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Mechanical and thermal characteristics of amaranth starch isolated by acid wet-milling procedure

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ABSTRACT

Effects of soaking conditions during acid wet-milling of amaranth grain on mechanical and thermal properties of amaranth starch were investigated. A factorial design based on temperature (40–60 °C) and SO_2 concentration (0.1–1.0 g/L) was used. Dynamic oscillatory tests involved heating and cooling cycles (25–90 °C) with tempering at 90 °C followed by a frequency sweep (0.1–20 Hz) at constant temperature. Thermal properties of starch suspensions were based on DSC tests. Viscoelastic modulus and thermal properties were affected by both factors, being significant the interaction effect. Maximum values of viscoelastic modulus at the end of heating and cooling steps were determined for samples corresponding to soaking conditions of 50 °C and 1.0 g/L SO_2. Based on Ross-Murphy index from frequency sweep analysis it was found that paste behavior occurs at 60 °C and 0.1 g/L, while at 47.4 °C and 0.72 g/L starch suspension gave a strong gel. Onset (61.7 °C) and peak (66.2 °C) temperatures showed a minimum at 50 °C and 1.0 g/L. As SO_2 concentration decreased, the end of gelatinization took place at lower temperature. Maximum gelatinization enthalpy (12.7 J/g) was found at 40 °C and 0.1 g/L SO_2.

1. Introduction

Grain amaranth is a pseudo-cereal with a longstanding tradition in Central and South America. The plant produces very small seeds with high nutritional benefits. Starch is the major component in amaranth grain accounting for 48–69 g/100 g of its weight, depending on the species (Teutonico & Knorr, 1985). Amaranth starch has received particular attention among investigators as a consequence of its small uniform granules, 0.5–2 μm in diameter (Baker & Rayas-Duarte, 1998; Radosavljevic, Jane, & Johnson, 1998) compared to rice starch, which represents the smallest produced starch available in the market, whose granules are between 3 and 8 μm. The extremely small starch granules of amaranth provide unique functional properties in numerous industrial and food applications. Despite the increasing commercial interest in amaranth starch, till present there is no effective wet-milling procedure for obtaining starch from amaranth grain.

Soaking is a key step that affects the properties of milling fractions. The soaking temperature and the concentration of chemicals (alkali or acid) have been subject of several research works, with different proposed procedures. Yanez and Walker (1986) used a diluted solution of sodium metabisulfite at 52 °C as a soaking medium to isolate starch from amaranth grain. Soaking of amaranth grain in sodium hydroxide solutions was carried out by Perez, Bahnassey, and Breene (1993), Myers and Fox (1994) and Zhao and Whistler (1994), among others investigators to separate protein from starch. A shortcoming of alkaline soaking is the high concentration of the compound required to leach the protein, with the consequent damage of starch granules and the loss of starch yield. An alternative procedure, which includes the use of enzymes to reduce alkali concentration during soaking, was proposed by Radosavljevic et al. (1998) to isolate starch of whole amaranth grain with high starch recovery and purity.

In contrast to research undertaken on alkaline starch isolation procedures, acid wet-milling of amaranth grain has been less investigated. Malinski, Daniel, Zhang, and Whistler (2003) used an aqueous solution of sodium metabisulfite for soaking amaranth grain for 24 h (the soaking temperature was not reported). Calzetta Resio, Aguerre, and Suarez (2006) found that temperature and sulfur dioxide concentration had a marked effect on the rate of water absorption by amaranth grain during soaking. They determined that the presence of sulfur dioxide in the soaking media not only increased the rate of water absorption (in comparison with the rate of absorption in pure water), but also the level of water saturation within the grain. More recently, Calzetta Resio, Tolaba, and Suárez (2009) investigated the effect of soaking temperature and sodium metabisulfite concentration on yield and composition of wet-milling fractions of amaranth grain. Among the results

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obtained by these workers, it was possible to determine the soaking conditions (temperature and sulfur dioxide concentration) that conduct to maximum starch yield with low protein content.

