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Physicochemical Properties of Grain and Starch from Kanihua (*Chenopodium Pallidicaule*) Compared with Quinoa (*Chenopodium Quinoa*) Originated from Peru

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Kanihua and quinoa are closely related Andean grains that are unconventional starch sources. Starch was extracted from two kanihua and three quinoa genotypes and their physicochemical properties (proximate analysis, scanning electron microscopy, distribution and particle size, X-ray diffraction, FTIR, solubility, swelling power, pasting and thermal properties) were investigated. Kanihua and quinoa grains presented spherical shapes and regular sizes (1.05 - 1.30 mm). The starch granules show asymmetric monomodal distribution for all cases. Meanwhile, Sauter diameter values in kanihua were smaller (0.961 µm) than quinoa (1.099 µm). Regarding structure, all samples showed Type A polymorphism and similar FTIR spectra behavior. In addition, amilose content was around 11-14% and 8-12% for varieties of kanihua and quinoa respectively. Starch solubilities were less than 13%, and kanihua starches had less swelling power than quinoa starches. However, the maximum swelling power values were reached around 70 °C. The variety and type of grain influenced pasting properties, with an inverse relationship between the breakdown and setback values for the evaluated starches. Analyzing the thermal properties, gelatinization enthalpy and retrogradation were similar for all starches. Even though the kanihua and quinoa starches present similar structural characteristics, the pasting properties and swelling power were different. The insight into the morphological, thermal, and pasting properties of native *Chenopodium* starches could be helpful in the preparation and development of new food formulations.

1. Introduction

Kanihua (*Chenopodium pallidicaule*) and quinoa (*Chenopodium quinoa*) are typical grains from the Andean region grown since ancient times, and were the food basis of the Inca empire (Repo-Carrasco et al., 2003). In the 1970s, the nutritional value of high-Andean grains generated international interest, specifically because of protein quantity and quality (15-19%) and due to the balance of essential amino acids. Quinoa grains are consumed worldwide (Velázquez-Barreto et al., 2021) as a superfood, while kanihua, a grain with similar characteristics, is less consumed, which is associated with a lack of knowledge of the chemical, nutritional and functional composition.Starch is the main component present in kanihua and quinoa grains (48-69%) and it is primarily located in the perisperm. Its properties are a function of its botanical origin, granule morphology and structure, composition, proportions of amylose / amylopectin chains, distribution molecular weights and thermal processing conditions (Li, Zhu, 2018). Studies in unconventional starch sources with attractive properties has increased in recent years, such as chemical characterization (Villa et al., 2014), evaluation of antioxidant capacity (Repo-Carrasco et al., 2010), starch extraction techniques (Villarreal et al., 2013) and chemical modification as source of biodegradable material (Ferreira-Villadiego et al., 2018)
In Peru, kanihua and quinoa were recognized as priority native crops, to be researched and for development of techniques for exploiting and adding value to these grains. Therefore, considering the importance of high-Andean grains, this study aimed to evaluate the structural characteristics, physicochemical, thermal and pasting properties of starches extracted from two types of kanihua and compared with three types of quinoa originated in Peru.

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2. Material and methods

2.1 Raw material

The varieties of kanihua (*Chenopodium pallidicaule*) and quinoa (*Chenopodium quinoa*) grains were selected from the Instituto Nacional de Innovación Agraria (INIA) and were named: Kanihua Illpa (KI), Kanihua Cupi (KC), Quinoa Salcedo (QS), Quinoa Passankalla (QP) and Quinoa Negra Collana (QNC). All selected samples were registered varieties and grew in Puno region. The chemical composition of the grains was determined as described in AOAC methods (2000) for moisture (934.06), protein (920.87), ash (Nº 923.03), fatty material (945.16), crude fiber (962.09) contents and carbohydrates were obtained by difference.

