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Physicochemical, thermal and rheological properties of isolated Argentina quinoa starch.

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ABSTRACT

The aim of the present study was to evaluate the physicochemical, thermal and pasting properties of the starch of six quinoa genotypes native to the northwest of Argentina. The genotypes belonging to two genotype groups, highlands and dry valley, were grown in Jujuy, Argentina. Significant differences among genotypes were observed ($P < 0.05$) in amylose content, swelling power, water-binding capacity, thermal and pasting properties. In the different genotypes, the starch was characterized by a typical A-type X-ray diffraction pattern, with relative crystallinity ranging between 26.1 and 28.5%. Granule-bound starch synthase (GBSS), which is the single enzyme responsible for amylose biosynthesis, was also identified, with the 67- and 58-kDa quinoa polypeptides corresponding to the full-length and mature GBSS proteins. Studies of the pasting properties showed that the starch of the genotypes from the highlands had lower peak viscosity and lower breakdown parameter than that of the genotypes from the dry valleys. The results showed that the genotypic background and the environment influence the pasting curves. The novel findings discussed in this study constitute a starting point for research focusing on incorporating innovative technologies in the food and biomaterials industry.

Keywords: starch; quinoa; amylose; pseudocereals; genotype; DSC

List of Acronyms

AC = Amylose content

AAM = Apparent amylose

BD = Breakdown

BR = Setback ratio

Con A = Concavalin A

CPV = Cool paste viscosity

DR = Degree of retrogradation

GBSS = Granule-bound starch synthase

HPV = Hot paste viscosity

PCA = Principal component analysis

PKT = Peak temperature

PT = Pasting temperature

PV = Peak viscosity

S = Solubility

SB = Setback

48 ~~SP = Swelling power~~

49 ~~SR = Stability ratio~~

50 ~~WBC = Water-binding capacity~~

51

52 ## 1. INTRODUCTION

53

54 Starch, which is a renewable biopolymer, is the most common carbohydrate in the human diet. Starch is constituted by
55 two different glucose polymers: amylose and amylopectin. Amylose is a mainly linear polymer consisting of long chains
56 of α1,4-linked glucose units, whereas amylopectin is a branched polymer in which linear chains of α1,4-linked glucoses
57 are joined together by α1,6 linkages (Smith, 2001). Starch synthesis is achieved through the coordinated interactions of
58 several biosynthetic enzymes, including: ADP-Glc pyrophosphorylases, starch synthases, starch branching enzymes
59 and starch debranching enzymes. Starch synthases can be divided into soluble starch synthases and granule-bound
60 starch synthases (GBSS) (Bahaji et al., 2014). In wheat, genetic analyses have indicated that amylose synthesis is
61 strictly dependent upon GBSS and does not specifically require any of the four starch synthases (Zi et al., 2018). G. Li &
62 Zhu, (2018a) reported that amylose content and amylopectin fine structure greatly influence the physicochemical
63 properties of starch, thus affecting grain and flour quality. Among the genera of the family Poaceae, the morphological
64 characteristics and composition of storage starch are known to vary considerably. In rice, barley, sorghum, and wheat,
65 this variation among genotypes has been shown to lead to considerable differences in the nutritional and industrial
66 properties of starch (Kong, Zhu, Sui, & Bao, 2015; Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003; Wani et al.,
67 2012). Quinoa (*Chenopodium quinoa* Willd.), which is a grain crop from the Andes region of South America belonging to
68 the family Amaranthaceae, has been cultivated for the last 7000 years and is well adapted to extreme environmental
69 conditions such as high altitude, low annual precipitation, high soil salinity and freezing temperatures. In the quinoa
70 grain, the main storage compound is starch, which accounts for 65–70% of its final dry weight and is synthesized in
71 perisperm cells during seed development, for 14 days after fertilization (López-Fernández & Maldonado, 2013). It is
72 important to highlight that the perisperm resembles the cereal starchy endosperm both morphologically and functionally
73 (Burrieza, López-Fernández, & Maldonado, 2014).

74 In the northwest of Argentina, Andean farmers sow quinoa in a wide range of altitudes, temperatures and rainfall. Native
75 quinoa crops are found from the western arid highlands area, passing through the dry valleys of the Quebrada de
76 Humahuaca and the Valles Calchaquıes to the eastern Cordillera. Highland and valley quinoa genotypes show

differences in the grain number and weight and in their sensitivity to temperature and photoperiod (Curti, de la Vega, Andrade, Bramardi, & Bertero, 2014).

Studies on new natural starches are crucial to find their best use and to increase the utilization of starchy flours (Jan, Panesar, Rana, & Singh, 2017). Thus, screening the quinoa genotypes from northwestern Argentina will benefit the identification of quinoa grains with different functional and compositional properties. So far, several studies have examined the physicochemical, thermal (G. Li, Wang, & Zhu, 2016; Lindeboom, Chang, Falk, & Tyler, 2005; Steffolani, León, & Pérez, 2013) and rheological properties of starch in different genotypes of quinoa (Jan et al., 2017; G. Li & Zhu, 2018b), but, to our knowledge, none has reported evidence about the influence of the genotype origin on its physicochemical, thermal and pasting properties.

Understanding the impact of the agro-climatic conditions and genotype origin on the properties of quinoa starch is strategic for food security and can facilitate the development of value-added utilizations. Thus, the aim of this work was to isolate and characterize starch from six quinoa genotypes from two different ecological areas of northwestern Argentina: the highlands and the dry valley. The relationship between the genotype origin and the variability in functional properties, including swelling power (SP), solubility (S), water-binding capacity (WBC), pasting characteristics, thermal transition temperatures, and amylose content were here evaluated. Due to the importance of the amylose pathway in altering the physicochemical properties in the starch biosynthesis, the GBSS accumulation pattern was also evaluated.