Even though the results obtained till present show that acid wet-milling procedure of amaranth grain affects the yield and purity of milling fractions, little is known regarding the effect of milling procedure on the functional properties of isolated fractions. Bejarano-Luján and Netto (2010) found that differences in the process of alkaline extraction of protein from amaranth grain produces changes in composition and functionality of the isolated product. Bejarano-Luján, Lopes da Cunha, and Netto (2010) also observed that amaranth protein isolation procedure modifies the structure and rheological properties of their gels. According to these authors such modifications might be induced by the presence of non-protein components, such as starch in the isolated protein.

However, to the best of our knowledge, there is no information concerning to the effect of wet-milling procedure on the functionality of the starch isolated from amaranth grain. The aim of the present work was then to assess the effect of soaking procedure (temperature and sulfur dioxide concentration) on the functional properties of amaranth starch obtained by acid wet-milling method.

2. Materials and methods

2.1. Material

Amaranth grain (*Amaranthus cruentus*) used in this study was harvested at the experimental station of the National University of La Pampa (Argentina). The grains were screened to remove foreign matter and stored in sealed containers at room temperature (25 °C) previous to their use. The moisture content of amaranth grain was 10.5 ± 0.1 g/100 g (AOAC, 1995, 943.01 method).

The proximate composition of amaranth grain was determined by official methods: starch (AACC, 1995, 76-01 method); protein (AOAC, 1995, 976.05 method); fat (AOAC, 1995, 920.39 method); ash (AOAC, 1995, 923.03 method).

2.2. Experimental design

The wet-milling experiments were conducted following a 3^2 factorial design (Table 1). Each wet-milling test was duplicated except the central point of the design that was tripled. The soaking factors were temperature (40, 50 and 60 °C) and SO_2 concentration (0.1 g/L, 0.55 g/L and 1.0 g/L).

Amaranth grains (100 g) were placed in screw-cap flasks containing the soaking solution, prepared by dissolving the appropriate amounts sodium metabisulfite in distilled water. For this purpose the equivalent quantity was calculated, resulting 67.37 g of SO_2 every 100 g of sodium metabisulfite. The ratio of soaking solution/grain was 3:1 (mL/g). The soaking time, dependent on soaking temperature, was that adequate to accomplish saturation of the grain (Calzetta Resio, Aguerre, & Suarez, 2003). Following this criterion, the soaking times were 140, 120 and 45 min at 40, 50 and 60 °C, respectively.

Table 1
Effect of soaking conditions on maximum values[a] of storage modulus (G'_{max}), loss modulus (G''_{max}) and temperature of occurrence (T_{max}) during heating sweep.

N°	Soaking conditions		G'_{max} (Pa)	G''_{max} (Pa)	T_{max} (°C)
	Temperature (°C)	SO_2 concentration (g/L)			
1	40	0.1	57.4	21.5	81.8
2	40	0.55	44.5	23.3	77.15
3	40	1.0	527	114	71
4	50	0.1	1099	135	80.3
5	50	0.55	479	111	75.2
6	50	1.0	2520	265	74.8
7	60	0.1	371	105	80.5
8	60	0.55	287	81.7	79.3
9	60	1.0	1955	268	76.6

[a] The values shown are the average of two replications with the exception of central point of design (N° 5) that was triplicated.

Subsequently the grains were ground in a Waring blender (Dynamic Corp. of America, New Hartford City, CT) with 100 mL of soaking solution at the maximum speed for 3 min. The homogenate was sifted in a Testing Equipment Zonytest, model EJR 2000 through sieves of 180 μm (80 mesh ASTM), 75 μm (200 mesh ASTM) and 53 μm (270 mesh ASTM). Residues retained in each sieve were washed with sulfur dioxide solution of a selected concentration (0.1, 0.55 and 1.0 g/L) until no more visible starch was released. The starch was then isolated from the filtrate by using a final centrifugation at 4000 × g for 20 min. The supernatant containing proteins and fat was discarded, and the top grayish layer of the precipitate was carefully removed with a laboratory spatula. The bottom white layer of starch was resuspended in distilled water and centrifuged as described above; the process was repeated three times. Thereafter, the isolated starch was dried in a vacuum oven at 40 °C for 48 h and stored at room temperature in a sealed container.

