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Comparing methods for extracting amaranthus starch and the properties of the isolated starches

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ABSTRACT

Starch was isolated from *Amaranthus cruentus* whole grain (WG) and whole grain flour (WGF) using both the alkaline method (AM) and AM combined with food degree protease digestion (AMP). The methods involved successive soaking in NaOH solution (0.25 g/100 ml in AM and 0.05 g/100 ml in AMP), fibrous fraction wet milling, enzymatic hydrolysis in AMP and multi-staged centrifugation. Milling the amaranth grains in both methods increased significantly starch yield, recovery, and purity when compared against WG and lowered soaking times as well. Starch yield and recovery were 116.7% and 123.6% higher in WGF while protein, fiber, and ash contents showed decreases of about 44.4%, 34.8%, and 30.4% respectively. The effect of the extracting methods was less notorious than that of the grain milling. The results suggest that both methods are suitable for extracting starch from previously milled grains despite the fact that the AM shows significant operative advantages. The starches extracted showed conservative structure, A-type difractrometric patterns with high crystallinity degrees (~39%) and T_g (gelatinization temperature) values (~74 °C). These properties not present significant differences as a consequence of the extracting procedure used.

1. Introduction

The starch content of the grains of the genus Amaranthus varies between 55 g/100 g and 60 g/100 g of their weight. This changes them into a potential resource for the commercial production of starch (Calzetta Rezio, Aguerre, & Suarez, 2006). The distinctive and unique features such as its very small size, high stability against freezing/defrosting, and retrogradation resistance in comparison with that of starches from other sources, make the starch granule a product of interest due to its potential applications in both the food and non-food industry (Baker & Rayas-Duarte, 1998; Kong, Bao, & Corke, 2009; Radosavljevic, Jane, & Johnson, 1998) and explain the special attention it has drawn for the last decades. The particular structure and morphology of the amaranth grain as well as the compartmentalized location of its main components (Bestchart, Irving, Shepherd, & Saunders, 1981; Irving, Betschart, & Saunders, 1981) make high yield and recovery starch extracting methods applicable. Thus alkaline treatments, enzymatic hydrolysis, wet milling, and dry milling for its isolation have been adapted and applied by different authors (Malinski, Daniel, Zhang, & Whistler, 2003; Mundliger, 1998; Myers & Fox, 1994; Perez, Bahnassey, & Breene, 1993; Radosavljevic et al., 1998; Uriyapongson & Rayas Duarte, 1994; Yañez & Walker, 1986; Zhao & Whistler, 1994). Its extraction however presents several drawbacks because of, on the one hand, the joint sedimentation of proteins and very fine fiber together with the starch (Zhao and Whistler), and, on the other, hand the small size of its granules brings about important losses at the several sorting and purification stages. These drawbacks determine low recovery percentages much alike those informed for rice by Lundubwong and Seib (2000).

Treatments either alkaline at concentrations higher than 1 g/100 g of NaOH, or enzymatic or combination of both allow obtaining good yield and recovery and low protein contents of the extracted starches. However, the former generate undesirable effluents (both alkaline and saline) along the process whose treatment and later removal increase additional costs; the second involve longer (more than 24 h) treatments for proteins and fibers removal and starch release that not only increase capital costs and intensify microbial problems but requires the use of mostly toxic preserving substances (Lundubwong & Seib, 2000; Perez et al., 1993).

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Selecting an appropriate starch extracting method is restricted to obtain a pure product with the maximum yield and recovery, the lowest cost, and the least amount of effluents possible out of the application of a series of interrelated stages allowing the non-starchy fraction to be removed without affecting the granule native structure and minimal incidence on its physicochemical and mechanical properties nor endowing it with associated toxicities (Correia & Beirao-da-Costa, 2012; Li, Shoemaker, Ma, Luo, & Zhong, 2009; Tester, Yousuf, Karkalas, Kettlitz, & Roper, 2008; Tester, Yousuf, Kettlitz, & Röper, 2007).

The requisites listed above provide elements that help research perfect and compare starch extracting methods from amaranth grains and determine their potential influence on the microstructure, crystalline, and thermal properties of the starch granules.

The objectives of this paper were to isolate amaranth starch from *Amaranthus cruentus* grains using an alkaline method, and the same alkaline method but combined with hydrolysis with a food degree protease; and to characterize such starches by comparing their respective yield, recovery, and purity.