2. MATERIALS AND METHODS

2.1. Genotypes

Quinoa (*Chenopodium quinoa* Willd.) genotypes from the northwest region of Argentina were studied. Genotypes, provided by the National Institute of Agricultural Technology (INTA- Abra Pampa, Argentina), are the result of the 5-year mass selection of the accessions CHEN 182,CHEN 252,CHEN 435,CHEN 420, CHEN 426 and INTA-Hornillos, sheltered in the Germplasm Bank. The genotypes were from different ecological areas: dry valleys and highlands, of the northwestern Argentina as described in (Curti et al., 2014)(Table 1). Quinoa grains were collected at Estación Experimental Agropecuaria Abra Pampa Hornillos –Instituto Nacional de Tecnología Agropecuaria-INTA, *(22,83° S, 65,85° O; 3484 m s.n.m,)* in April 2016. The highland genotypes were harvested at 90-110 days after sowing whereas dry valley genotypes were harvested 130-140 days after sowing (Curti et al., 2014).Experiments reported here were repeated at least three times.

2.2. Starch isolation

Quinoa grains (100 g) were passed through a laboratory rice mill Suzuki (MT-95,Suzuki Co,São Paulo, Brazil) to remove the pericarp and the embryo. Starch was isolated according to Jan *et al.*, (2017) with slight modifications by steeping milled quinoa grains (mostly perisperm) in 0.25 % aqueous NaOH solution (pH 12.6) and kept at 4 °C for 24h. Next, the samples were grinded in a Waring® blender for 1 min, the slurry was screened over 60 and 200 Tyler mesh, and centrifuged for 10 min at 3,500 ×g. Finally, the pellet was dispersed in distilled water and centrifuged, up to neutrality. The starch was freeze-dried (Heto Holten A/S, cooling trap model CT 110 freeze-dryer, Heto Lab Equipment, Denmark) and stored in hermetic sealed vials at 25 ºC until used.

2.3. Isolation of granule- bound starch proteins

Quinoa granule- bound starch proteins were isolated according to Lindeboom *et al.*, (2005) with slight modifications. Briefly, dried starch (20 mg) was dispersed in 250 µl of extraction buffer [55 mM Tris-HCl, pH 6.8; 2.3 % (w/v) SDS; 5 % (v/v) β-mercaptoethanol; 10 % (v/v) glycerol; 0.0005 % (w/v) bromophenol blue], and boiled (100 ºC) for 5 min. The suspension was centrifuged at 15,000 × *g* for 10 min at 4 ºC. The supernatant containing the granule- bound starch proteins was decanted from the gelatinized starch pellet and 7 µl of the resulting supernatant were electrophoresed using a Mini-Protean II (Bio-Rad Laboratories, Hercules, CA, USA). The extracts fractionated on SDS–PAGE gels were stained by Coomasie Brilliant Blue staining (0.1 % Coomassie R250, 10 % acetic acid, 40 % methanol) for 1 h at room temperature, and distained by several rinses with hot distilled water until proper contrast was achieved. The gels were photographed using G:Box GeneSnap software from Syngene.

2.4. Western blot and signal quantification analysis

The isolated protein extracts (7 µl), obtained as described above in 2.3 were electrophoresed using a Mini-Protean II (Bio-Rad Laboratories, Hercules, CA, USA). The protein extracts were separately analyzed on a 10% SDS -PAGE and electrotransferred at 4ºC onto a nitrocellulose membrane (Hybond Amersham Pharmacia Biotech, Freiburg, Germany) at 100 V for 1 h using a MiniTrans-Blot electrophoretic transfer cell (Bio-Rad Laboratories, Hercules, CA, USA). Additionally, prior to immunoblotting, membranes were stained with 0.1 % (w/v) Ponceau S and 5 % (v/v) acetic acid to ensure equal loading of protein. The membranes were immersed in 3 % BSA blocking solution in TTBS (50 mM Tris–HCl, 150 mM NaCl, pH 8, 0.05 % Tween-20) overnight at 4 ºC with gentle shaking. The blots were subjected to three 15-

min rinses in TTBS solution and were incubated with primary antibody raised against rice GBSSI (Fujita et al., 2006) diluted 1:1000 in blocking solution for 2 h on an orbital shaker at room temperature. Blots were then incubated with secondary antibody, Donkey Anti-Rabbit IgG (Thermo Fisher Scientific Pierce, MA, USA.) conjugated to alkaline phosphatase diluted 1:5000 in blocking buffer for 1 hour at room temperature and subjected to five 15-min rinses in TTBS solution. Immunoreactive bands were detected with the standard NBT/BCIP (Promega, Madison, WI, USA). The intensity of each band on the western blot was determined using ImageJ 1.46 software (NIH, http://imagej.nih.gov/ij/).

2.5. Physicochemical properties of quinoa starch

2.5.1. Amylose determination

Amylose content was determined using two methods: a extensively used colorimetry (Apparent amylase-AAP) and a new more accurate enzyme method (Amylose content -AC). Amylose content (AC) of quinoa samples was determined based on a concavalin A (Con A) precipitation procedure and apparent amylose (AAM) content was determined based on the iodine binding method (Li and Zhu, 2018b). For AC determination the K-Amyl kit (Megazyme International, Ltd., Wicklow, Ireland) was performed according to the manufacturer's instructions. Briefly, starch was dispersed in dimethyl sulphoxide, and the Con A addition precipitated amylopectin. Finally, amylose was hydrolyzed by amyloglucosidase and α-amylase. The absorbance was measured at λ = 510 nm.

For AAM content determination different amylose and amylopectin standards (Sigma, Merck KGaA, Darmstadt, Germany) were used for calibration. The AAM was determinate following Li *et al.*, (2016) procedure. The absorbance was measure at 600 nm using a JASCO V-630 UV–Visible spectrophotometer (JASCO International Co. Ltd. Tokyo).

2.5.2. Swelling power

Swelling power (SP) was analyzed as described by Li *et al.*, (2016) with some modifications. Briefly, starch sample (W0, 0.25 g, db) was weighed into a 15 mL centrifuge tube and resuspended in 8 mL deionized water. The tubes were heated over a temperature intervals range of 65–95 °C for 15 min with frequent shaking. Next, the samples were cooled and centrifuged at 2000 x *g* 20 min. The remained sediment in the centrifuge tube (Ws) were then weighted.

The SP of the starch samples was calculated using Eq.

$$SP\ \left(\frac{g}{g}\right) = \frac{Ws}{W0}$$

2.5.3. Water-binding capacity (WBC)

A suspension of 2.5 g starch (Md) in 37 ml distilled water was agitated for 1 h and centrifuged (3000 x g) for 10 min. The free water was removed from the wet starch. After draining for 10 min, the wet starch was weighed (Mw).