2.3. Starch yield and quality

Performance of each amaranth starch isolation test was evaluated on the basis of starch yield (ratio of extracted starch to the starch content of the grain), and purity of starch, measured in terms of the protein content of isolated starch. Nitrogen contents of isolated starch were determined by the micro-Kjeldahl. Protein contents were estimated at N × 5.85 (AOAC, 1995).

2.4. Differential scanning calorimeter (DSC)

Gelatinization parameters were measured by thermal analysis using a differential scanning calorimeter (DSC 822 Mettler Toledo, Switzerland), equipped with a thermal analysis data station (Mettler Star program), following the procedure by Barba de la Rosa, Paredes Lopez, Carabez Trejo, and Ordorica-Falomir (1989). Prior to experiments, DSC was calibrated with Indium. Starch (5 mg) was weighed directly in an aluminum DSC pan of 40 μL capacity. Deionized water was added with the help of a micro syringe to achieve a starch–water suspension containing 70 g/100 g water. Pans were hermetically sealed and equilibrated for 24 h at room temperature to allow complete hydration of starch. The pan with the sample was placed in the calorimeter and heated at a rate of 10 °C/min from 20 to 100 °C; an empty pan was used as reference. Peak temperature, initial and final temperatures and gelatinization enthalpy were calculated automatically.

2.5. Viscoelastic properties

Starch dispersions of 100 g/L of total solids were prepared by adding the powder to distilled water and suspending with a magnetic stirrer at room temperature for 15 min before loading on the bottom plate of the rheometer. Viscoelastic properties were measured under low amplitude oscillatory shear in a strain rheometer (Paar Physica MCR 300, Anton Paar, Austria), which was equipped with a parallel plate measuring geometry of 30 mm diameter and a gap of 1 mm, being the sample edges covered throughout with a thin layer of low-density silicon oil (Dimethylpolysiloxane; 50 cPs viscosity) to minimize evaporation. To determine the linear viscoelastic region, strain amplitude sweeps

were done between 0.1% and 20% at 25 °C and constant frequency of 1 Hz using gelatinized samples.

Once the linear viscoelastic region was found, the rheometer was programmed to run temperature sweeps consisting of heating (25–90 °C), tempering (90 °C for 20 min) and cooling (90–25 °C) steps at 10 °C/min, 1 Hz and deformation of 0.5%. Storage modulus (G') and loss modulus (G'') were recorded as a function of temperature.

Temperature sweep was followed by a frequency sweep performed at 25 °C using the gelatinized samples. The angular frequency varied between 0.1 and 20 Hz at constant strain of 0.01%. The mechanical spectrum of the sample was obtained in order to characterize the rheological behavior of the studied system.

2.6. Statistical analysis

To assess the effect of different soaking conditions on amaranth starch properties, a response surface methodology (RSM) was applied. The studied responses (Y_K, $K = 1, \ldots, p$) were modeled as function of process variables (x_i) by means of a polynomic model related to the experimental design (Khuri & Cornell, 1987):

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i=1}^{n} a_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{\substack{j=2 \\ i<j}}^{n} a_{ij} x_i x_j \quad (1)$$

Coefficients a_o, a_i and a_{ii} represent constant, linear and quadratic effects, respectively, whereas a_{ij} represents interaction effects between factors x_i and x_j. Results obtained were processed with Statgraphics® (Statistical graphics Corporation, U.S.A.).

