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Research article

Functional properties of starch cultivars of two Andean grains grown in Bolivia: Amaranth (*Amaranthus caudatus*) and canihua (*Chenopodium pallidicaule*)

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A R T I C L E I N F O

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A B S T R A C T

The functional properties of Andean grain starches of two species, amaranth (*Amaranthus caudatus*) and canihua (*Chenopodium pallidicaule*), three cultivars each, were studied. The study focused on chemical composition, pasting properties, thermal properties, water solubility index (WSI), swelling power (SP), and granule morphology. All amaranth starches were waxy starches, with amylose content less than 5 %, which had some differences in chemical composition ($p < 0.05$). The pasting properties differed between the species, canihua showed more resistance, than amaranth, to heat and shear stress (higher cool paste (CPV) and lower breakdown (BD), ranged between 1250 and 1600 cP and -30 – 10 cP respectively. The amaranth starches presented only similar CPV with 800–1000 cP, while canihua cultivars presented similar PT and BD, and both species presented similar PV, around 1000 cP. Thermal properties (T_o, T_p, T_c, ΔH, and ΔT) differed among cultivars and species. These differences could be related to the homogeneity molecular structure and content of amylose in canihua cultivars and possibly to genotype factor. Polygonal shapes were the predominant shape of starch granules, ranged 1.0–1.4 μm and 0.8–0.9 μm, for amaranth and canihua starches respectively. Amaranth starches swelled quickly to disintegrate partially at the end, contrary to canihua starches. The thermal and pasting properties were correlated between them. SB, CPV, HPV, CS, were correlated to the content of amylose in canihua starches. One amaranth cultivar was significantly different from the others. Thus, according the functional properties differenced both species and some cultivars in each species. Additionally, the amaranth starch has the potential to be used in the food industry where heat and stress are applied such as extrusion, while canihua starches can be used in desserts or in cosmetic uses, based on their functional properties.

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1. Introduction

Starch is the main component of cereals and often contributes with specific properties to both foods and non-foods, to which it can be added as a functional ingredient [1]. These multiple applications of starch are the result of its transformation by cooking (heat treatment) in the presence of water from a semi-crystalline granule to an amorphous state. The loss of native crystalline order occurs through the formation of a paste after the absorption of water and the development of viscosity (gelatinization), the formation of a gel after the formation of networks (gelation), and recrystallization to an insoluble and aggregated state (retrogradation) [2]. The functional properties describe these conditions and processes [1,3].

Starch can be derived from various different plant sources, ranging from the most well-known ones such as corn, cassava, or wheat [1,4,5] to new sources such as pseudocereals, i.e., amaranth, canihua, and quinoa [6,7]. Environmental and genetic factors can influence starch properties and functionalities. For example, the color, pH, ash, and protein content of cassava starch have been influenced by genetic and environmental factors [8]. In addition, some *Amaranthus* species were examined to evaluate differences in amylose content, thermal, and pasting properties [7,9]. They found diversity in starch properties within and among *Amaranthus* species although the starch granule morphology and size distribution were fairly constant. There is not a generic or functionally typical in *Amaranthus* starch, this represent a valuable resource for applications of this starch. In this study, the species *A. caudatus* was not represented. Konishi et al. [10] characterized starch granules from *Amaranthus*. They concluded that the starch of *A. caudatus* is waxy due to the amylose-deficiency in its starch (5–7 %) similar to that of waxy maize (2–5 %). Other studies focused on amaranth (*A. caudatus*) and canihua (*Chenopodium pallidicaule*) provided differences in amylose content, pasting and thermal properties, indicating good stability during cooking. Additionally, the amaranth starch was mainly amylopectin [6,11]. Although the nutritional potential of these grains is being investigated [12–14]. There is still lack of basic knowledge about the behavior of starch in Andean cultivars. This is an important information for industrialization as starch is a main component of the grains [15,16].

This study aims to describe and compare two Andean grains, amaranth (*Amaranthus caudatus*) and canihua (*Chenopodium pallidicaule*), at two levels: between species and amongst cultivars of each species, according to their pasting properties, thermal properties, solubility, and morphological characteristics of the starch.

2. Materials and methods

2.1. Materials

Six Andean cultivars, three of amaranth, *Amaranthus caudatus* (Oscar Blanco, Pucara, and Cotahuasi) and three of canihua, *Chenopodium pallidicaul*e (Illimani, Cañawiri, and Saigua L24) were studied. The samples were named as in Table 1 to better explain them. All samples and information were obtained from Bolivia through the "Fundación Promoción e Investigación de Productos Andinos", PROINPA (Foundation for the Promotion and Research of Andean Products), harvested in 2019. These amaranth and canihua grains have previously been studied with a focus on proximal, mineral and fatty acid contents [12,13]. In this study, the starch content in grains was 60.0, 61.0 and 63.0 % for amaranth species, and 51.0, 52.0 and 44.4 % for canihua samples, respectively. The appearance of these crops is shown in Fig. 1.

The amaranth samples were chosen from different location and altitudes, showed in Table 1 and Fig. 1a–c [12], while canihua samples were chosen from two different two growth habits (Fig. 1d–f), explained in a previous study [13].

2.2. Starch isolation procedure

Before the starch isolation the grains were pearled by friction, washing and drying at 30 °C for 12 h. This temperature was used to avoid damage to the granules, although other authors used 40 °C to dry *Amaranthus* [7]. Pearling was developed because previous trials (data not shown) resulted in low yield and impure starch when whole grains were used. The isolation of starch from amaranth and canihua was performed by combining two extraction methods to improve the purity of the obtained starch [11,17]. For starch isolation, the grains (1:10) were suspended at pH 10 (NaOH, 2 M) overnight at 4 °C. Then the liquid was divided into five parts and the grains were liquefied with one part of the liquid (Skymsen, Stainless Liquidifier TA 02, Brazil). It was then filtered through a homemade 20-mesh sieve, and repeated four times with the remaining parts of the liquid, there should be no uncrushed grains left. The extraction was carried out at a pH of 10, at 210 rpm, and, 30 °C for 2 h. The suspension was filtered through a gauze cloth, and the liquid fraction was filtered in stages using 125 μm, 75 μm, and 38 μm sieves (Retsch GmbH & Co KG, Haan, Germany). The filtrate was

Table 1
Information of cultivars, code, name, species, origin and altitude of growing place.