The WBC of the sample was calculated as follows:

$$WBC\ (\%) = \frac{(Mw - Md)}{Md} \times 100$$

2.6. Thermal properties

Thermal analysis was performed using a Differential Scanning Calorimeter (Mettler-Toledo, model 822, Mettler Toledo AG, Greifensee, Switzerland) with a STARe Thermal Analysis System version 8 software (Mettler Toledo AG, Ohio, USA).

The instrument calibration was performed using standard compounds (zinc and indium) of defined temperature and heat of fusion. Starch samples (10 mg) were accurately weighed into aluminum DSC pans. Deionized water was added by micropipette (20mg). The sample pans were sealed and equilibrated at 4 ºC for 24 h before analysis. An empty sealed pan was used as the reference. All the experiments were performed under nitrogen atmosphere. The samples were heated at 10 °C min^{-1} from 20 to 90 °C. The onset (To), peak (Tp), and the endset (Te) temperature were recorded.

Starch retrogradation was determined on the same gelatinized samples after storage at 4 °C for 7 days. The retrograded starch samples were re-scanned using the heating profile described for starch gelatinization. Gelatinization specific enthalpy of native starch (ΔH_G, J/g) and enthalpy change on reheating of retrograded starch gels (ΔH_R) were calculated by measuring the curve area of the corresponding thermograms. All measurements were performed in triplicate. Degree of retrogradation (%DR) i.e the percentage ratio between the enthalpy change of retrogradation and gelatinization was calculated according to the formula:

$$\%DR = \Delta HR/\Delta HG \times 100$$

2.7. Powdered X-ray diffractometry

Powder X-ray analysis of the starch samples was performed on a Phillips PW 1730/10 using Cu-Ka (1.54 Å) radiation. The accelerating voltage and current were set to 40 kV and 40 mA, respectively, in combination with a scan rate of 1/sec and a step interval of 0.02. The scanning regions of the diffraction angle 2θ were from 4° to 75°, covering all the significant diffraction peaks of starch samples. After recording the diffractograms, the degree of crystallinity was

186 calculated according to the method described by Nara and Komiya, (1983). First a smoothed curve was computed
187 connecting the baseline of the peaks in the diffractogram. Then, the area above the smoothed curve and the total area
188 were computed. The degree of crystallinity was calculated as a percentage as follows:

$$Degree\ of\ crystallinity = \frac{A_c}{A_T} \times 100$$

189
190 Where A_C is the area of the crystalline fraction and A_T is the total area (Roa, Santagapita, Buera, & Tolaba, 2014). The
191 smoothing baseline and the two areas under the curves were calculated using GNU Octave for windows ver. 4.4.1.

192

193 **2.8. Scanning electron microscopy**

194 Quinoa starch morphology was examined using a scanning electron microscope SEM Carl Zeiss NTS SUPRA 40
195 (Germany). The samples were sputtered with gold, and then observed and photographed. The micrographs were
196 recorded at 50,000× magnification.

197

198 **2.9. Attenuated Total Reflectance Fourier Transform Infrared Spectra (ATR/FTIR)**

199 Starch extracted from six different quinoa grains were scanned in a Fourier Transform Infrared spectrometer (FT-IR
200 Spectrum 400, Perkin Elmer Inc., Shelton, CT, USA) using an Attenuated Total Reflectance (ATR) accessory. A few
201 milligrams of each powdered sample were placed on a diamond/ZnSe crystal with one reflectance (PIKE Technologies
202 Inc, Madison, WI, USA) and pressed until desired pressure was reached. Spectra were scanned from 600 to 4000 cm^{-1}
203 with a resolution of 4 cm^{-1} until 64 scans were collected at 25 °C. Each sample was scanned in triplicate and the
204 obtained spectra were base line corrected and normalized using Spectrum Software (Perkin Elmer, Inc.). As the ATR
205 crystal showed absorbance peaks from 1800 to 2500 cm^{-1}, this region of the spectra was not considered for further
206 analysis.

207

208 **2.10. Pasting properties of quinoa starch**

209 A rheometer (Paar Physica MCR 300, Anton Paar GmbH, Austria) equipped with a 4.0 cm diameter (CP40) cone and
210 plate geometry was used. Temperature was controlled by a Peltier system (±0.1 ºC). A gap size of 0.5 mm was set, and
211 data points were recorded at a shear rate of 160 rpm. Before measurement, each starch suspension (10 % (w/w)) was
212 stirred manually to disperse the sample homogeneously. The pasting profile of the sample were monitored during

thermal treatment according to the method of Chantaro and Pongsawatmanit, (2010).Pasting temperature (PT), peak temperature (PKT), peak viscosity (PV), hot paste viscosity (HPV), cool paste viscosity (CPV), as well as, the derivative parameters breakdown (BD = PV – HPV), setback (SB = CPV – HPV), stability ratio (SR = 100×HPV/PV), and setback ratio (BR = CPV/HPV) were calculated for each genotype according Li *et al.*, (1997).

2.11. Statistical analysis

All determinations were performed at least in triplicate. The data were expressed as the mean values ± SD. Statistical tests were applied using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). When applicable, data were analyzed by one-way Analysis of variance (ANOVA), and differences between genotypes were determined following Tukey HSD post-hoc test, at $P \leq 0.05$. The relationships between different properties of starches were also determined using Pearson correlation coefficients. Statistical significance was defined as $P < 0.05$.Principal component analysis (PCA) was performed after normalization (using min-max algorithm) of the variables form pasting properties for each starch sample using Infostat/ p2011 software, Universidad de Córdoba, Córdoba, Argentina.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties

3.1.1. Amylose content

Considering differences in amylose content, cereals can be classified in three different categories, i) normal amylose (28%) corn, wheat starches, ii) high amylose (50-70%) corn starch and iii) low amylose (<2%) for waxy maize starch (Fennema´s Food Chemistry, 2008). Despite quinoa genotypes did not shown such a variability, slight statistical differences in their amylose content were found as reported by other authors (Jan et al 2017; Li et al 2016, 2018; Lindeboom et al. 2005, Steffolani et al 2013.) The AC differences ranged from 7.2 % (CHEN 426) to 10.1 % (INTA-Hornillos), and the AAM from 9.8 % (CHEN 252) to 12.3 % (CHEN 420) (Table 1). Similarly, AC values between 6.06 to 8.44 and an AAM range of 7.50 to 10.88 were reported for other quinoa genotypes (Li & Zhu, 2018a). The differences between methods may explain the AAM tendency to overestimate amylose content due to the formation of amylopectin-iodine complexes (Gibson et al., 1997). Also, the AAM relatively large standard deviation could be attributed to the quinoa grain low amylose content (Martinez, 1996)(Martinez et al., 1996). Many parameters, such as genetics, maturity, and growing conditions, may influence the grain amylose content (G. Li et al., 2016).