3. Results and discussion

3.1. Temperature sweep by dynamic oscillatory tests

The proximal composition of amaranth grain in percentage dry basis was: starch (71.5 ± 0.4 g/100 g), protein (16.19 ± 0.12 g/100 g; N × 5.85), fat (7.93 ± 0.12 g/100 g) and ash (3.30 ± 0.06 g/100 g); mean values of three repetitions.

The experimental procedure used to isolate amaranth starch allowed for a mean starch yield of 42.2 g/100 g, which contained 3.05 g/100 g protein in average (Calzetta Resio, Tolaba, & Suárez, 2006). Rheological test were performed within the linear viscoelastic region. It was found a linear behavior for amplitudes smaller than 5%, which falls within the range reported by others authors (6–10%) for starch suspensions (Rosalina & Bhattacharya, 2002; Sopade, Halley, & Junming, 2004).

Representative curves for the changes on the storage modulus (G') of starch suspensions obtained from starch samples isolated under different soaking conditions are shown in Fig. 1a and b. Similar behavior (not shown here) was found for loss modulus (G''). Fig. 1a shows the effect of soaking temperature on G' at constant concentration of soaking media (1.0 g/L SO_2). In Fig. 1b the effect of SO_2 concentration on G' at constant soaking temperature (40 °C) can be appreciated. In all cases, values of G' increased steeply to a maximum at temperatures above 71–82 °C and then decreased with continuous heating; G'' maximum values were also observed for the same temperature range. Such rapid increase of G' and G'' is usually attributed to the hydration and swelling of the starch granules to fill the entire available volume of the system and intergranule contact to form a network of swollen granules (Eliasson, 1986; Vasanthan & Bhatty, 1996).

A more detailed examination of soaking effect on the viscoelastic functions indicated that the temperatures (T_{max}) at which they reached the highest values of G'_{max} and G''_{max} decreased with

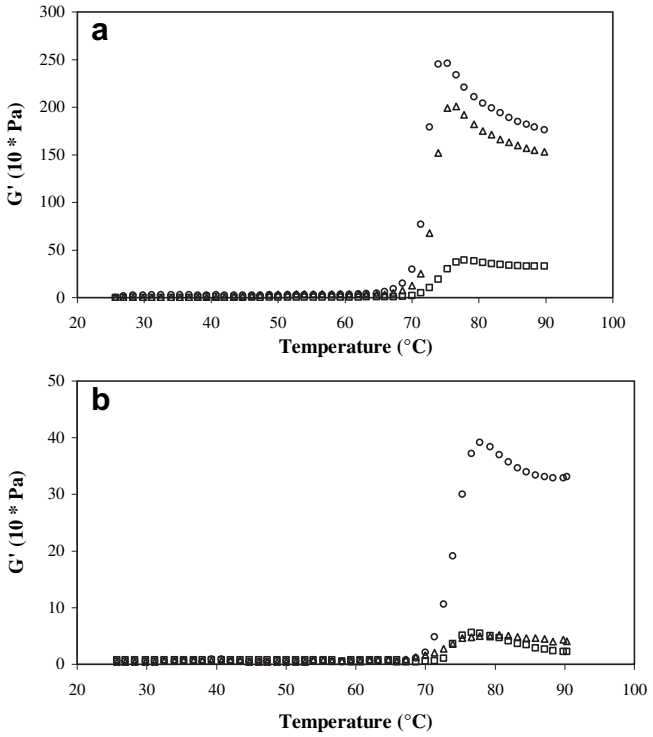

Fig. 1. Rheological properties of amaranth starch from different soaking conditions during a temperature sweep: a) changes of storage modulus (G') as affected by soaking temperature at constant SO_2 concentration (1.0 g/L): 40 °C (□), 50 °C (○), 60 °C (△); b) changes of storage modulus (G') as affected by SO_2 concentration at constant soaking temperature (40 °C): 0.1 g/L SO_2 (△), 0.55 g/L SO_2 (□), 1.0 g/L SO_2 (○).