2.2 Starch extraction

Starch extraction was carried out according to Tapia-Blácido et al. (2010) with some modification. Samples were placed in NaOH solution (0.25 % (w/v) (1: 5) at 4-7 °C for 24 h. After, they were blended and the slurry was run through sieves with 0.180, 0.075 and 0.053 mm opening to separate coarse fibrous material. This process was repeated up to three times. The suspension was centrifuged at a 10 ºC for 20 min with rotation speed of 6000 rpm in a refrigerated centrifuge (Eppendorf AG, model 5430 R), the supernatant was discarded. The sediment was resuspended in distilled water and the pH was adjusted to 7.0 with HCl solution (0.2 N). Recovered starch was dried at 38 °C in an air oven (Marconi MA035/1, Brazil) for 16 h. The product obtained was ground with a mortar and pestle and sieved through a100 mesh.

2.3 Starch characterization

Starch proximate composition was determined as described in AOAC methods (2000). Amylose-content was determined using starch samples (0.5 g) dispersed in 25 mL of dimethyl sulfoxide (DMSO, 90% w/w), placed under stirring in boiling water bath for 1 h, and stirred for further 16 h at room temperature. The starches were precipitated with approximately 75 mL of anhydrous ethanol and centrifuged at 12,000 g for 10 min. The precipitated starches were again dispersed in anhydrous ethanol, vacuum filtered and dried in oven with forced air circulation at 38 °C/24 h. The apparent amylose content was calculated as a ratio between iodine affinity of amylose (AIA) and 20%, which value corresponds to the iodine affinity of pure amylose.
Morphology of the kanihua and quinoa starch powders were analysed by scanning electron microscopy (SEM) (TM-3000, HITACHI, Japan) at an accelerated voltage of 15 kV. Before analysis, powders were conditioned under vacuum for 12 h and then fixed onto aluminum stubs by means of carbon tape. Particle size distribution was obtained using a laser diffraction particle size analyzer (SALD-201V, Shimadzu, Japan). Ethanol was used as solvent to disperse starch and improve granule distribution in an ultrasonic bath for 3 min. Sauter mean diameter values were calculated, $D_{[3,2]}$ (Eq. 1) These determinations were performed in triplicate.

$$D[3,2] = \frac{\sum_i d_i^3}{\sum_i d_i^2} \quad (1)$$

X-ray diffraction analyses were performed using an X-ray diffractometer RU200B (Rigaku Rotaflex, Japan), with copper anode, operating with 40 kV and current of 20 mA. The diffractograms were collected in 2Θ range of 3 to 40º, using a scan rate of 2 º/min in Scion Image software. Crystallinity index was determined calculating the area under the curve. Absorbance spectra were recorded on a Spectrum One Perkin Elmes (California, USA) equipped with attenuated total reflectance (ART) accessory. The infrared spectrum was obtained at a resolution 2 cm^{-1} (average 64 scan), in the range from 4000 to 600 cm^{-1}.
Solubility (S) and swelling power (PI) analysis of starches (2.5 % w/v) were performed in a temperature range from 50 - 90 ºC using the method described by Yu et al. (2012), and according to equations 2 and 3:

$$S\ (\%) = \frac{W_r}{W} \times 100 \quad (2)$$

$$PI\ (g/g) = \frac{W_t}{W - W_r} \quad (3)$$

Where, W = weight of sample (db, mg); Wt = weight of the wet sediment; Wr = weight of the dried supernatant.

Pasting properties were evaluated by a Rapid Visco Analyzer (RVA-4, Newport Scientific, Australia). Starch (~3 g, moisture content ~10%) was suspended in water (~25 mL). The suspension was stirred in the aluminum RVA sample canister by helix (960 rpm, 10 s) to avoid bubbles formation, and after 160 rpm until the end. First suspension was heated to 50 °C for 1 min and a programmed heating and cooling cycle was used: heating to 95 °C at 5 °C/min, holding at 95 °C for 4 min and 30 s, and cooling to 50 °C at the same rate (5 °C/min). Thermal

properties were obtained using a differential scanning calorimeter (DSC 2010, TA Instrument, USA). Distilled water was added to starch (~2.0 mg) in a DSC pan (ratio water:starch, 3:1). The samples were scanned from 5 to 105 ºC at 10 ºC/min using empty pans as reference. Heated pans were then cooled immediately and kept at 4 °C for 15 days in the BOD (MA415 Marconi, Brazil) oven. After this storage period, samples were scanned under the same conditions as the first scanning.