2. Materials and methods

2.1. Materials

The research was carried out on *A. cruentus* whole grains (WG) and whole grain flour (WGF). The former was supplied by the Food Technology Research and Development Center at the Regional Faculty of Rosario, National Technological University, Argentina; the latter was obtained by differential milling as described by Tosi and Ré (1999). A food-degree protease obtained from *Streptomyces griseus* (Type XIV, with a ≥0.35 units/mg of solid activity declared) was purchased at Sigma–Aldrich Co., Argentina and used in addition to chemicals of reactive degree.

WG downsizing was carried out using a table mill for 5 min, the material was then sorted granulometrically using the JR2000 Zonytest vibrating-screen and a set of American Society for Testing and Materials (ASTM) 8-18-35-45-60-80-120-140-170-200 sieves and openings of 200 mesh/inch. The most abundant fractions, namely particles ranging from 2380 to 250 μm (P8/R60) for WG, and those from 1000 to 177 μm (P18/R80) for WGF were used for starch extraction to avoid the very fine fiber from the smaller fractions.

2.2. Starch isolation

2.2.1. Alkaline method (AM)

Starches from the amaranth WG and WGF were extracted by the AM following the process depicted in Fig. 1. The method was redesigned according to the suggestions made by different authors (Myers & Fox, 1994; Radosavljevic et al., 1998; Uriyapongson & Rayas Duarte, 1994; Yañez & Walker, 1986). In comparing this to the above methods results that smaller amounts of alkaline solution (i.e. 1:5 ration against 1:10), shorter times at the soaking steps (i.e. 300 min against 1200 or 1440) and both slower speeds and shorter times at the centrifugation steps were used, and that protein controls along the successive extracting steps were added as well as a neutralizing step before decantation were incorporated.

The sample (50 g) soaking stage in a 0.25 g/100 g of NaOH solution was carried out in a magnetic shaking heater at room temperature at kind shaking for 1 h. The mixture was then centrifuged in a 323K Hermle-Z equipment and the supernatant was kept for determining residual proteins. The solids precipitated were re-extracted "*n*" times using fresh NaOH solution up to obtaining a supernatant whose protein content is less than 1 mg/ml

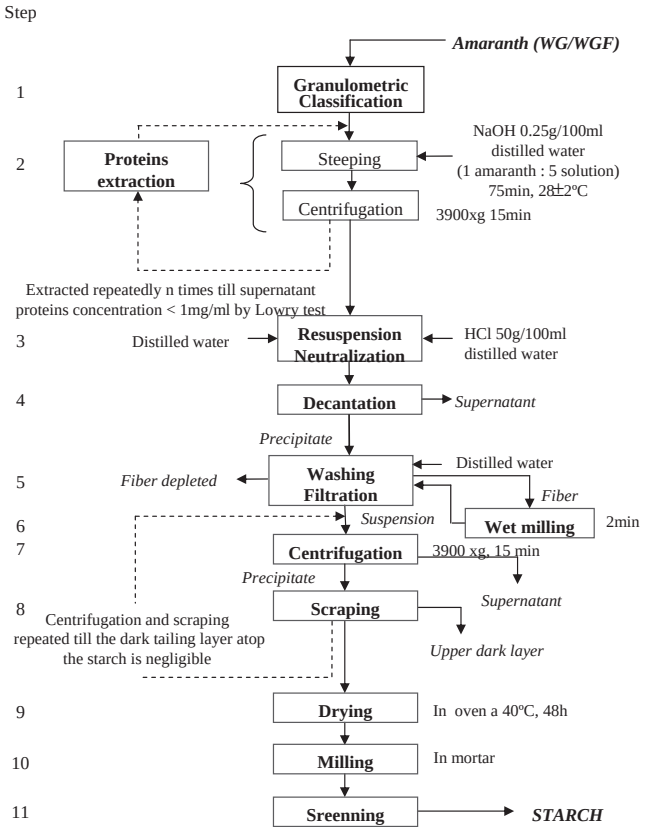

Fig. 1. Isolation of amaranth starch from alkaline method.

in the presence of the Folin Ciocalteu reagent (Lowry, Rosebrough, Farr, & Randall, 1951) (stage 2).