3.1.2. Swelling power (SP) and water-binding capacity (WBC)

The SP and WBC of quinoa starch genotypes are shown in Table 1. Significant differences in the quinoa genotypes SP were observed. As temperature raised the quinoa genotypes SP increased, disrupting the granule structure by weakening their internal associative forces in all quinoa genotypes starches. Since amylopectin/amylose ratio influences SP some authors looked for an amylose content-SP correlation having found it negative (Sasaki, Yasui, & Matsuki, 2000). Similarly, in the present work a negative correlation between the SP and the AC was observed at 65ºC, 75ºC and 85ºC (r= -0.856, -0.929, -0.904, respectively $P<0.05$). Other authors, have found that various parameters also influence starches SP: average granule size, gelatinization peak temperature (J.-Y. Li & Yeh, 2001), starch chains interaction, amylose and amylopectin molecular weight/distribution, degree and length of branching and conformation (Singh et al., 2003) (G. Li & Zhu, 2018a). Further studies on the quantity of short internal chains of the different genotypes here evaluated are necessary to elucidate the quinoa starch granule structure.

3.2. Thermal properties of Native and Retrograded starches

The DSC curves of starch gelatinization of the six genotypes studied are presented in Fig.1A. Thermal transition temperatures of native starches and retrograded starches determined by DSC are summarized in Table 2.The onset gelatinization temperature(To), the peak temperature(Tp) and the endset temperature(Te) values obtained range from 58.0 to 67.9, 62.2 to 75.1 and 74.6 to 85.8 °C, respectively. The transition temperature ranged (Te-To) from 15.2 to 22.1 °C. The highland genotype CHEN 420 and dry valley genotype INTA-Hornillos, had higher onset and endset temperatures. The gelatinization enthalpy (ΔH_G) ranged from 9.8 to 12.2 J/g. The To, Tp, Te, (Te-To) and ΔH_G differed significantly ($P < 0.05$) between genotypes. This is probably due to different quantities of longer chains in amylopectin (G. Li et al., 2016; Singh et al., 2003).The onset temperature correlated positively with the amylose content (r=0.90). No significant correlations were found between the amylose content and Tp or Te. Starch retrogradation was observed when the gelatinized samples were stored at 4 °C for 7 days (Table 2). The enthalpy of retrogradation (ΔH_R) and retrogradation percentage (% DR) for starches isolated from the different genotypes ranged from 1.9 to 3.5 J/g; and from 17.7 to 32 % up to 7 days storage, respectively. The quinoa starch retrogradation degree was positively correlated to amylose content. The ΔH_R and %DR increased as the gels become less soluble due to starch molecules recrystallization. According to Steffolani et al., (2013) quinoa starches exhibited lower %DR when compared to other pseudocereals. A thorough understanding of starch gelatinization and retrogradation is needed to control starch functional properties for food processing, human nutrition and industrial applications.

3.3. Crystalline properties of quinoa starches

The XRD diffractograms of these starches give the stronger diffraction peaks at around 15, 17, 18 and 23° (2θ), all exhibited a typical A type, indicating that they share similar crystalline patterns (Fig1). Crystallinity is affected by amylose content and proportions of different chain length in amylopectin (Cheetham & Tao, 1998). The relative crystallinity, calculated from the XRD patterns, were almost identical: 28.5, 28.4, 27.6, 27.5, 26.6 and 26.1 % for CHEN 426, 182, 252,435 420, INTA- Hornillos, respectively, these values were inversely proportional to the amylose content. Crystallinity values between 21.5 and 43 % have been previously reported for starch isolated from quinoa (G. Li & Zhu, 2018a).

3.4. ATR/FTIR spectra collected from the starch fractions of each quinoa genotypes

Fig.1 C shows the normalized ATR/FTIR spectra for CHEN 182 and CHEN 420genotypes. Spectra show a broad absorption band from 3010 to 3750 cm^{-1} associated mainly with hydroxyl groups present in carbohydrates, due to OH stretching. Moreover, a small band with at least two overlapped peaks is observed from 2800 to 3000 cm^{-1}, this band is associated mainly to C-H stretching. Besides, a small broad band from 1200 to 1500 cm^{-1} is represented by CH_2OH side chain related mode, C-O-H bending, CH_2 twisting, CH_2 bending and C-O-O stretching. Finally, spectra show a strong absorption band, from 900 to 1200 cm^{-1} associated to C-O and C-C stretching (1163 cm^{-1}), C-O-H bending (1094 cm^{-1}) and C-H bending (1067 cm^{-1}). Additionally, below 900 cm^{-1}, small bands can be observed due to skeletal modes of the pyranose ring (Warren, Gidley, & Flanagan, 2016). The spectra analyzed in Fig. 1 C shows no significant differences between the starches from the dry valley (CHEN 182) and the highland (CHEN 420) genotypes. The same pattern was observed for all the genotypes analyzed i.e. CHEN 252, CHEN 426, CHEN 435 and INTA-Hornillos (Spectra non-shown).