the increase of SO_2 concentration in the soaking media (Table 1). A similar effect of acid concentration on gelatinization temperature (T_p) from DSC can be observed in Table 5 at soaking temperatures of 50 °C and 60 °C. In Table 1 the positive effect of SO_2 on the values of G'_{max} and G''_{max} are also observed, being more marked for soaking at 40 °C. Values of G'_{max} were always larger than the values of G''_{max}, showing the elastic behavior of the studied system. Table 2 reproduces the final values of G' and G'' obtained at 90 °C (G'_{90}, G''_{90}) and during controlled cooling at 25 °C (G'_{25}, G''_{25}). It can be seen that differences in soaking procedure can impact on the material properties. So, during controlled heating the largest values of viscoelastic modulus correspond to amaranth grains soaked at 50 °C, while for lower and higher steeping temperatures the starch network resulted much weaker. This result reflects the combined effect of temperature–time used during grain soaking. Viscoelasticity increased during controlled cooling following sample gelatinization, as results from the values of G' and G'' at 25 °C given in

Table 2
Rheological characteristics of gelatinized amaranth starch at 95 °C and 25 °C (average values).[a]

N°	G'_{90} (Pa)	G''_{90} (Pa)	G'_{25} (Pa)	G''_{25} (Pa)
1	74.2	40.2	80.1	67.8
2	32.1	25	43.6	42
3	518	114	29,100	4410
4	689	123	723	145
5	491	95.55	515.5	121
6	2083	308.5	4910	2517.5
7	328	117.5	5905	3585
8	217	96.5	261	138
9	1062	131	937	143

[a] The values shown are the average of two replications with the exception of central point of design (N° 5) that was triplicated.

Table 2. It can be appreciated a non linear effect of acid concentration on viscoelastic modulus at constant temperature.

3.1.1. Effect of soaking procedure on temperature sweep

A RSM was applied to analyze the effect of soaking conditions (factors) on the viscoelastic modulus (responses) mentioned previously. In Table 3 the effects of soaking temperature and SO_2 concentration on T_{max}, G'_{max} and G''_{max} are shown in terms of coefficients of Eq. (1), together with the correlation coefficient of the regression, r^2. The table also includes the significant level of each coefficient of Eq. (1). The responses studied were suitably explained by a second-order model (Eq. (1)) and acceptable fitting ($r^2 \geq 0.88$) was obtained in all cases. Table 3 shows that temperature, acid concentration and interaction factor affected the values of T_{max}. At high levels of SO_2 concentration an increase in soaking temperature resulted in a significant increase in the values of T_{max}. However, at low SO_2 values a slight decrease of the T_{max} response with the increase of soaking temperature was observed. Such behavior highlights the significance of temperature–SO_2 concentration interaction during soaking procedure. In Table 3, a significant quadratic effect of temperature and SO_2 concentration on the values of G'_{max} and G''_{max} is also observed, as well as an increase of G'_{max} with the increase of acid concentration. The maximum value (2359 Pa) was obtained for a soaking temperature of 50 °C and 1.0 g/L SO_2. Temperature and acid concentration affected positively G''_{max}; the maximum value of G''_{max} (259 Pa) corresponded to the highest acid concentration tested.

The effect of soaking conditions on the final values of G' and G'' obtained at the end of the heating and cooling during a temperature sweep, were revealed by the coefficients of the second-order polynomials (Table 4). Significant effects of both factors on the viscoelastic modulus were observed; the interaction among them was only significant for G'_{25} and G''_{25}. The effect of acid concentration became more significant with the decrease of soaking temperature. In the case of the elastic modulus it was negatively affected by temperature. The highest values of G'_{25} and G''_{25} were 29.1 and 4.4 kPa, respectively, corresponding to a soaking temperature of 40 °C and 1.0 g/L SO_2. Viscoelastic modulus G'_{90} and G''_{90} were also affected by temperature and acid concentration (quadratic effects). The maximum values of G'_{90} (2085 Pa) and G''_{90} (309 Pa) corresponded to soaking temperatures and acid concentration of 50 °C and 1.0 g/L SO_2, respectively.