Upon extractions, the precipitate was re-suspended in 50 ml of distilled water and adjusted to pH 7 by adding 50 g/100 ml of HCl (stage 3); it was allowed to decant for 30 min (stage 4) and the solids precipitated were washed and filtered with distilled water (100 ml) through a 74 μm opening stainless steel mesh (stage 5). The fiber portion retained was milled for 2 min in a table blade mill, and washed and filtered using distilled water (100 ml). The resulting suspension was centrifuged (stage 7), the supernatant discarded as well as the scrapped starch dark top layer (stage 8); the precipitate was re-suspended in distilled water (50 ml) and stages 7 and 8 repeated up to obtaining the same conductivity than distilled water and an imperceptible dark layer. The starch was oven dried at 40 °C for 48 h, milled in mortar and sieved in a 74 μm mesh (stages 10, 11, 12 respectively).

2.2.2. Alkaline method combined with protease digestion (AMP)

The procedure for extracting starch out of *Amaranthus* WG and WGF by combining the alkaline method followed by hydrolysis using a food degree protease is depicted in Fig. 2.

The procedure reported by Radosavljevic et al. (1998) was taken as a reference though modified as follows: shorter soaking times (from 22 to 15 h) in the presence of antimicrobial agents, neutralization before the decantation stage, wet milling of the filtration retained, longer digestion time with less amount of enzyme and shorter times of centrifugation.

The sample (50 g) was steeped in a 0.05 g/100 ml NaOH solution with 0.1 g/100 g of sodium azide as an antimicrobial reagent using a magnetic heating-shaker at room temperature under mild shaking for 15 h (stage 2). The steeped suspension was neutralized using a 50 g/100 g of HCl solution, decanted for 30 min and the resulting

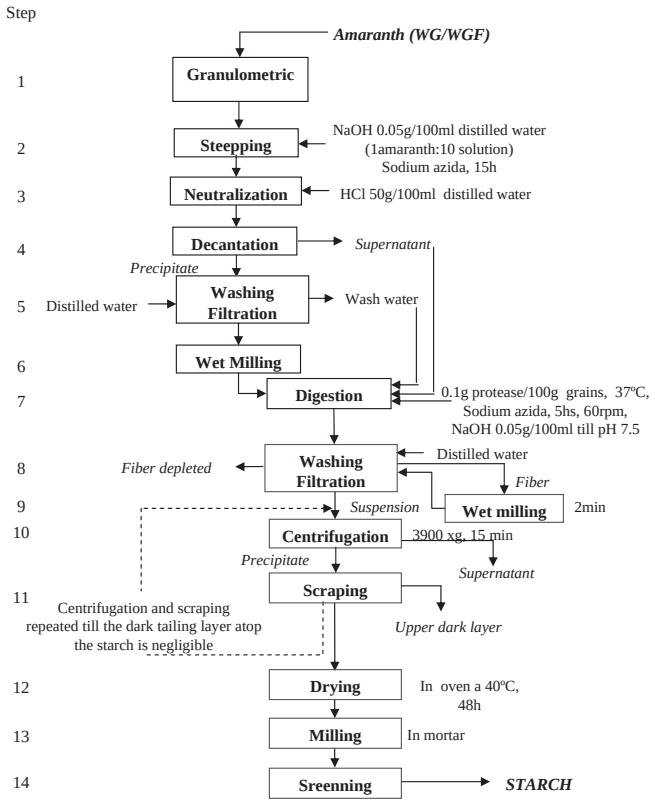

Fig. 2. Isolation of amaranth starch by alkaline steeping combined with protease digestion.

precipitate washed with distilled water (50 ml) on a 74 μm stainless steel mesh (stages 3, 4, and 5 respectively). The solids retained on the mesh were milled using a table blade-mill (stage 6) and later mixed with the supernatant and the water used for the washing. This mixture was added with the enzyme at 0.1 g/100 g as to the total grain processed. The mixture digestion took place in a magnetic thermo stirrer under controlled conditions of temperature ($37 \pm 1\,^{\circ}C$), shaking speed (30 rpm), pH (7.5 ± 0.1) and digestion time (5 h) (stage 7). The remainder stages were carried out in conditions identical to those for the AM procedure (stages 8–14).