Name	Cultivar	Species	Origin (Province, Department, Country)	Altitude (m.a.s.l.)
AC1	Oscar Blanco	*Amaranthus caudatus*	Zudañes, Chuquisaca, Bolivia	2339
AC2	Pucara	*Amaranthus caudatus*	Tomina, Chuquisaca, Bolivia	2102
AC3	Cotahuasi	*Amaranthus caudatus*	Tomina, Chuquisaca, Bolivia	2300
CP1	Illimani	*Chenopodium pallidicaule*	Ingavi, La Paz, Bolivia	3857
CP2	Cañawiri	*Chenopodium pallidicaule*	Ingavi, La Paz, Bolivia	3857
CP3	Saigua L 24	*Chenopodium pallidicaule*	Ingavi, La Paz, Bolivia	3857

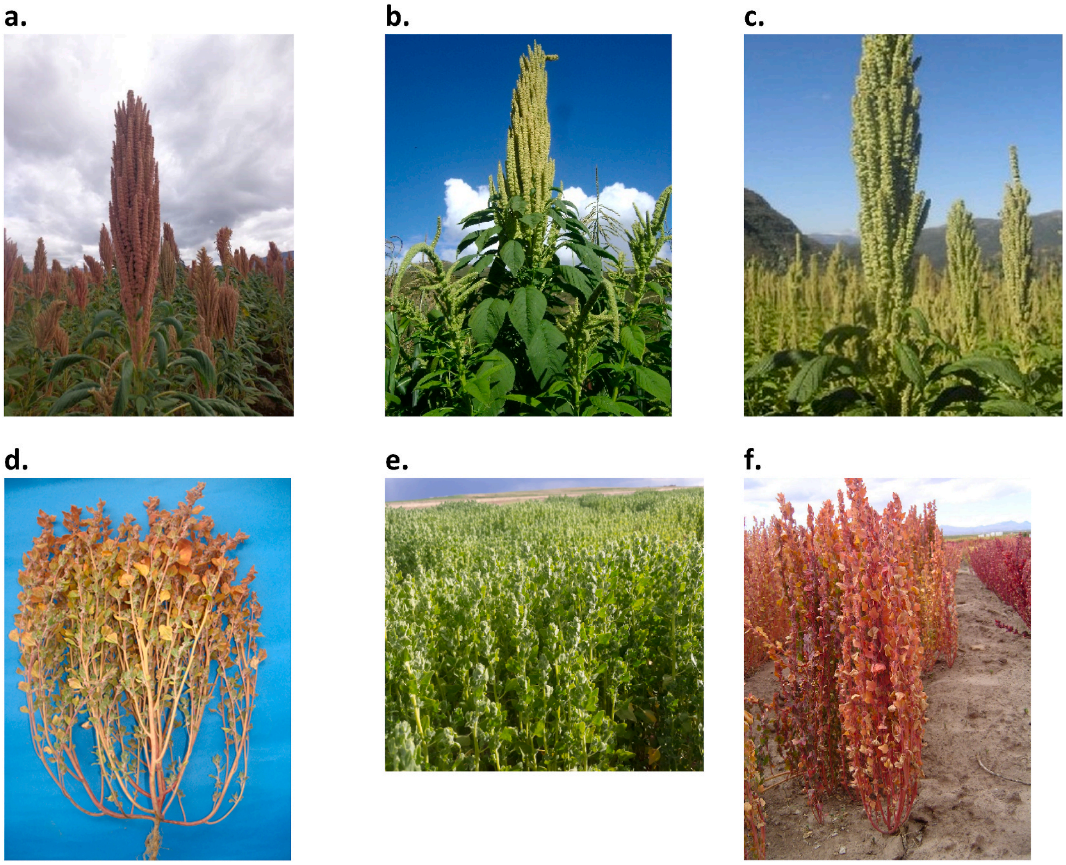

Fig. 1. Crops. Amaranth (*Amaranthus caudatus*): **a.** AC1 (Oscar Blanco), **b.** AC2 (Pucara), and **c.** AC3 (Cotahuasi). Canihua (*Chenopodium pallidicaule*), cultivars and growth habits are: **d.** CP1 (Illimani, lasta), **e.** CP2 (Cañawiri, lasta), and **f.** CP3 (Saigua L24, saigua).

centrifuged at 3600 rpm (Hettich, Rotina 38 Darmstadt, Germany) for 15 min, discarding the supernatant. The solid phase presented a yellow-brown-gray top layer, which was scraped off and discarded. The starch was re-dispersed in distilled water and centrifuged as described above until obtaining a layer of white starch on the bottom. This was done once for amaranth and four times for canihua. The starch obtained was dried in oven overnight at 36 °C.

2.3. Proximate analysis and chemical composition

The proximate composition of the starch isolated were analyzed according to the official AOAC methods [2]. For moisture content (925.10), the extracted samples were dried at 105 °C to constant weight. Ash content (923.03) was determined by ignition at 550 °C until light gray ash was obtained. Crude fiber (962.09) was determined by acid-alkali hydrolysis. Protein content (984.13) was determined by the micro-Kjeldahl method using a factor of 6.25 to calculate protein content. Fat content (963.15) was determined by Soxhlet hexane extraction.

2.3.1. Total starch

The total starch was determined enzymatically using a total starch analysis kit from Megazyme (Megazyme International, Bray, Ireland), according previous study [11].

2.3.2. Amylose content

The content of amylose was determined according to Perez-Rea et al. [18,19], this method has a limit of detection of 5–95 % of total starch content. 100 mg of starch, contained in a vial, was dissolved with 0.3 mL of ethanol (80 % v/v) using magnetic stirring for 3–5 min, then 3 mL of dimethyl sulfoxide (DMSO) was added and stirred for 5 min more, no lumps should be formed in the sample. The sample vial was capped and heated for 1 h in a boiling water bath during magnetic stirring at 160 rpm (magnetic stirrer with heating, IKA, RET control-visc, Bundesländer, Germany). The solution was thereafter cooled to room temperature and transferred into a 100 mL volumetric flask. 10 mL aliquot of this solution was transferred into a 100 mL flask, to which 50 mL of distilled water and 2 mL I_2-KI (2 mg I_2, 20 mg KI/mL) were added. Deionized water was added to reach the final volume of 100 mL. After 15 min of resting time, the absorbance was read using a spectrophotometer at 675 nm (GENESYS™ 180 UV–Vis, Wisconsin, USA). The amylose content was

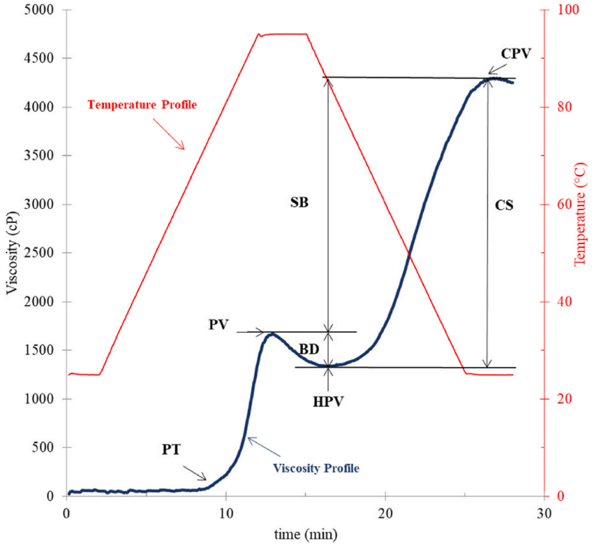

Fig. 2. Typical pasting curve. Pasting temperature (PT), peak viscosity (PV), hot paste (HPV), breakdown (BD), cool paste (CPV or final viscosity (FV)), consistency (CS= CPV – HPV), and setback (SB = FV – PV). Adapted from Bao & Bergman (2018) [20].

determined using a calibration curve prepared with a mixture of potato amylose (10130 Fluka, Sigma Aldrich, St. Louis, MO, USA) and potato amylopectin (A8515, Fluka, Sigma Aldrich, St. Louis, MO, USA) containing 0, 20, 40, 60, 80, and 100 % amylose. The solution of the mixture was treated the same as the samples. The analysis was carried out in triplicate.