3.5. Scanning electron microscope of quinoa starch granule

The starch granule morphology of the six genotypes was examined using SEM. The granules showed polygonal and irregular shapes (Fig. 2 A) The granule size ranging from 0.5 to 1.8 µm is smaller than cereal starches, providing exclusive properties for pharmaceutical and cosmetic industry. Starch granule structure suitability for a food manufacturing process or its nutritional qualities depends on i) the grain genetic ii) the environmental factors that control starch biosynthesis iii) and how the material is processed. For example, small starch granules were associated with high rupture resistance and high stability against shearing (Jan et al., 2017; Steffolani et al., 2013). Moreover, small sized starch is required for specific applications in food industry such as fat replacer (Lindeboom, Chang, & Tyler, 2004), flavor

encapsulation by spray drying" and as a thickener agent in cosmetic industry (Lindeboom et al., 2004; Peng, Wang, Chen, Pan, & Tu, 2018).

3.6. Quinoa Starch granule-bound proteins

Amylose is mainly produced via the activity of granule-bond starch synthase. The accumulation of the amylose fraction of starch is controlled by a single dominant gene in quinoa, *GBSS* (Brown, Cepeda-Cornejo, Maughan, & Jellen, 2015).

To characterize the accumulation of GBSS from different quinoa genotypes, protein extracts were separated by SDS–PAGE, as mentioned in methods (Fig 2 B upper panel). The electrophoresis showed the presence of two bands of a molecular weight of approximately ~63 and ~56 kDa for quinoa genotypes and one band of ~57 kDa for rice both polypeptides reacted with rice GBSSI (Fig 2 B lower panel). The proteins were transferred into a nitrocellulose membrane and antibodies specific to rice GBSSI were used. The western blot showed that both the ~63- and the ~56 kDa quinoa polypeptides reacted with rice GBSSI (Fig 2 B lower panel). The accumulation of CHEN182 and CHEN 426 GBSS, both genotypes with lower amylose content, was ~1/4 times lower than INTA- Hornillos mature GBSS (Fig 2 B lower panel).

Lindeboom et al. (2005) identified two quinoa GBSSI proteins with molecular masses of 62- and 56 kDa and showed that quinoa lines with high amylose (19.5%) had larger bands than that of intermediate amylose content (13.5%) by inmunoblot analysis. These authors also suggested these two proteins that immunoreacted with wheat GBBS were different isoforms. According to Brown et al. (2015) the two bands identified correspond to the full-length and mature GBSS proteins. The protein is localized within the central core of the starch grains and the mature *Chenopodium* GBSS consensus protein sequence was conserved across wheat, maize and rice (Brown et al., 2015).Investigation on the genetic variability of quinoa genotypes possessing higher and lower amylose content could be useful for novel technological and nutritional value of starch. For example, the reduction in amylose content via GBSS modification in cassava starch enhanced clarity and stability properties making it useful for paper and textile industries, also as making food products such dairy and noddles (Tappiban, Smith, Triwitayakorn, & Bao, 2019).For potato lines, suppression of GBSS demonstrates a combined rheological, crystalline and degradability functionalities (Wang & Copeland, 2015 and references therein).

3.7. Pasting properties

Significant differences in the pasting characteristics were observed between the starches of the quinoa genotypes studied (Table 3, Fig. 3A). Pasting temperature (PT) is the temperature at which starch molecules hydrate and the suspension viscosity increases abruptly. The PT values obtained, which ranged from 62 (CHEN 252) to 65.1 ºC (CHEN 426), were similar to the DSC T_0 values obtained as well as to other quinoa starch values previously reported (Jan et al., 2017; G. Li et al., 2016). PT and peak temperature (PKT) are highly dependent on the swelling granule behavior and WBC (Jan et al., 2017; Kong et al., 2015; G. Li et al., 2016). Both parameters are also associated with granule rigidity and with different degree of branching and crystallinity (Jan et al., 2017; Kim & Wiesenborn, 1996; Wang, Li, Copeland, Niu, & Wang, 2015 and references therein).

Peak viscosity (PV), which is related to starch swelling power (Kong et al., 2015), showed the maximum viscosity attained by quinoa starch samples while heating. In addition to PV, Hot Viscosity (HV-at 95ºC) and Cool Viscosity (CV-at 50ºC) are key factors for quinoa starch industrial processing, transport and applications. The INTA-Hornillos genotype showed the highest viscosity parameters (PV, HV, and CV), which can be related to its high AC, WBC, and SP values at 95ºC. On the other hand, the highland genotypes CHEN 420 and CHEN 426 showed lower PV and stable viscosity values, evidenced by their low breakdown parameter (BD). Both genotypes had the same pasting curve, a tendency that may be associated with their ecological origin. The highland genotypes are plants with shorter crop cycle (90 to 110 days from sowing to harvest) and greater temperature sensitivity influencing the rate of flowering, than the dry valley genotypes (130 to 140 days from sowing to harvest). The dry valley genotypes showed similar pasting curves, except INTA-Hornillos, which is from the inter-Andean Valley of Peru and has been cultivated in northwestern Argentina for more than 30 years. Thus, the effect of the genotypic background and environment became evident on the pasting curves. The composition of different starches is susceptible to environmental variation, especially growth temperature (Tester & Karkalas, 2001). The evaluation of these parameters may be a useful tool to discriminate between ecological area and quinoa genotypes and the differences among these genotypes and their desirable properties.

It is worth noticing that multiple factors, such as particle size distribution, internal and external chain length, and granule strength, influence the rheological analysis of quinoa starch (G. Li & Zhu, 2018b). Quinoa starch is a complex system and the pasting process implies different variables such as molecular diffusion, granule rigidity and degree of damage, amylose content, solubility, and degree of crystallinity (Jan et al., 2017). The analysis of differences in pasting parameters may allow identifying a quinoa genotype that provides desirable properties for a specific technological use,

as reported by (Kim & Wiesenborn, 1996) in a particular potato genotype whose starch had high setback ratio, providing excellent attributes for noodle preparation.

3.8. Principal component analysis (PCA)

PCA was performed to further explore the relationship between the pasting properties and the ecological origin of the genotypes. Figure 3B shows the biplot (scores and loadings) using the pasting properties (nine normalized variables) as the input variables for PCA. The two first principal components (PC1 and PC2) accounted for a total accumulated variance of about 94% of the original data set and clearly discriminate the quinoa samples into three well-defined groups: one composed of quinoa grains from the highlands (CHEN 420 and 426), another one composed of three of the four genotypes from the dry valley (CHEN 182, 252 and 435), and the last one composed of the INTA-Hornillos genotype. The possible reason of the third group arrangement could be the ancestor of this last genotype, which originated from a quinoa *var. CICA* grown in the dry valleys of Peru. According to the PCA results recorded with the nine variables used in this work, similar quinoa grains showed a similar pasting profile. The arrows in the biplot represent the relative weight of each variable to each component. The most relevant variables influencing to PC1 are PV, BD and SR, and for the PC2 are PT and PKT. Lastly, CV, HC and SB show low relevance and are highly correlated.