2.3. Chemical analysis, yield and recovery

Isolated starches moisture, ashes, fiber, and lipids were determined in accordance with Association of Official Analytical Chemists(AOAC) (1995) standardize techniques, namely 27.3.06, 32.10.5, 32.1.22 and 32.1.14 while proteins (N × 5.75) by the rapid method described by Hach, Brayton, and Kopelove (1985).

The total starch content in WG and fractions of WG (P8/R60) and WGF (P8/R80) was determined by the method described by Tovar, Bjorck, and Asp (1990).

Both the yield and recovery of the starches obtained by the AM and AMP were studied for every sample and estimated according to Equations (1) and (2) respectively:

$$\text{Yield}[\%] = \frac{\text{starch extracted}[g]}{\text{initial sample quantity}[g]} \times 100 \quad (1)$$

$$\text{Recovery}[\%] = \frac{\text{starch extracted}[g]}{\text{total starch sample}[g]} \times 100 \quad (2)$$

2.4. Measurement of color

The color of the starches extracted was analyzed using the CR300 Minolta Chroma meter and the results expressed using the Cartesian coordinates of the CIELAB color space, i.e. luminosity (L^*), red-green chromaticity coordinate (a^*), and yellow-blue chromaticity coordinate (b^*) (Pérez Álvarez, Fernández López and Sayas, 1999).

2.5. Microstructure

The microstructure of the starches extracted by both methods was observed using the 55VP-25-83 SUPRA scanning electron microscope at 1.20 kV of accelerating voltage. Micrographs at 46,000× were taken.

2.6. Relative crystallinity

The relative crystallinity of the WGF and of the starch extracted by both procedures was determined through the technique introduced by Priestley (1975) using $CaCO_3$ as the 100% of crystallinity internal standard. Flour/starches mixtures (5 g $CaCO_3$/100 g sample) were prepared and homogenized in mortar for 30 min. The analysis was performed in a powder diffractometer Philips PW 1710, equipped with exit beam crystalline graphite monochromator, radiation Kα of Cu, $\lambda = 1.5406$Å, 40 kV voltage and current 30 mA, divergence slit 0.5°. Data were collected in the range $5 \leq 2\Theta \leq 40°$, step with 0.02 with a counting time of 1 s. The crystallinity degree (CD) was calculated as the percent ratio between the height of the peak corresponding to $CaCO_3$ (h_1) and that of the most clearly defined peak in the X-ray spectrum of flour/starch (h_2) (Equation (3)):

$$\text{Crystallinity degree}[\%] = \frac{h_2}{h_1} \times 100 \quad (3)$$

2.7. Total amylose (TA) and apparent amylose content (ApA)

Total amylose (TA) and apparent amylose (ApA) contents were determined using the colorimetric method proposed by Juliano et al. (1981). Defatted and whole starches were thus prepared respectively. Potato amylose and amylopectin (SIGMA) were used as measuring standards. Their absorbance was measured at 620 nm using an UV/Vis Hitachi spectrophotometer.

2.8. Thermal properties

The thermal properties were measured using an 823e/500/512 METTLER-DSC Scanning Differential Calorimeter driven by the STARe Default DB V9.00 software (Mettler Toledo, Switzerland). Samples 70:30 (water: starch) g: g were prepared in 100 μl aluminum capsules. The starch was weighed and added distilled water using a micro syringe and left to rest for 24 h. The calorimetric scanning was made according to the following protocol: a) 25 °C isotherm for 2 min, and b) 25 °C–120 °C scanning at $\beta = 10$ °C/min. An empty capsule (air) was used as reference. The starting temperature (T_o), that of gelatinization (T_g), the enthalpy of gelatinization (ΔH_g); the cooperativeness of the gelatinization process ($\Delta T_{½}$) was obtained by measuring the gelatinization endotherm width at half the peak height (STARe Default DB V9.00 software).

2.9. Statistical analysis

The results were analyzed statistically using InfoStat (Di Rienzo et al., 2008). The data were evaluated using the one way analysis of

Table 1
Yield and recovery of *Amaranthus cruentus* starches isolated by different procedures.