2.4. Pasting properties

In high altitude countries such as the Andean ones, it is difficult to apply standardized methods for pasting properties because the boiling point of water is strongly affected. Therefore, it is most appropriate to adapt the methods. Pasting properties of the starch samples were evaluated following the method used and adapted by Fuentes et al. [11]. This method used a Physica MCR 301 rheometer (Anton Paar, Graz-Austria) equipped with the Physica Smart Starch analyzer accessory, which includes an electric heater and a special starch stirrer. For each starch sample, a 20 g starch suspension (8 % w/w) was prepared and stirred to homogenize. The starch suspension was kept at equilibrium at 20 °C for 1 min, then heated to 90 °C at 4.7 °C/min, kept at 90 °C for 5 min, cooled to 50 °C at 6 °C/min, and kept at 50 °C for 2 min. The stirring speed was 160 rpm. Pasting temperature (PT), peak viscosity (PV), hot paste viscosity (HPV or holding strength (HS)), breakdown (BD), cool paste viscosity (CPV or final viscosity (FV)), consistency (CS= CPV – HPV), and setback (SB = CPV – PV) were determined using Rheoplus/32 V2.65 supporting software (Anton Paar). Fig. 2 allows us to understand these parameters more easily. Measurements were performed in triplicate for each starch sample.

2.5. Water solubility index (WSI) and swelling power (SP)

Water solubility index (WSI) and swelling power (SP) were determined following methods described by Leach et al. Kong et al. and Li et al. [21–23] with some modifications, the last evaluation temperature was 90 °C rather than 95 °C, due to the vapor pressure in the highlands. Starch (100 mg, dry weight ($m_{tot,dry}$)) was weighed directly into conical centrifuge tubes, and 10 mL of distilled water was added. The tubes were placed on a vortex mixer for 10 s and incubated in a hot water bath at 55 °C, 65 °C, 75 °C, 85 °C, and 90 °C for 30 min with frequent stirring at 2-min intervals. The tubes were thereafter cooled to room temperature in an ice water bath and centrifuged at 2000 g (Hettich, Rotina 38 Darmstadt, Germany) for 30 min, and the supernatant was aspirated. The solid sticking to the bottom and the wall including whitish material was considered the sediment and weighed wet ($m_{sed,wet}$). The supernatant was dried to constant weight ($m_{sup,dry}$) at 100 °C. The water solubility index (WSI) and swelling power (*SP*) were calculated using equations (1) and (2).

$$WSI\,\% = \left(m_{sup,dry} / m_{tot,dry}\right) \cdot 100 \tag{1}$$

$$SP\ [g_{wet} / g_{insoluble\ starch}] = m_{sed,wet} \,/\, \left(m_{tot,dry} - m_{sup,dry}\right) \tag{2}$$

2.6. Thermal properties

Thermal properties of isolated starches were analyzed using a Differential Scanning Calorimeter 6200 (Seiko Instruments, Shizuoka, Japan), in the same way as described by other authors [18,24]. The analyzes were carried out with a scanning rate of 10 °C/min from 20 °C to 140 °C in excess of MilliQ-water 1:3 (m/v) starch/water ratio. The onset (T_o), peak (T_p), conclusion (T_c) temperatures, and the enthalpy (ΔH) of gelatinization was collected using the DSC software (SII EXSTAR6000 Muse, Shizuoka, Japan). The thermal transition (ΔT) was calculated following equation (3). The measurements were evaluated at least in triplicate.

$$\Delta T = T_c - T_o \tag{3}$$

2.6.1. Scanning electron microscopy (SEM)

The morphology of starch granules was observed by scanning electron microscopy (SEM) according to other authors [24]. Starch samples were dehydrated with ethanol in 99.8 % ethanol and spread on double-sided adhesive tape attached to an aluminum stub. They were then coated with gold using a sputter coater (Q150R Plus-Rotary Pumped Coater, East Sussex, UK) at 20 mA for 120 s and analyzed using SEM (Tescan VEGA3, Kohoutovice, Czech Republic) at 20 kV. About 200 random were considered for the normal distribution of the particles, and each starch particle was considered as a sphere in terms of its diameter and volume. The diameter data were collected by visualization and measurement using the software Tescan Vega, then classes were established with width of 0.2 μm and the number of granules was numbered in class i ($f_{number,i}$). After that, the volume of each class was calculated assuming spherical granules with equation (4). To make the y-axis comparable, the volume of each granule was divided by the total volume of all particles to obtain the relative volume fraction of class i ($rf_{volume,i}$), presented in equation (5). Finally, a volume-weighted diameter was obtained equivalent using equation (6), this will be called d_{volume}.

$$f_{volume,i} = \left(f_{number,i} * d^3 * \pi\right) \Big/ 6 \tag{4}$$

$$rf_{volume,i} = f_{volume,i} \Bigg/ \left(\sum_{j=1}^{j} f_{volume,j}\right) \tag{5}$$

$$\overline{d}_{volume} = \sum_{j=1}^{n} d_j \cdot rf_{volume,j} \tag{6}$$

2.7. Statistical analysis

Two types of Andean species with three cultivars from each species were evaluated. Data analysis has been performed both on species level – two samples (amaranth and canihua), and on the cultivar level – three samples (AC1, AC2, and AC3 for amaranth and CP1, CP2, and CP3 for canihua). For the comparisons between cultivars in each species, one-way ANOVA was performed with Tukey analysis using SPSS Statistics 24 package (SPSS Inc., IBM Corporation Armok, USA). For inter-species comparison, the mean and SD were calculated using SD with all data for each grain, followed by a t-test for independent samples using the same program. Lowercase letters were used to compare cultivars in each species and uppercase letters were used to compare values between species. Additionally, Pearson's correlation coefficients (r) were developed to compare the various physicochemical properties and principal component analysis (PCA) was carried out to provide the visualization of the differences and similarities among starches from different cultivars and species.

3. Results

Representative starch samples were isolated from the 3 amaranth (*Amaranthus caudatus*) and the 3 canihua (*Chenopodium pallidicaule*) cultivars according to the description in the method section. The chemical composition and the most important impurities of starch isolated from amaranth and canihua cultivars are shown in Table 2. All amaranth samples are characterized by low amylose content (<5 %) while the canihua starches had an amylose content between 21 and 23 %. Finally, the purity of the isolated starches ranged between 97 and 98 % for amaranth and 95–96 % for canihua starches with protein, lipids and fibers as the major impurities.

