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REGULAR ARTICLE

Chemical and nutritional characterization of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) and cañihua (*Chenopodium pallidicaule* Allen) are native Andean food plants of high nutritional value used as food by the Incas and previous cultures. An extensive analytical study was done on three samples for each species for all amino acids, sterols, fatty acids and mineral determination. The aim was to evaluate the chemical and nutritional characterization of cañihua and quinoa in relationship with wheat, corn, rice, rye, as sources of dietary fiber and other bioactive compounds in human and animal. *C. quinoa* and pallidicaule present an excellent nutritional value with high (14-18%) protein content, balanced amino acid composition, trace elements and vitamins and contain no gluten. This food species presented rich flavonol and triterpene glycosides fractions that include different compounds. *C. quinoa* and pallidicaule are an excellent example of functional foods that aims to prevent the risk of various diseases.

Key words: *Chenopodium quinoa*, *Chenopodium pallidicaule*, Chenopodiaceae, South-American crop, Nutritional value

Introduction

Quinoa (*Chenopodium quinoa* Willd.) and cañihua (*Chenopodium pallidicaule* Allen) are native food plants of high nutritional value grown in the Andean region and used as food by the Incas and previous cultures.

Cañihua has long been considered a variety of quinoa, cañihua in 1929 was ranked as distinct species, are annual herbaceous plants, differ in height between 20 and 60 cm for the cañihua and 2 m for quinoa, significant differences are also found in inflorescences and flowers. They are pseudograin that formed a major part of the diet of the Incas. Unlike other Andean crops such as beans, maize, and potato, quinoa and cañihua have not been cultivated in recent years on a wide scale in other countries. Recently, there has been a renewed interest in these crops. This interest is due partly because of its high (14-18%) protein content and balanced amino acid composition and partly because of the tolerance of the plant to a wide range of unfavorable climatic conditions (Rastrelli et al., 1998). These crops have a remarkable adaptability to different agro-ecological regions. They can grow at relative humidity from 40% to 88%, and withstands temperatures from -4°C to 38°C. Is tolerant and resistant to lack of soil moisture, and produces acceptable yields with rainfall of 100 to 200 mm (FAO/WHO, 2011).

Because of the execellent nutritional value, quinoa and cañihua served as a substitute for scarce animal proteins and are still one of the principal protein sources of the region. They contain all the essential amino acids, trace elements and vitamins and contain no gluten. The importance of these proteins is based on their quality, with a balanced composition of essential amino acids similar to the composition of casein, the protein of milk. They are high in lysine, considered to be deficient in most cereal grains, making their protein profile incomplete (Jacobsen et al., 1997; Valencia et al., 2009).

Andean cereals may be promising food as regard to their content of phenolic secondary

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metabolites such as polyphenols, synthesized to counteract adverse climatic and growing conditions. These metabolites are linked to the reduction of the risk of major chronic diseases when consumed via diet (Liu, 2004). For example, plant polyphenols, natural antioxidants, are ideal candidates linked to the protective effects of vegetables and fruits against cancer and cardiovascular diseases (Arts and Hollman, 2005).

The nutritive and antioxidant properties of cañihua and quinoa could be extended to animal nutrition field where several experimental studies have been conducted on the use of quinoa in feed for chickens (Jacobsen et al., 1997; Improta et al., 2001; Munoz, 1980) and pigs (Cardozo et al., 1979; Van der Peet-Schwering et al., 1993), yielding excellent results. Cardozo et al. (1979) mentioned that although the main objective in the cultivation of quinoa is the production of grain for human consumption, it has been considered a second-class grain from the by-products of a crop that can be used in feeding poultry, pigs and cattle in special conditions (Jacobsen, 2003; Rosero et al., 2010). Nevertheless, it has been reported the low uptake of quinoa by-products for livestock feed among local farming communities (Rosero et al., 2010). In turn, cañihua is mainly grown for families by their own consumption in instance of its high nutritive value (Valencia et al., 2009). Also cañihua by-products are used as forage in the Altiplano areas where the agriculture is limited, moreover, these are used as a supplement for fattening poultry such as chickens and ducks (Rosero et al., 2010).