Samples	Yield (%)		Recovery (%)	
	AM	AMP	AM	AMP
WG	17.56 ± 0.13[a]	24.47 ± 0.35[b]	25.93 ± 0.19[a]	36.14 ± 0.52[b]
WGF	45.29 ± 0.21[c]	46.18 ± 0.18[c]	69.55 ± 0.33[c]	70.93 ± 0.27[c]

Means values ± standard deviation ($n = 3$).
Value in columns and files with different superscript for identical parameter are significantly different ($P \leq 0.05$).

variance (ANOVA) for a significance level of $P \leq 0.05$. The multiple comparisons test by Tukey was used to establish the significant differences among treatments.

3. Results and discussion

The starch content of the WG, of its fraction P8/R60, and of the fraction P8/R80 of the WGF used in the extractions was: 68 g/100 g ± 1.3; 67.7 g/100 g ± 1.1 and 65.1 g/100 g ± 0.97 (d.b.) respectively.

3.1. Starch yield, recovery and purity

The performance of the methods for extracting starch from *A. cruentus* WG and WGF was evaluated in terms of their yield; recovery; ash, fiber, and protein contents; and color.

Table 1 shows WG and WGF yields and recoveries achieved in both procedures. For WGF, its yield and recovery in both methods were higher than those reported by Malinsky et al. (2003); Myers and Fox (1994) and Uriyapongson and Rayas Duarte (1994). These results might be due to both the fact that the grain was ground before the alkaline treatment which favored protein release in a shorter soaking stage than those taken up by Radosavljevic et al. (1998): 5 h against 24 h in the AM and 15 h against 22 h in the AMP and that the fibrous fraction entered the wet milling stage with starch still adhered to it (stage 6 in AM, stages 6 and 9 in the AMP) as well as to the successive centrifuging and scraping (stages 7 and 8 in AM, stages 10 and 11 in the AMP).

In this work and in order to prevent starch gelatinization and highly alkaline discharges (Mitchell, 2009), more diluted solutions of NaOH (0.25 g/100 ml in AM and 0.05 g/100 ml in AMP) and smaller quantities than those used by Myers and Fox (1994), Perez et al. (1993) and Radosavljevic et al. (1998) and the same that those considered as optimal in the AM proposed by Correia and Beirao-da-Costa (2012) in the enzymatic method were used. This condition may possibly have resulted in the lower yields of WG for both procedures as to those in the method by Radosajvljevic et al. (1998).

Table 2 depicts purity and color of the starches extracted from WG and WGF by the treatments under study. WG and WGF starches purity was higher than that informed by Myers and Fox (1994) and Zhao and Whistler (1994). This may probably happen because of the bigger and heavier fractions used in stage 1, the special care taken at the washing and filtration stages (5 in AM, and 8 in AMP), the repeated centrifugations and scrapping stages (7 and 8 in AM, 10 and 11 in AMP) that altogether lowered starch protein, ash, and very fine fiber contents. Uriyapongson and Rayas Duarte (1994) reported protein and fiber lower than those in this work, possibly due to the previous abrasive separation of the protein-fibrous embryo that instead improving subsequent starch extraction and purity reduces yield.

As to color, it is noticed that both luminosity (L^*) and chromaticity coordinates (a^*, b^*) of the WGF starch extracted using AM are quite closer to those of the blank. This attests that the final product quality associates to higher purity. In turn, the chromaticity coordinates of the WGF starch extracted by AMP showed good luminosity even though they move away from the reference towards the greenish yellow which might be associated to the its higher ash content. The starches extracted from WG presented significantly lower luminosities and chromaticity parameters slightly moving towards the greenish yellow (AMP) and reddish yellow (AM).

Table 3 summarizes a comparison between the methods under study and grain milling in terms of the yield, recovery, and purity of the starches isolated. The results show that milling the grains contributes to a large extent to a higher yield, recovery and purity than the extracting methods. Yield increased 117.6% because of the grinding effect and 12.4% because of the enzyme treatment. Alike results occurred in recoveries: WGF recovered 126.3% more than WG while the recovery using AMP was 12.1% more than that using AM.

Concerning purity, it is observed that the extracting methods had non-significant effect upon the final ash and fiber contents of the starches extracted while the protein content in the starches extracted using AMP was 48% lesser than those of the starches isolated by AM. Sample grinding caused significant increase in purity showing diminution of about 44.4%, 34.8%, and 30.4% in protein, fiber, and ash contents respectively as to those in the starches extracted from WG. Contrary to what Correia and Beirao-da-Costa (2012) reported for chestnut and acorn flours, the purity shown by the starch isolated from WGF and WG did not show significant differences related to the extracting methods.