Table 2
Chemical composition of starch isolates from Andean grain cultivars. All results are given as mass fraction counted on dry matter.

	Ash (%)	Crude fiber (%)	Protein (%)	Fat (%)	Amylose (%)	Total starch (%)
AC1	1.52 ± 0.02^{a}	0.22 ± 0.01^{b}	0.21 ± 0.02^{a}	0.19 ± 0.01^{b}	<5 %	97.3 ± 0.5^{a}
AC2	2.05 ± 0.05^{b}	0.13 ± 0.01^{a}	0.24 ± 0.01^{a}	0.18 ± 0.01^{b}	<5 %	97.1 ± 0.4^{a}
AC3	1.55 ± 0.02^{a}	0.25 ± 0.02^{b}	0.25 ± 0.02^{a}	0.11 ± 0.01^{a}	<5 %	97.8 ± 0.5^{a}
Amaranth	1.71 ± 0.26^{A}	0.20 ± 0.05^{A}	0.23 ± 0.17^{A}	0.16 ± 0.04^{A}	$<5\%^{A}$	97.4 ± 0.7^{B}
CP1	1.24 ± 0.05^{a}	0.24 ± 0.02^{a}	0.46 ± 0.01^{b}	0.46 ± 0.02^{b}	21.6 ± 0.62^{a}	96.0 ± 0.7^{a}
CP2	1.64 ± 0.05^{ab}	0.38 ± 0.01^{b}	0.25 ± 0.01^{a}	0.48 ± 0.01^{b}	21.2 ± 0.24^{a}	96.1 ± 0.8^{a}
CP3	1.91 ± 0.04^{c}	0.28 ± 0.02^{a}	1.61 ± 0.06c	0.21 ± 0.01^{a}	23.3 ± 0.60^{b}	95.4 ± 0.6^{a}
Canihua	1.60 ± 0.30^{A}	0.30 ± 0.07^{B}	0.77 ± 0.64^{B}	0.38 ± 0.13^{B}	22.0 ± 1.08^{B}	95.8 ± 0.9^{A}

The results are presented in relative percentage as mean ± standard deviation (n = 3 for each cultivar and n = 9 for each specie). For each compound, different lowercase letters in columns indicate significant differences between starches of cultivars ($p < 0.05$) in each species. Uppercase letters indicate significant differences between the species amaranth and canihua ($p < 0.05$).

The viscosity profiles of different cultivar starches are shown in Fig. 3, amaranth starches (Fig. 3a) and canihua starches (Fig. 3b); each line represents the mean of triplicate measurements with its standard deviation for each cultivar. The data is reported in Table 3, pasting temperature (PT), peak viscosity (PV), hot paste (HPV or holding strenght (HS)), breakdown (BD), cool paste (CPV or final viscosity (FV)), consistency (CS= CPV – HPV), and setback (SB= CPV – PV). For all amaranth cultivars, an abrupt increase in viscosity was observed, and AC3 cultivar presented the lowest viscosity values except for HPV and SB. In addition, amaranth cultivars were similar only in HPV in two cultivars (AC2 and AC3). Canihua showed similarities only in PT (CP2 and CP3). Both species showed differences in pasting properties except for PV.

Fig. 4 (4a – 4c) gives the water solubility index (WSI) and the swelling power (SP) of amaranth and canihua starches in the temperature range of 55–90 °C. The WSI for amaranth starches was similar, having a non-linear behavior with a main increase at 75 °C in all samples. A much higher WSI was observed for amaranth compared to canihua starches.

The SP had a wide variation in amaranth cultivars, while in canihua cultivars showed similar SP at each temperature. Comparing the SP versus the WSI (Fig. 4c), temperature-dependent increased in both amaranth and canihua. Canihua showed very similar curves between samples, while amaranth had different slope between samples. AC1 and AC3 had a small variation in SP up to 75 °C, then they behaved differently. AC2 and AC3 were similar only at 75 °C and 85 °C.

The thermal properties (T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy; and thermal transition, ΔT) are shown in Table 4. AC2 showed the highest T_o, T_p, and T_c, but the lowest ΔT amongst amaranth cultivars. ΔH was similar for all amaranth cultivars. Canihua had some similarities among cultivars, but they were different according to ΔT. Finally, all the thermal properties were different between both species.

Fig. 5 shows that the amaranth granules are larger than the canihua granules. The starches of both species presented polygonal shapes, slightly indented faces, rough surfaces without fissures, and some granules with sphere form. The mode value of the volume-weighted distribution of the amaranth starch size ($rf_{volume,i}$) was between 1.0 and 1.6 μm for the three samples. The volume-weighted average diameter had the following trend AC3>AC1>AC2, with 1.39, 1.36, and 1.04 μm, for amaranth cultivars. The mode value of the volume-weighted distribution of the canihua starches were 0.8 and 1 μm. Finally, volume-weighted average diameter was 0.90, 0.89, and 0.80 μm for CP1, CP2 and CP3, respectively.

For Pearson and PCA analysis the thermal, pasting, solubility and swelling properties were considered. The Pearson's correlation coefficient for the relationship among the various physicochemical properties of the starches is shown in Table 5. Amylose content showed a significant positive correlation with HPV and CPV and strong negative correlations with size granules (d_{volume}), PT, BD, SB,

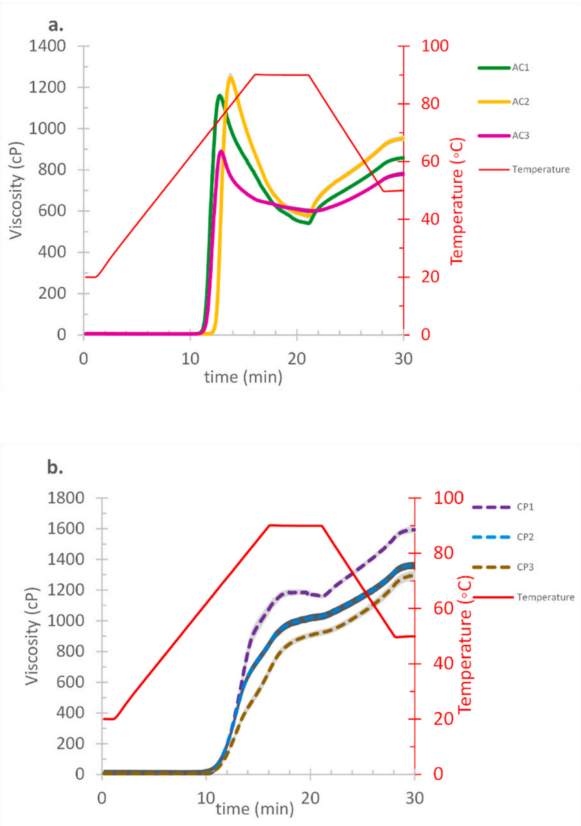

Fig. 3. Pasting properties of **a.** *Amaranth caudatus* (AC, continuous line) and **b.** *Chenopodium pallidicaule* (CP, dash line) of starches from Andean grain cultivars. Each curve is presented with a standard deviation (n = 3).