3.2. Frequency dependence

The frequency sweep test was applied to further determine whether the gelatinized starch isolated after different soaking procedures formed gel or paste. According to Ross-Murphy (1995) it is possible to relate the storage modulus (G') and the oscillatory frequency (ω) by means of the expression:

Table 3
Regression coefficients of the second-order polynomial model for the response variables G'_{max}, G''_{max} and T_{max}: effect of temperature (x_1) and SO_2 concentration (x_2).

Coefficient	T_{max} (°C)	G'_{max} (kPa)	G''_{max} (kPa)
a_o	105.0	−19.62	−1.65
a_1	−0.886*	0.838*	0.070*
a_2	−239.4*	−58.45*	−4.80*
a_{11}	8.01×10^{-3} NS	-8.38×10^{-3}*	-0.67×10^{-3}*
a_{22}	–	428.1*	40.048*
a_{12}	3.43*	0.521 NS	0.038 NS
r^2	0.8801	0.9088	0.9338

*Significant at $p < 0.05$; NS: non significant coefficient; –, eliminated coefficient.

Table 4
Regression coefficients of second-order polynomial model for the response variables G'_{90}, G''_{90}, G'_{25} and G''_{25}: effect of temperature (x_1) and SO_2 concentration (x_2).

Coefficient	G'_{90} (kPa)	G''_{90} (kPa)	G'_{25} (kPa)	G''_{25} (kPa)
a_o	−14.17	−2.15	59.34	2.53
a_1	0.589*	0.088*	−2.94*	−0.263 NS
a_2	−18.95*	−0.204*	624.9*	138.4 NS
a_{11}	-5.75×10^{-3}*	-0.83×10^{-3}*	0.036 NS	4.92×10^{-3} NS
a_{22}	252.72*	32.92*	2.89×10^{3}*	855.6*
a_{12}	–	−0.046 NS	−17.01*	−4.40*
r^2	0.9352	0.8026	0.7808	0.7063

*Significant at $p < 0.05$; NS: non significant coefficient; –, eliminated coefficient.

$$G' = K\omega^n \tag{2}$$

or the linearized form of equation:

$$\log G' = n \log \omega + \log K \tag{3}$$

Plots of $\log G'$ versus $\log \omega$ were then used to obtain values of n (slope) and K from the intercept ($\log K$) for each of the soaking procedures used to isolate the starch from amaranth grain. The values of n vary in a relative wide range (from 0.057 to 0.41), similar to that found in literature for starchy materials (Chun & Choo, 2004; Navarro, Martino, & Zaritsky, 1997; Yoneya, Ishibashi, Hironaka, & Yamamoto, 2003), which suggests the remarkable effect that soaking conditions have on the viscoelastic characteristics of the isolated starch. So, depending on the combination of temperature and acid concentration it is possible to have amaranth starch which behaved as gels, small values of n parameter and independent of frequency, or pastes, relatively higher n values and largely frequency dependant.

3.2.1. Effect of soaking procedure on mechanical spectrum

The effect of the operating variables (temperature and acid concentration during soaking) and their interaction on the n parameter of Eq. (3) were estimated using a quadratic polynomial model (Eq. (1)). The values of the coefficients were obtained by RSM method with a correlation coefficient $r^2 = 0.81$ and a significant level of 5% ($p < 0.05$). The effect of soaking temperature (x_1) and different SO_2 concentrations (x_2) on the response are revealed by the values of the polynomial coefficients given by the following equation:

$$n = 3.235 - 0.1326x_1 + 6.564x_2 + 1.410 \times 10^{-3}x_1^2 - 9.917x_2^2 - 0.1395x_1x_2 \tag{4}$$