The results provide information that allows asserting that the method has scarce incidence upon yield, recovery and purity exception made as to the significant decrease of the protein content recorded in the AMP. These suggest that either of both methods is appropriate for extracting starch out of WGF. However, the AM presents important operational advantages concerning total times (Table 4), lesser risk of starch fermentation/degradation and of toxicity along the various stages of the process and in the products, and lower costs of reagents (Lundubwong & Seib, 2000; Tester et al., 2007). On the other hand, it can be stated that the Radosavljevic et al. (1998); Uriyapongson and Rayas Duarte (1994); Yañez and

Table 2
Purity and color of starches isolated of WG and WGF *Amaranthus cruentus* by AM and AMP.

		WG starch		WGF starch	
		AM	AMP	AM	AMP
Purity (g/100 g d.b.)	Protein	0.722 ± 0.125a	0.277 ± 0.075b	0.301 ± 0.103b	0.254 ± 0.211b
	Fiber	0.054 ± 0.013a	0.039 ± 0.012b	0.029 ± 0.014c	0.031 ± 0.020c
	Ash	0.795 ± 0.035a	0.698 ± 0.077b	0.500 ± 0.052c	0.541 ± 0.036c
Color[a]	L^*	89.66 ± 0.01a	89.28 ± 0.01a	96.64 ± 0.02b	96.06 ± 0.01b
	a^*	+0.46 ± 0.01	−0.25 ± 0.02	+0.16 ± 0.04	−0.19 ± 0.01
	b^*	+2.19 ± 0.05	+2.58 ± 0.02	+1.95 ± 0.02	+2.75 ± 0.02

Means values ± standard deviation ($n = 2$).
Value in columns and files with different superscript for identical parameter are significantly different ($P \leq 0.05$).
[a] Color white calibration ($L^* = 96.82$, $a^* = +0.19$, $b^* = +1.95$).

Table 3
Effect of different methods of starch extraction and grinding of the sample on yield, recovery and purity.

Effect	Yield	Recovery	Purity		
			Protein	Fiber	Ash
Extraction method					
AM	31.42[a]	47.74[a]	0.512[a]	0.042[a]	0.648[a]
AMP	35.32[b]	53.53[b]	0.266[b]	0.035[a]	0.620[a]
Sample grinding					
WG	21.02[a]	31.04[a]	0.500[a]	0.046[a]	0.746[a]
WGF	45.74[b]	70.24[b]	0.278[b]	0.030[b]	0.520[b]

Different letters in the same column for each effect separately means significant differences ($P \leq 0.05$).

Table 4
Times used at different stages of AM and AMP to isolate starch from WG *Amaranthus cruentus*.[a]

Step	Description	Time AM (min)	Description	Time AMP (min)
1	Granulometric classification	5	Granulometric classification	5
2	Proteins extraction[b]	75 (×4)	Steepping	900
3	Neutralization	2	Neutralization	2
4	Decantation	30	Decantation	30
5	Washing-filtration	15	Washing-filtration	15
6	Wet milling	2	Wet milling	2
7	Centrifugation[c]	15(×3)	Digestion	300
8	Scraping[c]	5(×3)	Wash-filtration	15
9	Drying	2880	Wet milling	2
10	Milling	10	Centrifugation[c]	15(×3)
11	Screening	5	Scraping[c]	5(×3)
12	–	–	Drying	2880
13	–	–	Milling	10
14	–	–	Screening	5
		Total time:3309		Total time:4226

[a] Estimated times for 50 g of WG/WGF. In the case of WGF is added milling step (5 min).
[b] Repeat step 4 times.
[c] Repeat steps 3 times.

Walker (1986) procedure modified as indicated above improved extraction yield, purity, and total times.

3.2. Granule morphology and crystalline structure

Scanning electron micrographs of the starches extracted by the AM and the AMP procedures are shown in Fig. 3. They are polygonal in shape with faceted sides and with diameters varying from 0.5 to 1.5 μm. They showed no protein bodies or empty spaces that may suggest they would have been extracted neither are pits brought about by the use of protease (Tester et al., 2008) nor hollowness characteristic of tightly packed systems (Willet, 2001) seen on their surface. Both granules size and shape observed were concordant with those reported by previous research (Irving et al., 1981; Kong et al., 2009; Marcone, 2001; Willet, 2001). The presence of multigranular aggregates might be due to the way in which starches aggregate natively in the starchy perisperm.