The main producers are Bolivia, Peru and the United States, quinoa production is expanding to other continents and it is currently being cultivated in several countries in Europe and Asia with interesting yields. Quinoa and cañihua could have a significant potential in the world as a new species of cultivation imported from South America. The main uses of this Chenopodiaceae are for cooking, products for people allergic to gluten; animal feed, green fodder, and pellets; modified food products such as breakfast cereals, pasta, and cookies; industrial use of starch, protein, and saponin; and as a game-cover crop. In developing countries of Africa and Asia, quinoa may be a crop able to provide highly nutritious food in extreme conditions.

This paper deals with the relative amounts of nutrient such as carbohydrates, lipids and proteins and also the amino acid, fatty acid and sterol composition of quinoa and cañihua in relationship with major crops like wheat, corn, rice and rye.

Material and Methods

Material

C. quinoa and *pallidicaule* were supplied by Central Peruviane de Servicios and was collected in Ayavaca (D. of Piura), Peru, in 2009. A voucher specimen is deposited in the Herbario del Museo de Historia Natural "J. Prado" Un. H. S. Lima (Peru).

Methods

Moisture content was determined by slicing and powdering the seeds in a blender and drying them in an oven at 130°C to constant weight by the AOAC 934.06 method (AOAC, 2000).

Fat was determined by weighing the dichloromethane extracts. The nitrogen content was established in a Kjeldahl apparatus, following the 920.87 AOAC method; the factor N × 6.25 was used to convert nitrogen into crude protein. Amino acid contents were determined after hydrolysis with 6N HCI for 4 h at 145°C in vacuum hydrolysis tubes from Pierce (Product N. 29560) (Dini et al., 1994).

The analyses were done by reverse-phase HPLC using a system of Hewlett-Packard HP 1050 series modules with quaternary pump, an autosampler provided with injector programme, a variable wavelength detector, an HP 3396A integrator and a Spherisorb ODS-2, 5 /zm 250 mm x 4 mm column. The derivatization procedure was automated, withdrawing from different vials suitable amounts of sample and reagents OPA from Sigma (50 mg) in CH_3OH (1 ml), FMOC from Aldrich and buffer, mixing for a minute and injecting into the column [Dini et al., 1994]. The eluents were MeOH(A) and a 50 mM AcONa solution (B) (flow rate 1.0ml/min; Gradients: 18-23% A (10 min linear); 23-27.2% A (12 min linear) held at 27-2% A for 4 min; 27-2-50% A (12 min linear), held at 50% A for 10 min: 50-80% A (10 min linear), held at 80% A for 10 min).

Fatty acids were analysed as methyl esters after hydrolysis in 2N KOH by using a Hewlett-Packard 5890 apparatus and an HP-5 column, a gas chromatograph fitted with an HP 5970B mass detector (helium was used as a carrier gas, flow 6-845 kPa (10 psi) and an HP 59970 MS Chemstation. Conditions here as in Table 3.

Sterols were examined after purification on a silicagel column (eluent: CH_2C1_2) of the insaponifiable matter by gas chromatography using the same apparatus as the methyl ester; conditions as in Table 4.

Mineral ions were examined using a Varian AA-475 flame photometer and a Varian AA-475 atomic adsorption spectrophotometer.

Starch and hydrolysable carbohydrates were examined by the AOAC 948.02 method (AOAC, 1990). Ash was determined by the AOAC 923.03 method (AOAC, 1990), and the crude fiber content by the Bellucci method (Bellucci, 1932) 3 g of powder were boiled for 25 min with 50 ml of AcOH (80%) and conc. HNO_3 (45/5, v/v), filtered and, after a wash with boiling water (10 ml), ethanol (20 ml), ethyl ether (20) and boiling water to neutralize, the precipitate was dried in an oven for 3 h at 105°C, weighed, burned on flame in a crucible and re-weighed.

