.62
Opacity	6.45 ± 0.90

[a] Films were conditioned at 25 °C and 58% of relative humidity for 48 h.

3.2. Properties of Amaranth flour film at the best formulation

Properties of films made with the optimal formulation with a process temperature of 82 °C, dried at 40 °C and 55% relative humidity, are presented in Table 2. The tensile stress (1.45 MPa) was lower than that obtained for film of pure amylose (20 MPa) and amylopectin (8 MPa) (Myllärinen, Partanen, Seppälä, & Forssell, 2002b) but tensile elongation was greater (83.74%) than 5% for amylose and 2% for amylopectin films. Pure starch films thus seem to be more brittle and with poorer elongation properties, although Amaranth starch films are less resistant.

Mixtures of amylose and amylopectin present a different behavior. Lourdin et al. (1995) studied the main characteristics of films made with blends of amylose (from pea starch) and amylopectin (potato starch), plasticized with glycerol (20% d.b.). Although the film thickness ranged from 30 to 60 μm, the tensile strength of pure amylopectin film ranged from a low value of 5 MPa, to a high of 24 MPa for those with 40% of amylose content. Tensile stress, however, remained constant with even further addition of amylose. The elongation varied inversely with tensile strength with greater plastic deformation observed for pure amylopectin film (25% of strain) decaying to 7% when amylose content reached 40%. The authors concluded that the amylopectin fraction was more sensitive to glycerol content than the amylose, thus resulting in more ductile material. Amaranth flour films have a more complex composition including not only glycerol, amylose and amylopectin

Table 3
Barrier properties of films

Material	Film thickness (μm)/(glycerol content)	O_2 Permeability 10^{10} ($cm^3\,m^{-1}\,s^{-1}\,Pa^{-1}$)	Water permeability 10^{10} ($g\,m^{-1}\,s^{-1}\,Pa^{-1}$)
Corn starch[a]	104.34 (34.4%)	4.61	2.57
Amylomaize[a]	128.7 (34.4%)	3.21	2.14
Yam starch[b]	110 (40%)	–	1.3
Whey protein[c]	110 (33.3%)	–	13.3
Hydroxypropyl starch/gelatin[d]	800 (15%)	0.0002	0.5
Amaranth flour[e]	83 (22.5%)	0.00065	0.025
LPDE	–	0.16	0.00316
Myofibrilar protein[f]	40 (40% sorbitol)	–	0.049

[a] García et al. (2000).
[b] Mali et al. (2002).
[c] Shaw et al. (2002).
[d] Arvanitoyannis et al. (1998).
[e] This work.
[f] Souza (2001).

but also protein and fat. Glycerol, fat and water behaves as plasticizers and this may explain the greater strain and low tensile stress obtained.

Nevertheless, these films furnish better barrier properties than other biofilms. This was an unexpected fact as Amaranth is a waxy starch. Rindlav-Wetsling, Standing, Hermansson, & Gatenholm (1998) report a value of 5 $g\,mm\,m^{-2}kPa$ for the water vapor permeability of amylopectin film, but the value of Amaranth is much lower: 9.3×10^{-3} $g\,mm\,m^{-2}kPa$. Oxygen permeability is also lower: 6.5×10^{-14} $cm^3\ m^{-1}\ s^{-1}Pa^{-1}$. Table 3 shows the water and oxygen permeability of several materials and Amaranth film can be seen to present advantageous barrier properties.

4. Conclusions

Amaranth flour seems to be a very interesting material for the formulation of coatings and edible films. The presence of native lipids and proteins of its formulation provide it with better barrier properties if compared with other biofilms. The glycerol content as usual is the most important parameter influencing on mechanical properties but due to the complex composition of Amaranth flour also these properties and film solubility are function of pH and temperature. Statistical methodology used allowed to find the optimal condition of casting process: pH: 10.5–11.5, Cg: 22–35 and Tg: 76–85. The process developed here, produced films slightly yellowish with moderate opacity, good flexibility and barrier properties although mechanical resistance was relatively low.

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