The WGF X-ray diffraction spectrum showed a small peak at 17.9 while that of the starches extracted from it presented the distinctive A type pattern of the cereal starches with peaks of 2θ at 15; 23 and an overlapped double peak at 17 and 17.9° (Fig. 4) being the last two coincident with those reported by Kong et al. (2009) and Marcone (2001). In turn, The WGF diffractogram showed the characteristic starch profile masked by amorphous areas brought about by other non-crystalline components which become apparent as the different degrees of crystallinity shown by the samples (27.15% for WGF; 39.48% and 39.37% for the starch extracted by AM and AMP, respectively). Not observed incidence methods of insulation on the crystalline structure of starch.

The degree of crystallinity obtained in the starches from amaranthus is as higher as those reported by Iturriaga, Lopez de Mishima, and Añon (2004) for waxy rice starches, Ao and Jane (2007) for barley waxy starches, and Buléon, Colonna, Planchot, and Ball (1998) for waxy corn starches. These results are associated to the amylopectin fraction, a predominant crystalline component in the granules of waxy starches, as argued by Jane, Wong, and Mcpherson (1997) and Buleon et al. (1998).

3.3. Amylose content and thermal properties

The starch granule TA and ApA contents on dry basis were of 3.66% and 3.33% respectively. The results showed non-significant differences between them ($P \leq 0.05$) which might be assumed to the low lipid content (0.05 g/100 g d.b) of the starches. These results place the various amaranthus starches as of the waxy type which coincides with what informed Becker et al. (1981); Irving et al. (1981); and Marcone (2001) even though Kong et al. (2009) reported figures of up to 12.5 g/100 g of amylose in starches from *A. cruentus*.

Table 5 shows the WGF thermal properties and those of the starches isolated by the AM and AMP procedures in presence of water in excess (70:30 water/sample). The thermal properties (T_o, T_g and ΔH_g) of the starches extracted by the AM and the AMP procedures showed non-significant differences which would point it out the scarce or null influence of the extracting procedures upon them.

The starches isolated from WGF by both methods presented high T_g values (~74 °C), much alike to those obtained by Kong et al. (2009) for low amylose content starches from different species of

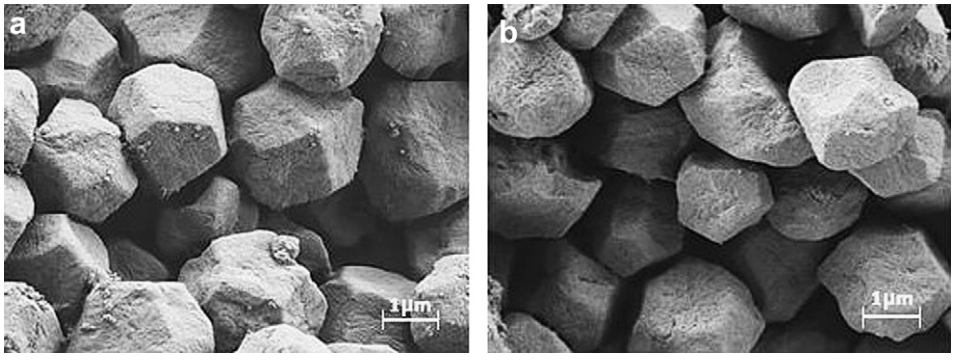

Fig. 3. SEM of starch granules isolated by AM (a) and AMP (b) of *Amaranthus cruentus* (46,000×, bar:1 μm).