Statistical analysis

Three independent analyses were done on three sample for each species for all amino acids, sterols, fatty acids and mineral determination. Statistical analysis was performed by Mann - Whitney U Test.

Results and Discussion

C. quinoa and *C. pallidicaule* (quinoa and cañihua) have been cultivated as a food crop for centuries in Latin America. Still today, descendants of the Inca Empire still use its seeds as an important component in their diet, and by-products are partially used in animal nutrition. The chemical analysis showed a chemical composition in protein, lipids and carbohydrates comparable to that of the most common cereals. The carbohydrates constitute 59.9% in cañihua and 55.3% in quinoa, the lipids 7% (cañihua) and 12.4 % (quinoa), the proteins 12.8% (cañihua) and 11.7% (quinoa) (Table 1). The seeds showed high protein content and average 12-18% on a fresh basis. Moreover, this protein are of an exceptionally high quality and are particularly rich in essential amino acids, such as sulfur amino acids, lysine and aromatic amino acids, higher than those recommended by the FAO-WHO (Table 2) and which are deficient in most grain crops but necessary for proper nutrition in humans. This fact results in protein content comparable to that of whole dry milk. The seeds are nutritionally very interesting also in comparison with other cereals such as wheat, corn, rice and rye. Furthermore, the nutritive value in quinoa and cañihua by-products could provide advantage in animal performance through diet supplementation, encouraging animal health and production cualities.

The analysis of the mixture of fatty acids after saponification showed a high content of unsaturated fatty acids (71.4% for cañihua and 72.5% for quinoa), with high concentrations of linoleic acid (39.2% for cañihua and 38.9% for quinoa), the most distinctive polyunsaturated fatty acid of both seeds studied and oleic (28.6% for cañihua and 27.7% for quinoa) (Table 3). For both species polyunsaturated fatty acids were the highest, followed by monounsaturated and saturated. Some authors was also mentioned the importance of a proper relationship between saturated and unsaturated fatty acids feeding; in fact, while a high saturation may promote the onset of hepatic steatosis and were hypercholesterolemic and atherosclerotic excess unsaturation may result in harmful consequences such as liver necrosis and nutritional encephalomalacia.

The sterol fraction, which is very useful in characterising the source of vegetable oils, shows Δ7-stigmasterol (46.6% in cañihua), (43.9% in quinoa) as the main component, followed by $\Delta^{7,22}$-stigmastedienol acetate (29.4% in cañihua), β-sitosterol acetate (10.7 in cañihua) and (15.0 in quinoa) (table 4). $\Delta^{5,22(28)}$-Avenasterol was present in quinoa but not in cañihua whereas Δ^{7}-campesterol and $\Delta^{7,22}$-stigmastedienol acetate were found only in cañihua. In addition, quinoa and cañihua seeds are rich in Ca, Fe, K, (Table 5).

Table 1. Analytical Composition of *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (quinoa) seeds and other cereals*.

%	Cañihua	Quinoa	Wheat**	Corn**	Rice**	Rye**
Water	10.8 ± 0.2	14.7 ± 0.3	12.0	12.0	12.0	12.0
Proteins	12.8 ± 0.3	11.7 ± 0.2	12.2	9.2	7.4	11.1
Lipids	7.0 ± 0.2	12.4 ± 0.1	2.3	3.9	0.5	1.9
Hydrolyzable carbohydrates	59.9 ± 1.7	55.3 ± 0.6	71.8	73.7	80.0	73.1
Whole fibre	6.3 ± 0.1	2.2 ± 0.1	2.1	1.6	0.4	-
Ash	3.1 ± 0.1	3.0 ± 0.1	-	-	-	-

* Mean ± SD of three determinations; **from Documenta Geigy

Table 2. Aminoacid composition of *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (Quinoa) seeds*