4. CONCLUSIONS

Screening the natural variability present in germplasm banks allows for the identification of interesting and unusual genotypes properties. The characterization of the physicochemical and functional starch properties provides tools to identify genotypes with industrial and commercial potential.

The physicochemical, thermal, and pasting properties of the starch of six quinoa genotypes native to the highlands and dry valleys of northwestern Argentina were here studied. Significant differences were observed among the six quinoa genotypes studied in AC, SP, WBC, thermal and pasting properties. The pasting properties led to a good clustering of the genotypes according to their ecological origin and genotype background. This finding suggests that the higher PKT values and lower PV and BD values of the highland genotypes might be related to higher environmental temperatures during grain filling.

To the best of our knowledge, this is the first report of the characterization of quinoa starch properties related to their ecological origin and genotype background. The current research is part of our broader goal of completing the characterization of a wider set of quinoa starches from different germplasm and environments, with the final objective of

379 attaining useful information regarding desirable new industrial applications. Likewise, promoting the development of
380 cultivation in historically neglected regions by expanding the agricultural frontier.

381

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477

Table 1. Amylose content, swelling power and water binding capacity of the starches of the quinoa genotypes studied

a (Curti et al., 2014), *b* provided by the National Institute of Agricultural Technology (INTA- Abra Pampa, Argentina) (INTA Abra Pampa – Argentina. Values are means ± SD. Values in the same column followed by the same letter are not significantly different (P< 0.05, n=3)

Table 2. Thermal transition temperatures of the six quinoa starch samples.

Values are means ± SD. Values in the same column followed by the same letter are not significantly different (P < 0.05, n=3). *To, onset temperature; Tp, peak temperature; ΔH, gelatinization enthalpy; Te, conclusion temperature; Te-To,*

Table 3. Pasting properties of the starches of the quinoa genotypes studied.

Values in the same column followed by the same letter are not significantly different (P<0.05) (n=3). *PT pasting temperature; PKT peak temperature; PV peak viscosity; HV hot viscosity; CV cool viscosity; BD breakdown (PV-HV); SB setback (CV-HV); SR stability ratio (100*HV)/PV; BR setback ratio (CV/HV).*

513 **Table 1. Amylose content, swelling power and water binding capacity of the starches of the quinoa genotypes**

Genotypes	Genotypes group ecoregion	Genotype origin	AC (%)	AAM (%)	Swelling Power at				Water Binding (%)
					65 ºC	75 ºC	85 ºC	95 ºC	
CHEN 182[a]	Dry valleys	QQ 95-NSL 106394, Humahuaca (Jujuy)	7.7 ± 0.2^{bc}	10.5 ± 0.5^{a}	6.5 ± 0.3^{ab}	9.0 ± 0.2^{a}	11.3 ± 0.3 ^{a}	10.7± 0.1^{c}	90.4 ± 0.4^{a}
CHEN 252 [a]	Dry valleys	Maimará, Tilcara (Jujuy)	8.3 ± 0.2^{bc}	9.8 ± 0.6^{a}	7.0 ± 0.1^{a}	8.5 ± 0.5^{ab}	10.9 ± 0.1^{a}	11.8 ± 0.2^{b}	81.3 ± 0.1^{c}
CHEN 435 [a]	Dry valleys	Cangrejillos, Yavi (Jujuy)	8.9 ± 0.0^{ab}	11.1 ± 0.1^{a}	5.8 ± 0.1^{bc}	8.5 ± 0.1^{ac}	10.7 ± 0.1^{a}	9.0 ± 0.1^{d}	85.1 ± 0.1^{b}
CHEN 420 [a]	Highlands - Puna	Antofallita, Los Andes (Salta)	9.4 ± 0.0^{a}	12.3 ± 0.5^{a}	6.0 ± 0.2^{abc}	7.6 ± 0.2^{bc}	8.8 ± 0.2^{b}	11.1 ± 0.1^{c}	81.0 ± 0.3^{c}
CHEN 426 [a]	Highlands - Puna	Santa Rosa de los Pastos Grandes, Los Andes (Salta)	7.2 ± 0.0^{c}	10 ± 1^{a}	6.6 ± 0.1^{ab}	9.2 ± 0.2^{a}	11.0 ± 0.3^{a}	13.0 ± 0.1^{a}	90.2 ± 0.8^{a}
INTA-Hornillos [b]	Dry valleys	Hornillos (Jujuy)	10.1 ± 0.4^{a}	12 ± 2^{a}	5.3 ± 0.3^{d}	7.6 ± 0.4^{bc}	8.6 ± 0.1^{b}	12.4 ± 0.3^{b}	88.3 ± 0.3^{a}
Mean			8.6	11.01	6.2	8.2	10.2	11.3	86.06

514 **studied**

515

516

517 **Table 2. Thermal transition temperatures of the six quinoa starch samples.**

518

	Gelatinization					Retrogradation	519
Genotypes	**ΔH_G (J/g)**	**T_o (ºC)**	**T_p (ºC)**	**T_e (ºC)**	**T_e-T_o (ºC)**	**ΔH_R (J/g)**	**DR (%)** 520
CHEN 182	11.7 ± 0.1^{ab}	61.2 ± 0.2^{b}	74.3 ± 0.3^{bc}	83.3 ± 1.3^{a}	22.1^{a}	2.4 ± 0.2^{ab}	20.6^{ab}
CHEN 252	12.2 ± 0.8^{a}	65.7 ± 0.4^{a}	72.0 ± 0.0^{b}	82.7 ± 0.7^{a}	17.0^{ab}	3.1 ± 0.3^{ab}	25.3^{ab}
CHEN 435	9.8 ± 0.5^{b}	65.7 ± 0.3^{a}	72.5 ± 0.1^{bc}	80.8 ± 1.6^{ab}	15.2^{b}	2.4 ± 0.2^{ab}	24.3^{ab}
CHEN 420	10.1 ± 0.5^{ab}	67.9 ± 0.3^{a}	74.0 ± 1.1^{bc}	85.8 ± 1.7^{a}	17.9^{ab}	3.2 ± 0.3^{ab}	31.6^{a}
CHEN 426	10.4 ± 0.2^{ab}	58.0 ± 0.2^{c}	64.2 ± 0.1^{d}	74.6 ± 0.5^{b}	16.6^{ab}	1.9 ± 0.2^{b}	17.7^{b}
INTA-Hornillos	10.9 ± 0.3^{ab}	67.3 ± 1.5b^{a}	75.1 ± 0.0^{a}	83.6 ± 1.2^{a}	16.3^{ab}	3.5 ± 0.4^{a}	32^{a}