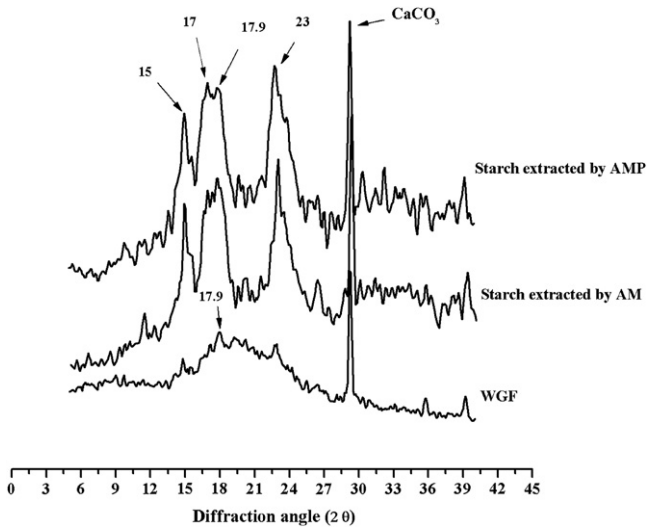

Fig. 4. X-ray diffraction patterns to blends of $CaCO_3$ and *Amaranthus cruentus* starch extracted by AM and AMP and WGF.

amaranthus which proves the higher resistance to gelatinization of these high crytallinity degree starches compared to non-waxy starches (Fujita, Yamamoto, Sugimoto, Morita, & Yamamori, 1998). The cooperativity ($\Delta T_{1/2}$) showed high values for the starch obtained by AMP respect to the one obtained using AM, meaning that the cooperativity of the process was lower in the former. Considering that this property is related to the destabilizing effect of the amorphous zones (branched regions) that absorb water prior that the crystalline zones (rich in amylopectin), the presence of the alkali used in the AM could affect the crystalline domains, becoming the starch more amorphous and hence helping to the melting of the crystallites occurs in a narrower range of temperatures, what means making the process more cooperative. However the significant differences showed in the cooperativity in the two extraction methods, didn't modify temperatures and enthalpies (Biliaderis, Page, Maurice, & Juliano, 1986; Iturriaga et al., 2004).

The WFG T_o and T_g values were higher as to those of the starches. This would be explained by the competitiveness for water between the high fiber and protein contents of the flour (13.9 g/100 g and 19 g/100 g in WGF, respectively). These components absorb water, diminish its availability for the starch to gelatinize, and increase transitional temperatures (Eliasson, 1992; Huang, Chang, Chang, & Lii, 1994; Iturriaga et al., 2004). The same as what was observed for temperatures, the WGF enthalpy of gelatinization (ΔH_g) was significantly lower ($P \leq 0{,}05$) than that of the starches extracted by both methods and show a higher structural order that make them require higher energies in order to destabilize their amorphous and crystalline areas (Lund, 1983).

Table 5
Gelatinization temperatures and enthalpies of *Amaranthus cruentus* starches and WGF.

Properties	Starches		WGF
	AM	AMP	
T_o [°C]	69.22 ± 0.28[a]	70.40 ± 0.43[a]	71.83 ± 0.49[b]
T_g [°C]	73.97 ± 0.35[a]	74.83 ± 0.49[a]	77.62 ± 0.22[b]
ΔH_g [J/g dry sample]	4.18 ± 0.21[a]	4.38 ± 0.15[a]	2.86 ± 0.15[b]
Cooperativity [°C]	7.44 ± 0.12[a]	8.98 ± 0.05[b]	11.54 ± 0.85[b]

Means values ± standard deviation ($n = 2$).
Values with different superscript within the same row are significantly different ($P \leq 0.05$).

4. Conclusions

Compared to WG, the milling of the amaranths grains in both methods prior to soaking them in a dilute NaOH solution increased starch yield and recovery significantly (namely 11.76% and 126.3%) as well as reduced soaking times as to those reported by other authors. The protein content of the starches isolated by the AMP was 48 g/100 g lower than those of the extracted by AM. However no significant effect upon the ash and fiber contents was observed as a consequence of the extracting methods employed. The results suggest that either of the two methods is appropriate to extract starch out of previously ground grains. However, the AM presents important operational advantages on the basis of the shorter total times required, lower risk of starch fermentation/degradation and of toxicity along the various stages of the process and in the final products due to the use of antimicrobial agents and lower costs of reagents.

The starches isolated by both methods showed a conservative microstructure without damaged starch and/or pits on their surfaces; A-type diffractograms similar to those of waxy starches and those of high crystallinity degree (~39%), associated to their waxy features; high T_g values (~74 °C), and a low cooperativeness. The properties analyzed showed non-significant differences because of the extracting procedure applied.

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