3. Results and discussion

3.1 Proximate analysis and apparent amylose content

Table 1 shows the chemical composition of kanihua and quinoa grains, highlighting a high protein content, fiber and ash, especially in kanihua grains.

Table 1. Proximate composition of kanihua (K) and quinoa (Q) grains and starches

	Parameters	KI	KC	QS	QP	QNC
Grains	Moisture (%)	6.04 ± 0.01^{a}	5.64 ± 0.18^{a}	5.10 ± 0.51^{a}	5.86 ± 0.01^{a}	5.47 ± 0.07^{a}
	Protein (%)	19.52 ± 0.59^{b}	18.56 ± 0.24^{ab}	17.69 ± 0.79^{ab}	15.62 ± 0.68^{a}	17.19 ± 0.53^{ab}
	Fatty material (%)	0.58 ± 0.01^{a}	3.47 ± 0.05^{b}	3.60 ± 0.05^{b}	4.53 ± 0.17^{b}	3.06 ± 1.06^{ab}
	Crude fiber (%)	11.13 ± 0.21^{c}	9.22 ± 0.18^{b}	5.82 ± 0.06^{a}	7.00 ± 0.01^{a}	9.92 ± 0.42^{bc}
	Ash (%)	4.04 ± 0.02^{b}	4.69 ± 0.08^{c}	2.88 ± 0.06^{a}	2.78 ± 0.04^{a}	2.87 ± 0.01^{a}
	Carbohydrates (%)*	62.46 ± 0.40^{a}	61.92 ± 0.09^{a}	68.40 ± 1.24^{b}	68.21 ± 0.91^{b}	64.60 ± 1.25^{ab}
Starch	Moisture (%)	10.73 ± 0.45^{a}	10.93 ± 0.10^{a}	12.20 ± 0.01^{a}	11.50 ± 0.93^{a}	10.36 ± 0.02^{a}
	Protein (%)	3.72 ± 0.30^{c}	1.24 ± 0.06^{a}	2.46 ± 0.13^{b}	1.83 ± 0.25^{ab}	1.68 ± 0.05^{ab}
	Fatty material (%)	1.14 ± 0.15^{ab}	1.74 ± 0.13^{b}	0.82 ± 0.24^{ab}	0.63 ± 0.20^{a}	0.81 ± 0.03^{ab}
	Crude fiber (%)	0.23 ± 0.02^{a}	0.61 ± 0.20^{a}	0.31 ± 0.04^{a}	0.26 ± 0.04^{a}	0.11 ± 0.08^{a}
	Ash (%)	1.95 ± 0.01^{b}	1.01 ± 0.16^{a}	2.05 ± 0.02^{b}	1.71 ± 0.04^{b}	3.18 ± 0.03^{c}
	Amylose content (%)	11.80 ± 0.15^{a}	13.90 ± 0.35^{b}	10.97 ± 0.61^{a}	8.75 ± 1.27^{a}	11.92 ± 1.24^{a}

Mean ± SD. Mean with different superscript along the rows are significantly different ($p<0.05$)
*Total carbohydrate calculated by difference K Illpa; K Cupi; Q Salcedo; Q Passankalla; Q Negra Collana

High fiber content and physical configuration in the grain can influence retention of water molecules on the grain surface and the chosen process for the extraction of these compounds, resulted in no significant difference in the moisture content values. These results corroborate those obtained by Repo-Carrasco et al. (2003) and Villa et al. (2014) and may influence the technological characteristics. The grains have a spherical shape with an outer fiber layer which seals the cubic or polygonal appearance with size range from 1.05 to 1.25 mm diameter. On the surface of the grains some starch granules can be observed due to breakage and mechanical damage. The apparent amylose content varied between 13.6-14.5 g/100 g solids and 8.8-11.9 g/ 100g solids for kanihua and quinoa starches, respectively. KC sample had the highest amylose content. Amylose results for quinoa starch agreed with values reported by Qian and Kuhn (1999) and no studies were found in the literature for the amylose content of kanihua starch. Significant variety differences in amylose content for kanihua and quinoa were observed. Quinoa starch presents short branched chains of glucose with molar mass of 11.3 x 10^{6} g/mol (Praznik et al., 1999).