521 **Table 3. Pasting properties of the starches of the quinoa genotypes studied.**

Genotypes	PT (ºC)	PKT (ºC)	PV (Pa.s)	HV (Pa.s)	CV (Pa.s)	BD (Pa.s)	SB (Pa.s)	SR	BR
CHEN 182	62.8± 0.1^{b}	66.8 ± 0.2^{b}	60 ± 7^{b}	16 ± 2^{bc}	28 ± 9^{b}	44 ± 9^{b}	12 ± 7^{bc}	26 ± 6^{d}	1.7 ± 0.3^{a}
CHEN 252	62.0± 0.1^{c}	68 ± 1^{b}	41 ± 6^{c}	18 ± 2^{b}	28 ± 7^{b}	23 ± 4^{c}	11 ± 5^{bc}	43 ± 2^{c}	1.6 ± 0.2^{a}
CHEN 435	62.5± 0.2^{b}	66 ± 1^{bc}	44 ± 7^{c}	17 ± 4^{b}	28 ± 2^{b}	27 ± 3^{c}	11 ± 2^{b}	30 ± 7^{d}	1.7 ± 0.3^{a}
CHEN 420	63± 1^{b}	88 ± 10^{a}	12 ± 3^{d}	7 ± 3^{d}	12 ± 4^{c}	5 ± 2^{d}	5 ± 1^{c}	65 ± 9^{b}	1.7 ± 0.2^{a}
CHEN 426	65.1± 0.3^{a}	92 ± 2^{a}	14 ± 1^{d}	14 ± 1^{c}	27 ± 2^{b}	0 ± 2^{d}	12 ± 3^{b}	102 ± 11^{a}	1.8 ± 0.2^{a}
INTA-Hornillos	62.1± 0.1^{c}	65.7 ± 0.2^{c}	127 ± 1^{a}	52 ± 1^{a}	89 ± 2^{a}	75 ± 1^{a}	36 ± 1^{a}	41 ± 1^{c}	1.7 ± 0.1^{a}

522

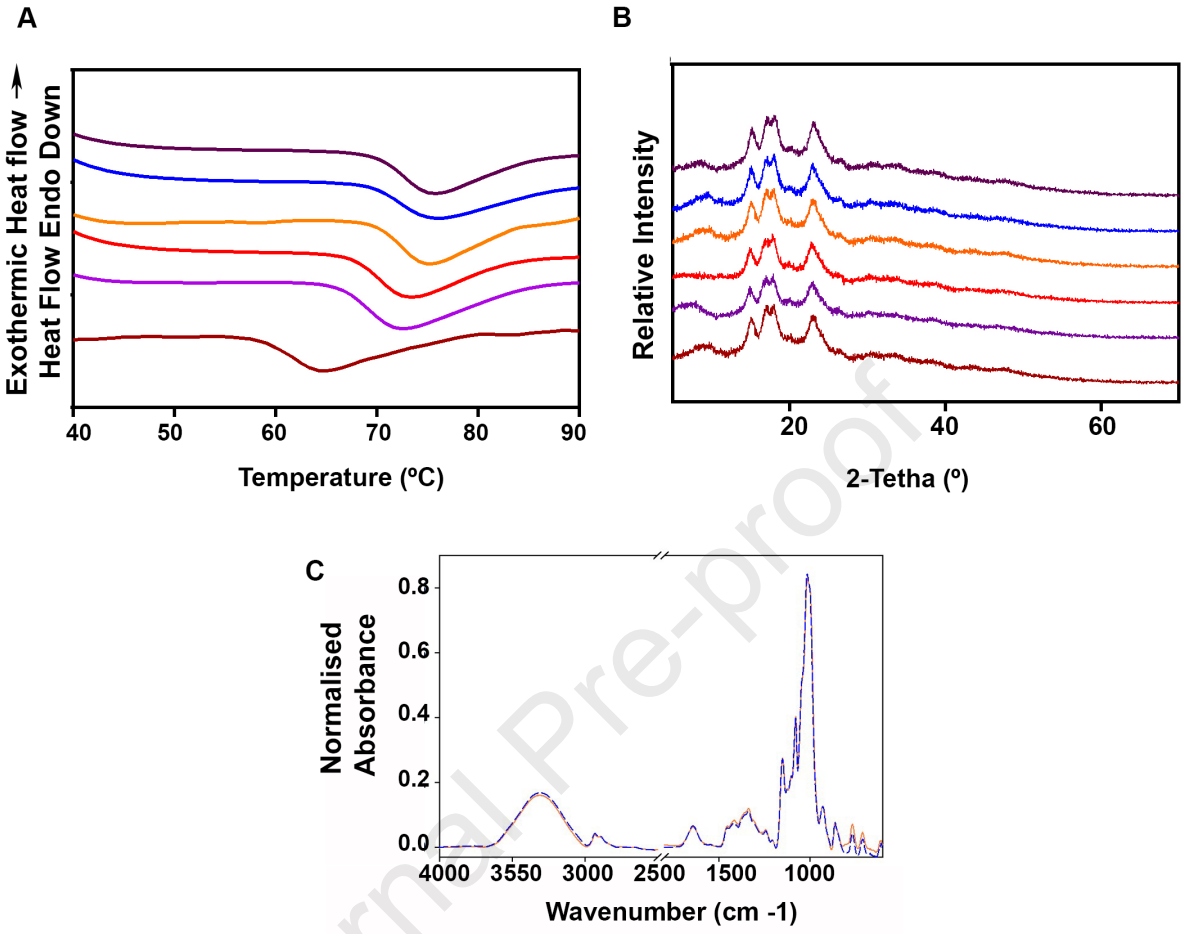
523 **Figure 1. DSC thermograms (A) XRD patterns (B) and FTIR spectra (C) of the quinoa starches studied.** In A and
524 B, INTA -Hornillos **(**purple), CHEN 420 (blue), CHEN 182 (orange), CHEN 435 (red), CHEN 252 (light purple), CHEN
525 426 (brown). In C, CHEN 420 blue dotted line, CHEN 182 continuous line.