Table 3
Pasting properties of starches of cultivars from amaranth and canihua.

	PT (°C)	PV (cP)	HPV (cP)	BD (cP)	CPV (cP)	CS (cP)	SB (cP)
AC1	62.2 ± 0.1^{b}	1002 ± 5^{b}	549 ± 9.9^{a}	453 ± 12^{b}	855 ± 4^{b}	306 ± 10^{b}	−147 ± 1.3^{b}
AC2	64.5 ± 0.4^{c}	1245 ± 22^{c}	591 ± 13^{b}	659 ± 20^{c}	964 ± 13^{c}	373 ± 4^{c}	−286 ± 16^{a}
AC3	61.2 ± 0.2^{a}	780 ± 8^{a}	609 ± 9^{b}	171 ± 2^{a}	787 ± 7^{a}	178 ± 2^{a}	6.9 ± 1.9^{c}
Amaranth	62.7 ± 1.7^{B}	1010 ± 203^{A}	584 ± 29^{A}	427 ± 212^{B}	869 ± 77^{A}	286 ± 86^{A}	−141 ± 127^{A}
CP1	56.8 ± 0.1^{b}	1179 ± 13^{c}	1169 ± 8^{c}	10 ± 2^{b}	1589 ± 25^{c}	421 ± 29^{c}	410 ± 29^{c}
CP2	55.6 ± 0.2^{a}	973 ± 18^{b}	996 ± 14^{b}	−22 ± 4^{a}	1336 ± 20^{b}	341 ± 6^{a}	363 ± 6^{b}
CP3	55.6 ± 0.1^{a}	856 ± 20^{a}	883 ± 19^{a}	−27 ± 1^{a}	1262 ± 31^{a}	379 ± 15^{b}	352 ± 15^{a}
Canihua	56.0 ± 0.6^{A}	1002 ± 143^{A}	1029 ± 119^{B}	−13 ± 20^{A}	1396 ± 150^{B}	380 ± 38^{B}	372 ± 38^{B}

The results are presented as mean ± standard deviation (n = 3 for each cultivar and n = 9 for each species). For each compound, different lowercase letters in columns indicate significant differences between starches of cultivars ($p < 0.05$) in each species. Uppercase letters indicate significant differences between species amaranth and canihua ($p < 0.05$). PT pasting temperature, PV peak viscosity, HPV hot paste, BD breakdown = PV – HPV, CPV cool paste, CS (consistency) = CPV – HPV, and SB (setback) = CPV – PV.

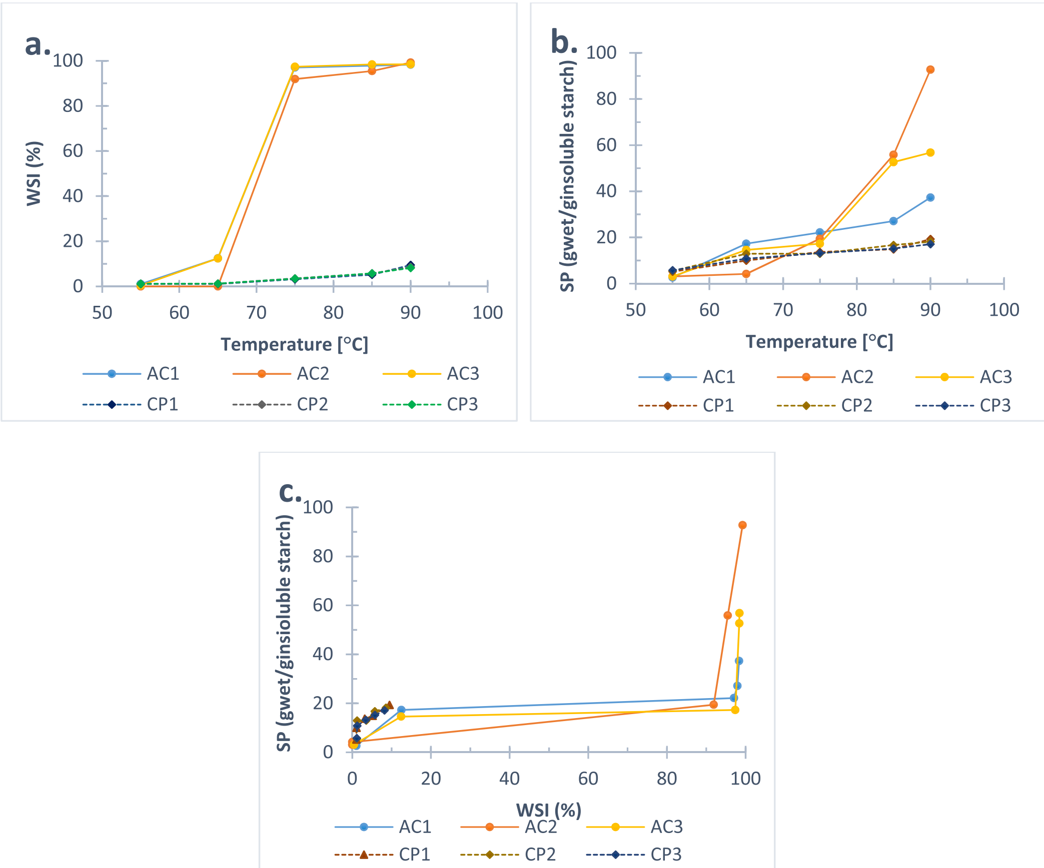

Fig. 4. Water solubility index (WSI) and swelling power (SP) of amaranth (*Amaranth caudatus*) and canihua (*Chenopodium pallidicaule*). **a.** WSI; **b.** SP; and **c.** SP versus WSI. Amaranth is presented with a continuous line and canihua with a dash line.

thermal properties except ΔT. Correlations were found between pasting and thermal properties, as well as correlations between WSI and SP with thermal properties. PV was independent of cultivars and species.

The PCA plots (Figs. 6 and 7) provide an overview of the similarities and differences between cultivars of each species and between species as well as the interrelationships between properties. The canihua starches (CP1, CP2, and CP3) were different from amaranth starches (AC1, AC2, and AC3) with highly negative scores in the plot. The canihua starch cultivars presented some similarities among themselves. Unlike amaranth starches because they disperse on the right side of the plot. The first (PC-1) and the second (PC-2) principal components described 76 % and 17 % of the variance, respectively. Together they represent 93 % of the total variability. The loading plot provided information on the correlations between the measured physicochemical properties (Fig. 7). Properties close to

Table 4
Thermal properties of starches of cultivars from amaranth and canihua.