The RSM analysis showed that the quadratic effect of temperature, the linear effect of acid as well as the interaction between temperature and acid concentration were significant. The response surface for n index is given in Fig. 2 to aid visualization of the effect of factors. It can be seen that the effect of acid concentration on the isolated starch varied with soaking temperature and became more marked as temperature increased. In the same figure it is observed that different soaking conditions conduct to a relative wide range of n parameter. For instance, the maximum value ($n = 0.4$) which is characteristic of paste behavior occurs at 60 °C and 0.1 g/L acid concentration, while at 47.4 °C and 0.72 g/L SO_2 concentration the n value was representative of strong gel ($n = 0.057$).

3.3. Thermal properties

Starch samples obtained from different soaking conditions were analyzed by DSC and the thermal parameters were determined, as shown in Table 5. Enthalpy values were similar to those reported in

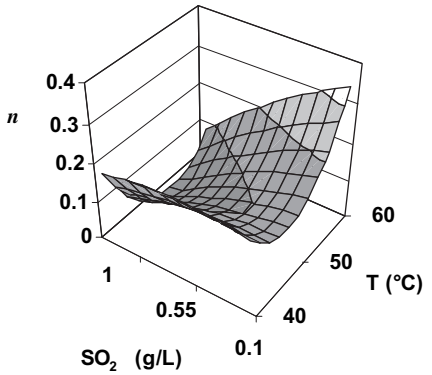

Fig. 2. Predicted response surface of n parameter as affected by soaking conditions obtained from Eq. (2). Different levels of index "*n*": ■ 0–0.1; ■ 0.1–0.2; ■ 0.2–0.3; □ 0.3–0.4.

literature for starches (Abdel-Aal, Hucl, Chibbar, Han, & Demeke, 2002; Huang, Chang, & Lii, 1994; Perez, 2000; Song & Jane, 2000; Tapia Blacido, 2006). However, gelatinization temperatures found here resulted significantly lower than those reported from Tapia Blacido (2006) and Calzetta Resio and Suarez (2001) for amaranth starch obtained by alkaline isolation method, which were 73.1 °C and 73.3 °C (peak temperatures) respectively. It should be noted that amaranth starch obtained by Tapia Blacido (2006) contained 13 g/100 g amylose while the one used in this work has 6.57 g/100 g. These results show that factors such as isolation method (alkaline or acid wet-milling), amaranth variety and amylose content have a significant influence on thermal behavior that make more difficult the comparison among literature data.

3.3.1. Effect of soaking procedure on thermal parameters

To assess the effect of soaking conditions on thermal parameters RSM analysis was performed, the results are shown in Table 6. A significant effect of soaking temperature on gelatinization temperatures (quadratic effect) can be observed, being the interaction effect among studied factors significant for gelatinization enthalpy, onset and peak temperatures. The effect of acid concentration was evident for T_e values (positive effect) and gelatinization enthalpy (negative effect). The minimum value of peak temperature (66.2 °C) was for soaking at 53.9 °C and 1.0 g/L SO_2. For the onset temperature, T_o, the minimum value was 61.7 °C, corresponding to soaking temperature of 50 °C and 1.0 g/L SO_2 concentration. According to Table 6 the interaction effect was more marked for T_p than for T_o, as results from the respective a_{12} coefficients. Likewise, the T_e showed a minimum (75.1 °C) for intermediate soaking temperature and 0.1 g/L SO_2, rising as temperatures approach to the extreme values tested. It was observed that gelatinization ended earlier for more diluted acid solutions.

Table 5
Thermal parameters and protein content of amaranth starch isolated following different soaking conditions.