526 **Figure 2**. **A) Scanning electron microscopy (SEM) micrograph at 50.000× magnification, of the isolated starch**
527 **granules.** (Scale bar = 600 nm) **B) Quinoa GBSS from the isolated starch was subjected to SDS- PAGE (upper**
528 **panel) and western blotting, probed with antibody specific for GBSS rice (lower panel).**

529 **Figure 3. A) Pasting properties of the starches of the quinoa genotypes studied.** CHEN 182 (orange solid line),
530 CHEN 252 (purple dotted line), CHEN 435 (red solid line), CHEN 420 (blue dash line), CHEN 426 (brown dotted line)
531 and INTA- Hornillos (purple solid line). Viscosity and temperature in function of time of analysis are showed. **B) Biplot**
532 **from the principal component analysis for the six quinoa grain genotypes** using the pasting properties variables as
533 input. The percentage of variance associate to each principal component is between parentheses**.** CHEN 426 (red),
534 CHEN 252 (purple), CHEN 426 (black), CHEN 435 (green), CHEN 182 (blue), INTA-Hornillos (grey)

535

536



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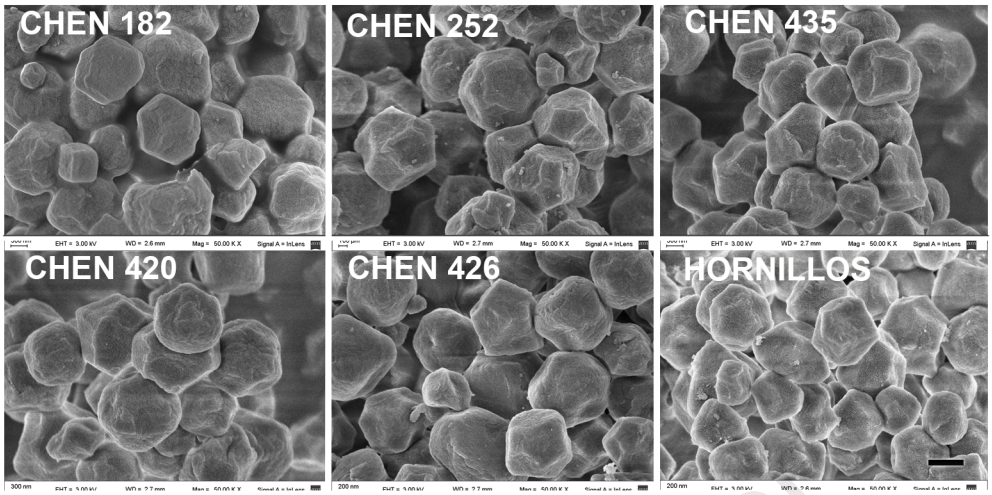

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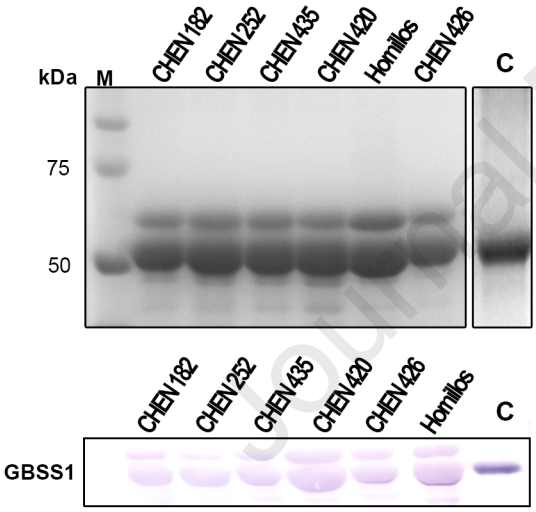

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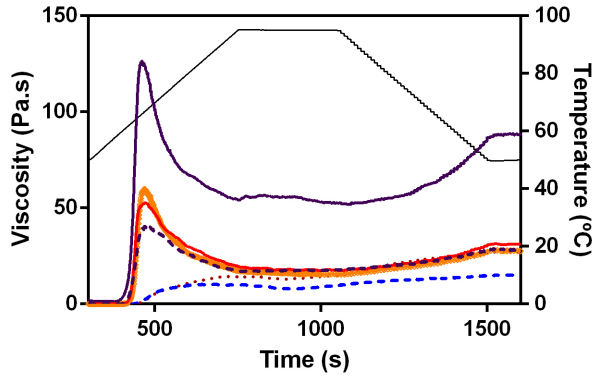

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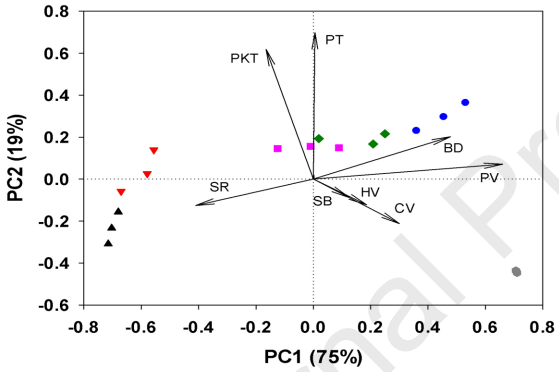

Highlights

- The genetic diversity in the physicochemical and pasting properties was evaluated.
- Quinoa NWA genotypes are valuable for food and industrial applications.
- Highland genotypes showed stable viscosities and low breakdown values
- PCA lead to a good clustering of genotypes depending on their ecogeographic origin.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.