	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (mJ/mg)	ΔT (°C)
AC1	61.5 ± 0.7^a	68.0 ± 0.1^a	75.6 ± 0.2^a	12.3 ± 0.6^a	14.0 ± 0.9^b
AC2	67.7 ± 0.9^b	72.6 ± 0.1^c	79.2 ± 0.4^b	11.5 ± 0.3^a	11.5 ± 0.5^a
AC3	60.0 ± 0.7^a	69.3 ± 0.3^b	75.5 ± 0.6^a	10.9 ± 1.1^a	15.5 ± 1.1^b
Amaranth	63.1 ± 3.6^B	69.9 ± 2.0^B	76.7 ± 1.9^B	11.6 ± 0.9^B	13.7 ± 1.9^A
CP1	53.3 ± 0.5^b	59.7 ± 0.1^b	69.3 ± 0.5^a	7.3 ± 0.1^a	16.0 ± 0.6^a
CP2	50.8 ± 0.2^a	58.2 ± 0.3^a	69.0 ± 0.6^a	8.2 ± 0.7^b	18.2 ± 0.7^b
CP3	52.0 ± 0.8^{ab}	59.3 ± 0.1^b	72.9 ± 0.6^b	6.9 ± 0.1^a	20.8 ± 1.1^c
Canihua	52.1 ± 1.2^A	59.1 ± 0.7^A	70.4 ± 2.0^A	7.5 ± 0.7^A	18.3 ± 2.2^B

The results are presented as mean ± standard deviation (n = 3 for each cultivar and n = 9 for each grain). For each compound, different lowercase letters in columns indicate significant differences between starches of cultivars ($p < 0.05$) in each species. Uppercase letters indicate significant differences between species amaranth and canihua ($p < 0.05$). T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy; and thermal transition, ΔT.

each other on the plot are positively correlated, while those in opposite directions are negatively correlated.

4. Discussion

Understanding the relationships between the structures and functional properties of starches is essential for developing new applications in food and other industries. The starches in the present study differed at two levels: between species (amaranth and canihua) and between cultivars of each species.

The composition of the isolated starch obtained was similar to previous studies [5,6]. The starch content of the starch isolates exceeded 95 %, indicating high purity [7]. Steffolani et al. [6] has reported low amylose content for canihua ecotypes (Local, 381, 081, and 300) ranged 10.7–17.4 % than we found (21.2–23.3 %). Other authors found lower amylose content in *hypochondriacus* and *cruentus*, species of amaranth (5–11 %) [7] or much lower (2 %) [25], which is much lower than common standard cereals such as corn, wheat or rice (20–22 %) [4,5,26]. While other authors have not detected amylose in the *A. caudatus* specie [11]. Amaranth, due to its low amylose content (<5 %), can thus be characterized as a waxy starch [10]. Regard to impurities of the starch isolates, the minor components analyzed in Table 2, the canihua starch presented more remaining crude fiber, protein and fat than amaranth reflecting the more challenging purification of starch with very small granules (0.7–0.8 μm for canihua compared to 1–1.6 μm for amaranth).

Although the pearled process of the grains was not studied, previous study concluded that was no significant effect of pearling on morphological, physicochemical and rheological characteristic of barley starch [27]. Fig. 8 illustrates the changes that occur in starch granules in presence of sufficient amount of water and heating. The swelling of the starch granules increases after 65 °C, as already observed in waxy and non-waxy starches [28]. In amaranth starch, the swelling (SP) is quite rapid and followed by a rapid dissolution (WSI), as observed by Lin et al. in waxy corn [28]. The starch swelling pattern of canihua indicate a two-stage swelling, as previously observed on quinoa [29]. The amylose reinforces the internal network within the granule, therefore restricts swelling, as observed by Lorenz et al. [29]. In contrast to the starch granules of canihua, the starch granules of amaranth disintegrate partially after swelling, leading to a decrease in cold paste viscosity (CPV) and final viscosity (FV). The viscosity during cooling may also be due to the molecular reassociation of the low amylose content (<5 %), overcoming the effect of continuous shearing, as was found in an aqueous paste of a waxy starch in a previous study [28]. In this study the waxy starches had fast swelling, strong degradation and low consistency, as observed in our study. The retrogradation effect (CS > BD) was quite clear in the case of the canihua starches. The weak gel properties of waxy starches could be used in foods that need to maintain their consistency without hardening after cooling, such as creamy instant desserts [30]. Additionally, a high amylopectin content may be ideal for use in extrusion expanded products, where the low amylose content leads to expanded, light, elastic and homogeneous textures, as concluded Moraru & Kokini [31].

Canihua showed slow swelling, possibly due to the strong and uniform binding forces between the starch granules and the higher content of amylose, as proposed in previous study on rice starch [32]. Another possible explanation is that the large amount of impurities delayed the swelling. These granules showed significant swelling of about 20 times their weight. The strong inter-granules forces resulted in no change dramatically in solubility, swelling and pasting properties at 90 °C. The canihua granules showed partial disintegration but dissolved, resulting in consistent viscosity when cooled. Furthermore, small starch granules observed in the granule size distributions (Fig. 5) are expected to display comparably high breaking strength, and thus, comparably high shear stability, as concluded by Steffolani et al. in a study of canihua and quinoa ecotypes [6]. These starches could have specific applications in the food industry, such as a fat substitute in desserts and pastries, biofilms, pharmaceuticals, or the cosmetic industry [33].

The onset temperature (T_o) for thermal properties was higher for species grown in Andean valleys (amaranth, ~2200 m. a.s.l.) than for species grown in the high plateau of the Andes (canihua, >3000 m. a.s.l.), as previously described in the study on Andean plants [11]. Lower T_o could indicate less perfect crystalline structure of starch [34]. The conclusion temperature (T_c) was negatively correlated with the amylose content (Fig. 7). The starches exhibited a single thermal transition (ΔH). However, in canihua starch second small was observed at ~88 °C, similar to previously found (85 °C) [11]. The higher enthalpy values in amaranth than in canihua might be related to the dominance of amylopectin and the structure of the starch granule [6,35]. Longer branching chains in amylopectin promote higher enthalpy values [36]. A large delta (T_c – T_o) in canihua starches indicates a large dispersion of starch structures, inhomogeneous and more branched structures (Fig. 6) [37,38].