T (°C)	SO_2 (g/L)	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)	Protein content (g/100 g)
40	0.1	62.5	67.4	76.9	12.7	3.56
40	0.55	62.7	67.4	75.8	12.5	2.53
40	1.0	63.1	69.9	76.6	12.8	3.99
50	0.1	62.5	67.1	75.1	12.7	1.79
50	0.55	62.3	66.7	74.7	12.7	1.97
50	1.0	61.5	65.9	75.9	13.0	0.69
60	0.1	64.0	69.1	76.5	11.9	0.68
60	0.55	64.2	68.4	76.9	12.7	0.71
60	1.0	62.7	66.8	77.8	13.5	1.87

Table 6
Effect of soaking conditions on thermal parameters of amaranth starch.

Coefficient	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g)
a_o	84.6	99.9	114.2	11.79
a_1	−0.99*	−1.46 NS	−1.53 NS	0.0725 NS
a_2	65.5 NS	118.8 NS	−73.9 NS	−41.5*
a_{11}	0.011*	0.016*	0.015*	−0.0012 NS
a_{22}	−172.8 NS	98.77 NS	329.2*	65.8 NS
a_{12}	−1.06*	−2.67*	0.89 NS	0.83*
r^2	0.9210	0.8686	0.9718	0.9139

From Table 6 enthalpy values became higher as acid concentration decreased ($a_2 = -41.5$), while temperature effect depended on SO_2 concentration ($a_{12} = 0.83$). The maximum value of gelatinization enthalpy calculated from RSM analysis was 12.7 J/g which corresponded to soaking temperature of 40 °C and SO_2 concentration of 0.1 g/L.

3.4. Correlations among starch quality and functional properties

In an attempt to determine to what extent changes in the rheological and thermal characteristics of starch were due to its protein content, the following analysis was carried out. Protein contents of starch resulting from the different steeping procedures are given in Table 5. The correlation analysis among protein content and the rheological properties of starch was performed, using for such purpose the Ross-Murphy index, n (Section 3.2). The correlation coefficients (r) among n values and protein contents were obtained from the Pearson product-moment matrix (Snedecor & Cochran, 1980). The correlation coefficient ($r = -0.2865$) and p-value ($p = 0.45$) were calculated with Statgraphics software® (Statistical graphics Corporation, U.S.A.). Given that statistically independent variables have an expected correlation coefficient of zero, it can be said that there was no significant correlation between n index and starch protein content. This lack of correlation (similar results were obtained for G_{95} and G_{25}) revealed that the amount of protein present in the starch fraction is not enough to alter its rheological properties.

A similar analysis was also performed to evaluate the incidence of protein content on thermal parameters of starch: onset temperature (T_o), peak temperature (T_p) and gelatinization enthalpy (ΔH). The correlation coefficients, r, and p-values obtained were the following:

Protein content and T_o: $r = 0.0431$; $p = 0.9124$
Protein content and T_p: $r = 0.4598$; $p = 0.2150$
Protein content and ΔH: $r = -0.1042$; $p = 0.7896$

As can be seen from the present results, it can be concluded that there is no significant correlation between starch protein content and thermal parameters.

4. Conclusions

Milling conditions (SO_2 concentration and soaking temperature) affected thermal and mechanical properties of amaranth starch, provoking significant changes in the consistency of starch suspensions (paste or gel), as well as in the temperature and enthalpy of gelatinization.

Soaking temperature and SO_2 concentration affected characteristic viscoelastic parameters during heating sweep (G'_{max}, G''_{max}, T_{max}), viscoelastic modulus at the end of heating and cooling stages (G'_{90}, G''_{90}, G'_{25}, G''_{25}) and mechanical spectrum of starch suspensions.

It was found significant interaction effects between temperature and acid concentration on T_{max}, G'_{25}, G''_{25} and index n. Strong gel resulted for soaking temperature of 47.4 °C and 0.72 g/L SO_2.

Gelatinization temperatures were affected by soaking temperature and significant interaction effect was found for T_o, T_p, and gelatinization enthalpy. Ending of gelatinization (T_e) and gelatinization enthalpy were also influenced by acid concentration.

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