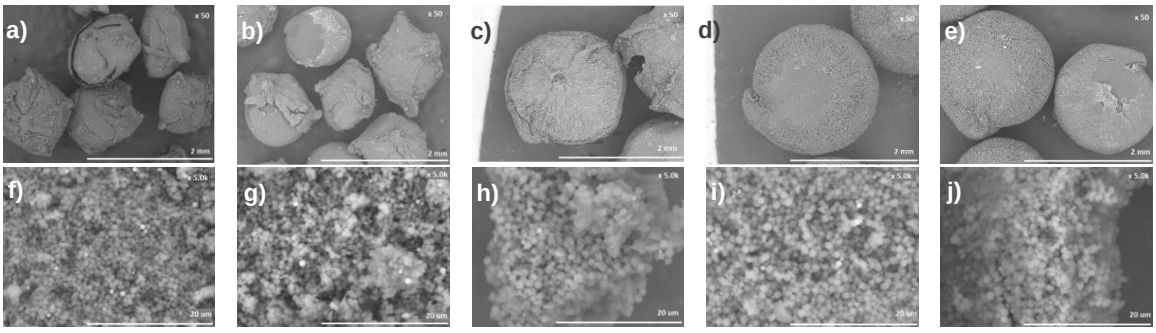

Figure 1. Representative micrograph of grains (a - e) and starches isolated (f - j) from kanihua (K) and quinoa (Q): K Illpa (a - f); K Cupi (b - g); Q Salcedo (c - h); Q Passankalla (d - i) and Q Negra Collana (e - j)

3.2 Scanning electron microscopy

Microscopic observation shows that the kanihua seeds have circular or ellipsoid shapes (Figure 1/a-b). The grains are surrounded by a layer of cellulosic material and this explains the high content of crude fiber in the compositional analysis. Starch granules are spherical in general appearance creating a polynomial structure (Figure 1/f-j) with sizes around 0.86 - 1.02 µm. Similar results were obtained by Abugoch (2009) for quinoa starch.

3.3 Distribution and particle size

The size distribution based on the number of particles or starch granules of kanihua and quinoa has an asymmetric monomodal distribution Figure 2/a. There is a significant difference between the particle's sizes, quinoa starch granules are larger than kanihua, QS has larger granules than other samples. $D_{[3,2]}$ was calculated between 0.937-1.204 µm for KI and QS, respectively. These calculated diameter values concur with the sizes obtained by SEM and also with those reported by Tang et al. (2002).

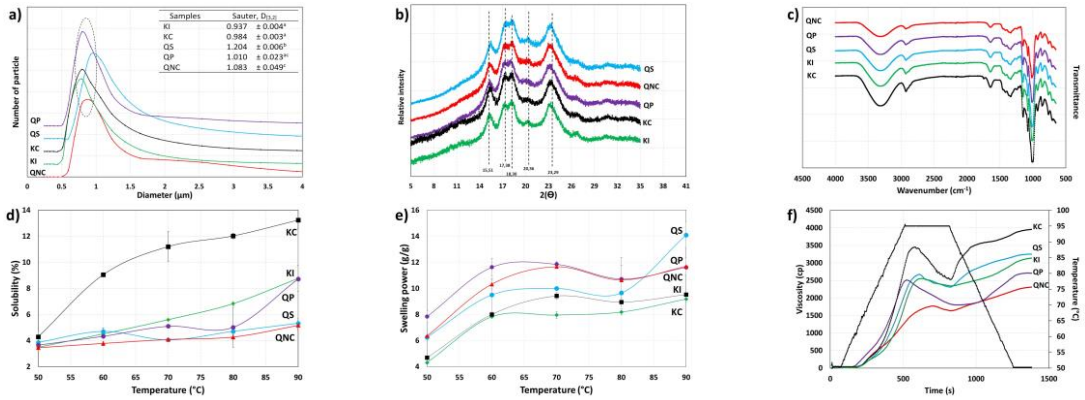

Figure 2. Starches of kanihua (K) and quinoa (Q): a) Distribution and size average of particles (Sauter's diameter); b) X-ray Diffractograms; c) FTIR spectra. d) Solubility and e) swelling power and f) pasting properties. K Illpa; K Cupi; Q Salcedo; Q Passankalla; Q Negra Collana