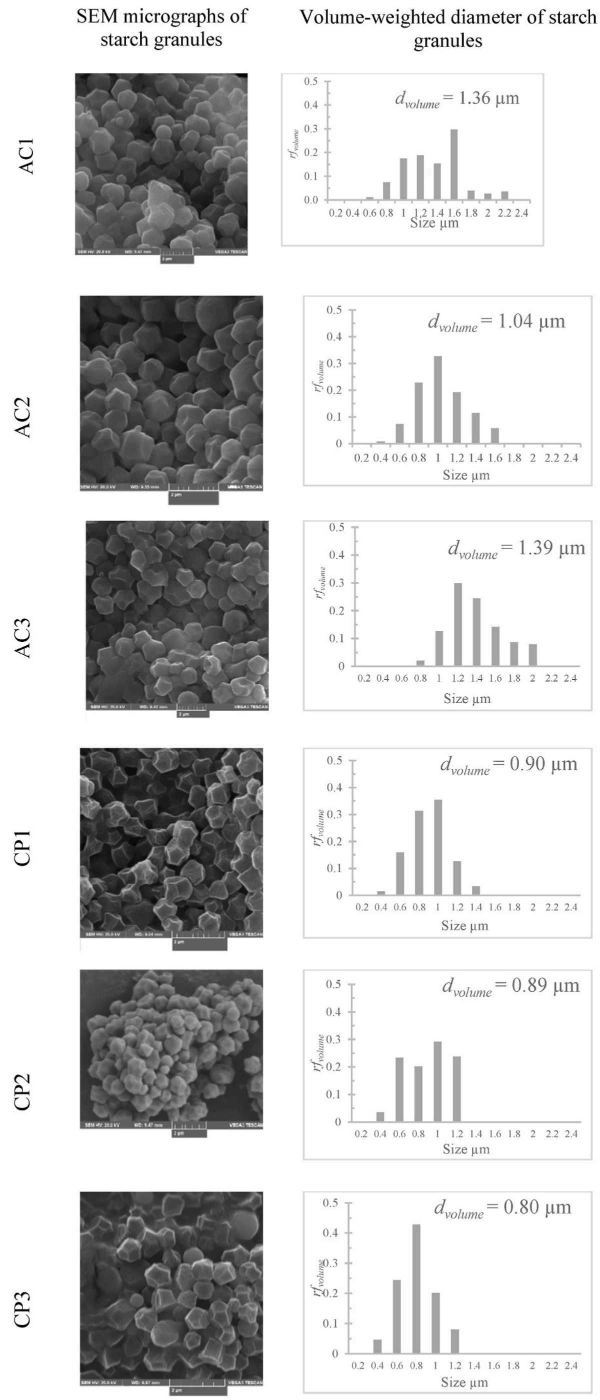

Fig. 5. Scanning electron microscopy (SEM) micrographs and distribution normal according to volume, each one with the volume weighted average diameter according the volume of the granules. Bar 2 μm. A. was added for amaranth cultivars and C. was added for canihua cultivar, n = 150–200.

Canihua cultivars showed arbitrarily different proximate composition possibly caused by the challenge of separating the impurities (ash, crude fiber, protein, and fat). Benesi et al. [8] found that the genotype of the plant could affect the proximal composition of cassava starch more than the growth environment. In our study, amylose content was related to plant branch shape during growth. Differences in plant architecture are referred to as growth habit, a factor described in a previous study [13]. Therefore, the amylose content varied between canihua cultivars. CP1 cultivar had the highest pasting property values and the lowest thermal transition. Both behaviors could respond to a wider range of normal granules size distribution up to 1.4 μm. Granule size distribution could affect some properties, such as thermal transition (ΔT), as found in previous studies in quinoa and potato [39,40]. In addition, the enthalpy may

Table 5
Pearson correlation coefficients among different properties of the different cultivar starches.

	Amylose	HPV	CPV	CS	d_{volume}	Ash	Fiber	Protein	Fat	TS	PT	PV	BD	SB	To	Tp	Tc	ΔH	WSI	SP
HPV	0,841*																			
CPV	0,908*	0.933**																		
CS	0,61	0.895*	0.764																	
d_{volume}	−0.870*	−0.851*	−0.844*	−0.810																
Ash	−0.178	−0.187	−0.334	0.087	−0.218															
Fiber	0.658	0.352	0.425	−0.071	−0.338	−0.283														
Protein	0.586	0.314	0.331	0.349	−0.58	0.333	0.184													
Fat	0.741	0.848*	0.869*	0.582	−0.629	−0.460	0.574	−0.093												
TS	−0.947*	−0.866*	−0.849*	−0.758	0.934**	−0.054	−0.470	−0.719	−0.608											
PT	−0.954**	−0.684	−0.788	−0.360	0.700	0.291	−0.823*	−0.549	−0.678	−0.838*										
PV	−0.41	0.458	0.325	0.689	−0.279	0.035	−0.533	−0.343	0.353	−0.075	0.305									
BD	−0.841*	−0.468	−0.664	−0.106	0.506	0.418	−0.825*	−0.475	−0.558	0.658	0.950**	0.480								
SB	0.945*	0.689	0.841	0.367	−0.68	−0.409	0.759	0.483	0.716	−0.801	−0.981**	−0.235	−0.962**							
T_o	−0.910*	−0.631	0.741	−0.271	0.603	0.381	−0.867*	−0.478	+0.674	0.768	0.991**	0.366	0.963**	−0.973**						
T_p	−0.963**	−0.781	−0.841*	−0.466	0.723	0.332	−0.797	−0.491	−0.761	0.864*	0.985**	0.179	0.897*	−0.965**	0.977**					
T_c	−0.855*	−0.715	−0.825*	−0.338	0.549	0.591	−0.807	−0.180	−0.865*	0.667	0.908*	0.116	0.879*	−0.935**	0.931**	0.934**				
ΔH	−0.973**	−0.746	−0.881*	−0.541	0.851*	0.163	−0.580	−0.644	−0.640	0.908*	0.927**	0.094	0.863*	−0.944**	0.875*	0.905*	0.797			
ΔT	0.810	0.412	0.504	0.138	−0.560	−0.056	0.781	0.748	0.322	−0.747	−0.908*	−0.600	−0.890*	0.814*	−0.899*	−0.847*	−0.678	−0.811		
WSI	−0.998**	−0.862*	−0.927**	−0.631	0.882*	0.184	−0.652	−0.538	−0.780	0.940**	0.941**	−0.010	0.822*	−0.937**	0.897*	0.957**	0.863*	0.965**	−0.774	
SP	−0.910*	−0.616	−0.799	−0.361	0.789	0.126	−0.677	−0.502	−0.641	0.787	−0.901*	0.181	0.882*	−0.920**	0.858*	0.852*	0.791	0.953**	−0.786	0.911*

*p ≤ 0.05; **p ≤; 0.01; HPV, hot peak viscosity; CPV, cold peak viscosity; CS, consistency; d_{volume} volume weighted diameter; TS, total starch; PT, pasting temperature; PV, peak viscosity; BD, breakdown; SB, setback; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy of gelatinization, and ΔT thermal transition.

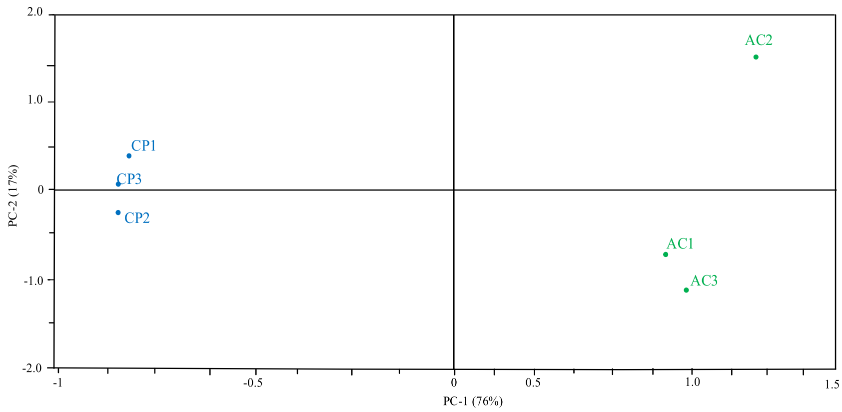

Fig. 6. Principal component analysis: score plot of first principal component (PC-1) and second principal component (PC-2) describing the overall variation among starches from different cultivars and species.

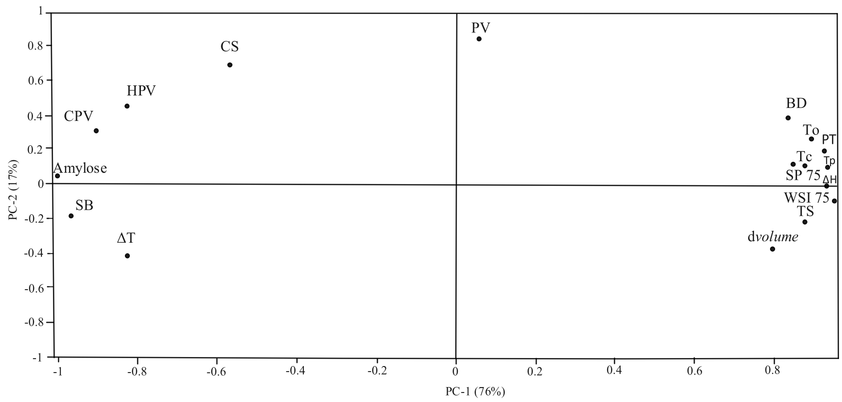

Fig. 7. Principal component analysis: Loading plot PC-1- and PC-2 describing the variation among properties of starches from different starches of cultivars and species. HPV, hot peak viscosity; CPV, cold peak viscosity; CS, consistency; d_{volume} volume weighted diameter; TS, total starch; PT, pasting temperature; PV, peak viscosity; BD, breakdown; SB, setback; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, enthalpy of gelatinization, and ΔT thermal transition.

have a relationship with amylopectin unit chains, as studied in potato and in rice cultivars [22,36,39,41]. In contrast, we did not find any differences between cultivars in WSI and SP.

Among the amaranth cultivars, AC2 had the highest values for pasting properties (PT, PV, HPV, BD, CPV, CS) and also for T_o, T_c, and T_p for thermal properties. This fact could differentiate it from the other amaranth cultivars (Fig. 6). The behavior of pasting properties of amaranth was in contrast to the previously studied waxy rice starches [22]. Pasting properties and thermal properties were negatively related to the relative size distribution of the granules (Figs. 5 and 6). AC3 had the largest volume weighted diameter (1.39 μm) and AC2 the smallest (1.04 μm). The small size of AC2 granules may have favored water absorption and swelling under endothermic conditions, related with pasting and thermal properties.

The significant increase in swelling and solubility, especially in amaranth starch, occurs above of 75 °C, as found by other authors in amaranth starches [7,29]. Therefore, for Pearson and PCA analysis, the WSI and SP data were considered only at 75 °C. According this analysis, the behavior of starch depends on starch composition in terms of amylose and amylopectin. Furthermore, cultivars within species could have different starch properties as well as between starches of different species. One cultivar (AC2) was not correlated with the others in amaranth cultivars. The starches from both species were totally opposite to each other. The higher concentration of amylose effects more rapid gelation and stronger gels, as previously has been observed in no-waxy-starches [28]. It is reflected in their positive correlation of HPV and CPV with the canihua starches. Unlike amaranth starches were correlated with thermal properties. It can support high temperatures and shear forces such as extrusion and this starch with mainly amylopectin content could expand more than starches containing amylose [31].

5. Conclusion

Amaranth and canihua starches exhibited different properties. The amaranth granules swelled strongly very quickly, then disintegrated and lost viscosity. Consequently, the WSI and SP were higher for amaranth than for canihua. Canihua showed slow swelling

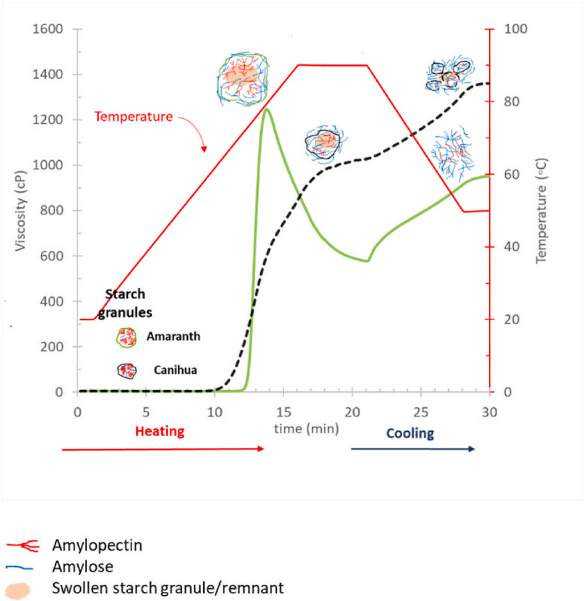

Fig. 8. Illustration of changes in starch granules over time in the presence of water and heating.

with a cool paste of good consistency with some granules still intact. Canihua cultivars showed more differences in thermal properties, which could be due to inhomogeneous molecular starch. Amaranth cultivars showed significant differences between them according the pasting and thermal properties. Correlation analysis indicated that he amylose content affected the functional properties of these starches. The differences between cultivars and species suggest diverse applications in the industry. Future work is needed to characterize the gel texture and its relationship to functional properties. Research is being done on the applications of these grains in processed foods.

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Data availability statement

The data associated with this study has not been deposited into any publicly available repository. The data associated are included in the article/supp. material/referenced in the article.

CRediT authorship contribution statement

Jenny Mérida-López: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cinthia Carola Rojas:** Visualization, Supervision, Methodology, Conceptualization. **Björn Bergenståhl:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jeanette K. Purhagen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Jenny Merida Lopez reports financial support, article publishing charges, equipment, drugs, or supplies, statistical analysis, and travel were provided by Swedish International Development Cooperation Agency. Cinthia Carola Rojas reports a relationship with Universidad Mayor de San Simon that includes: employment. Bjorn Bergenstahl reports a relationship with Lund University that includes: employment. Jeanette K. Purhagen reports a relationship with Lund University that includes: employment. None has patent pending to None. None If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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