These starch granules are considered very small due to their diameters less than 5 µm (Lindeboom et al., 2004). Unimodal distribution granules allow greater dispersion and homogeneous incorporation in water to produce films (Araujo-Farro et al., 2010). The small size of starch granules affects their processing, as smaller particle sizes require more energy to break the granule structure to release its contents and to promote gelatinization.

3.4 X-Ray Diffraction and FTIR

The X-ray diffraction patterns of kanihua and quinoa (Figure 2/b) showed similar A - type crystalline patterns. The peaks obtained at, 15.51; 17.38; 18.30; 20.36 e 23.29 (2θ), were very close to those reported by Qian and Kuhn (1999). The diffraction pattern of type A is associated with grain starches (except for the varieties with high amylose), short amylopectin chains and the presence of water-soluble organic acids and alcohols (Eliasson and Gudmundsson, 2006). The crystallinity of the samples was similar, mean estimated at 31.8% ± 4.5. The pattern of crystallinity obtained for amaranth and quinoa starches (type A) was 45.5% for amaranth and 35.4% for quinoa (Villarreal et al., 2013), similar to those obtained in the present study.
Mid-infrared region (600 - 4000 cm^{-1}) is useful to determine fundamental vibrations and rotational vibrational structure of kanihua and quinoa starches (Figure 2/c). Oyeyinka et al. (2015) reported the amylose/amylopectin ratio can influence absorbance of starch in this region and the peak intensity could be related to this ratio, the highest amylose content showed a weak peak compared to other samples. However, in Table 1 amylose content was significantly different for QP variety and therefore it presents smaller intense peaks in the FTIR spectrum, but this behavior was not observed in all samples studied. FTIR of starches exhibited complex vibrations in the peaks reported in bands smaller than 1000 cm^{-1} as a result of the skeletal mode vibration of glucose pyranose ring in accordance with other studies (Zeng et al., 2011).

3.5 Solubility and swelling power

Solubility and swelling power of both, kanihua and quinoa starches increased with the increase of temperature from 50 °C to 90 °C (Figure 2/d-e). The solubility is related to the leaching of amylose chains in solution. KC starch samples showed higher solubility than the other samples, it could be because the amylose content is

higher in KC. The increase in temperature affects the solubility proportion of starch in all samples, especially in kanihua, reaching maximum values of 8.7 - 13.2% solubility for KI and KC, respectively at 90 °C. The maximum solubility values for quinoa were in the range 5 - 8%. At temperatures from 60 to 80 °C, kanihua samples have lower swelling power; significant differences in this property were observed between kanihua and quinoa. The maximum swelling power values were reached around 70 °C, above this temperature fewer variations happened (exception QS). This behavior can be explained by the fast saturation with small increase in temperature of chemical terminations which bind water to starch.

3.6 Pasting and thermal properties

The pasting properties profile can be observed in Figure 2/f and in Table 2 were summarized data of pasting and thermal properties of starches. The maximum viscosity values were found for KC, however, the lowest viscosity values were observed for QP and QNC, these characteristics could be influenced by short starch chains (Paredes-López et al. 1994).

Table 2. Pasting and thermal properties of kanihua (K) and quinoa (Q) starches

	Parameters	KI	KC	QS	QP	QNC
Pasting temp. (°C)		79.3 ± 0.27^{a}	66.6 ± 0.22^{b}	74.3 ± 0.50c	63.0 ± 0.9^{d}	66.4 ± 0.1^{b}
Max. Peak (cp)		2558 ± 10^{a}	3448 ± 24^{b}	2672 ± 13^{a}	2510 ± 105^{a}	1785 ± 52^{c}
Breakdown (cp)		215 ± 25^{ad}	921 ± 9.5^{b}	364 ± 12^{a}	710 ± 63^{c}	152 ± 32^{d}
Setback (cp)		792 ± 14^{ac}	1426 ± 42^{b}	943 ± 21^{a}	905 ± 26^{a}	675 ± 43^{c}
Gelatini-zation	$T_{maximum}$ (°C)	63.1 ± 0.2^{a}	63.0 ± 0.2^{a}	58.7 ± 0.2^{b}	57.2 ± 0.4^{b}	59.3 ± 0.7^{b}
	T_{onset} (°C)	54.6 ± 0.5^{a}	55.7 ± 0.3^{a}	49.4 ± 0.5^{b}	48.8 ± 0.0^{b}	49.98 ± 0.7^{b}
	Range Temp (°C)	25.0 ± 1.2^{a}	22.0 ± 0.6^{a}	27.9 ± 0.6^{a}	27.2 ± 1.9^{a}	25.3 ± 0.3^{a}
	ΔH (Jg^{-1})	11.7 ± 0.3^{a}	11.6 ± 0.5^{a}	10.8 ± 0.5^{a}	12.6 ± 0.6^{a}	11.6 ± 0.0^{a}
Retrogra-dation	$T_{maximum}$ (°C)	45.2 ± 0.5^{a}	46.0 ± 0.7^{a}	46.2 ± 0.0^{a}	45.6 ± 0.6^{a}	45.0 ± 0.2^{a}
	T_{onset} (°C)	34.0 ± 0.0^{a}	35.8 ± 3.1^{a}	35.8 ± 0.1^{a}	34.8 ± 0.6^{a}	35.1 ± 0.2^{a}
	Range Temp. (°C)	29.0 ± 0.2^{a}	25.3 ± 2.7^{a}	25.3 ± 0.9^{a}	26.5 ± 1.4^{a}	26.2 ± 0.6^{a}
	ΔH (Jg^{-1})	8.5 ± 0.2^{a}	8.3 ± 0.4^{a}	6.5 ± 0.3^{a}	7.4 ± 0.3^{a}	6.8 ± 0.7^{a}
	Retrogadation (%)	73.3 ± 2.9^{a}	71.8 ± 6.6^{a}	59.8 ± 0.4^{a}	59.2 ± 5.3^{a}	58.5 ± 6.3^{a}

Mean ± SD. Mean with different superscript along the rows are significantly different ($p<0.05$). K Illpa; K Cupi; Q Salcedo; Q Passankalla; Q Negra Collana

Samples of KI, KC and QS had greater cohesive forces (hold) that means stability to temperature and shear forces. The low viscosity could be caused by possible damage produced by the alkaline solution during extraction (Qian and Kuhn, 1999). Samples of native kanihua starch had higher values T_{onset} and $T_{maximum}$ when compared to native quinoa starch. Further energy requirements were necessary to reach the melting temperature transition in kanihua starch granules. According to DSC analysis and pasting properties kanihua had a higher gelatinization temperature. Among quinoa samples, QNC has the lowest values due to the ability of its starch granules to quickly change the crystalline structure into amorphous, in addition to the lowest enthalpy variation. All gelatinized starches, after storage for 15 days at 4 °C showed similar behavior in retrogradation. The retrogradation values of kanihua (≈70%) were higher than quinoa (≈60%). Studies by López-Fernández et al. (2021) reported retrogradation values of around 35% for quinoa stored at 4 °C for 7 days. These differences can be attributed by the different varieties of the samples, alkaline extraction of the starch, and the refrigerated storage period (Velásquez-Barreto et al., 2021).

4. Conclusions

Starches extracted from quinoa and kanihua grains show predominantly spherical shape and very small size. Apparent amylose content was different between quinoa and kanihua samples, with kanihua Cupi starch presenting the highest amylose content. The structural characteristics of kanihua and quinoa starch were similar in type of polymorphism and percentage of crystallinity. FTIR spectra of kanihua and quinoa starches presented a similar behavior, despite differences in peak intensity in the band 2900 which can be attributed to differences in amylose content. These differences influence the pasting properties, solubility and swelling power of starches. Kanihua represents a non-traditional source of starch with potential comparable to quinoa, because both share similar structural characteristics and behavior of some properties.

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