Rt	Aminoacid	mg/g protein in Cañihua		mg/g protein in Quinoa		Essential aminoacid pattern (FAO-WHO)	Chemical Index Cañihua	Chemical Index Quinoa
2,8	Aspartic acid	67.5		66.8		-	-	-
3,9	Glutamic acid	169.1		16 166.7		-	-	-
9,8	Serine	36.3		38.3		-	-	-
14,2	Histidine	16.7		19.9		-	-	-
15,1	Glycine	63.5		60.9		-	-	-
15,6	Threonine	37.2		34.9		40	93	87
22,4	Arginine	87.4		84.3		-	-	-
24,5	Alanine	568		57.1		-	-	-
25	Tyrosine	29.0	} 68.8	31.3	} 74.7	60	115	125
37,8	Phenylalanine	39.8		43.4				
	Cystine	20.3	} 41.7	22.1	} 44.6	35	119	127
30,8	Methionine	21.4		22.5				
31,1	Valine	48.2		60.0		50	96	120
35,7	Isoleucine	37,5		743		41	94	81
37,8	Leucine	67.2		75.0		70	96	107
46,9	Lysine	58.3		45.8		55	106	83
	Tryptophan	n.d.		n.d.		10	n.d.	n.d.
42,9	Proline	18.4		22.6		-	-	-
Chemical score 93.3 Threonine limiting								

*Data are the means of five experiments performed in triplicate. Standard deviations were below 10 %

Quinoa is an excellent example of 'functional food', it give a significant contribution to human nutrition, protecting cell membranes, with proven good results in brain neuronal functions. In our study C. *quinoa* and *pallidicaule* presented a rich flavonol glycosides fraction that includes different compounds. They contain the aglycons of quercetin, isorhamnetin, and kaempferol and oligosaccharide moieties as disaccharides and trisaccharides linked at the C-3 position. The quantitative content (1.09 and 1.33 g/kg, respectively in quinoa and cañihua) as well as the structural variability appears to be very interesting for the alimentary and taxonomic properties ascribed to flavonol glycosides. Moreover, flavonol apiosides appears to occur frequently in glycosides of the *Chenopodiaceae* (Rastrelli et al., 1995; De Simone et al., 1990).

One of the problems associated with the use of quinoa and cañihua for production of food products is the bitter taste due to the presence of saponins, are a vast group of glycosides, the surfactant properties are what distinguishes these compounds from other glycosides.

Most saponins have haemolytic properties and are toxic to most cold-blooded animals, have pharmacological properties and used in phytotherapy, cosmetic industry and folk medicines. Animal nutritionists have generally considered saponins to be deleterious compounds. In ruminants, some saponins are considered to have detrimental effects on protozoa through their binding with sterols present on the protozoal surface; furthermore, in other domestic animals the dietary saponins have significant effects on all phases of metabolism, from the ingestion of feed to the excretion of wastes (Francis et al., 2002). Saponins can have effects on animal growth and feed intake. In turn, chickens feeded with higher levels of bitter quinoa (with saponin) have been reported a deficiency of vitamin A (Ward, 2000).

Table 3. Fatty acids as methyl ester derivatives, present in *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds.

Carbon	Fatty acids	% of methyl ester mixture in Cañihua	% of methyl ester mixture in Quinoa	Rt (min)
$C_{12:0}$	dodecanoic (lauric)	1.3		16.8
$C_{14:0}$	tetradecanoic (myristic)	1.5	1.3	17.6
$C_{15:0}$	pentadecanoic	1.2	0.4	21.4
$C_{16:1}$	9-esadecenoic (palmitoleic)	0.9	1.1	22.0
$C_{16:0}$	esadecanoic (palmitic)	22.8	24.3	22.2
$C_{18:3}$	linolenic	1.2		23.7
$C_{18:2}$	9, 12-octadecadienoic (linoleic)	39.2	38.9	24.6
$C_{18:1}$	9-octadecenoic (oleic)	29.8	27.7	24.8
$C_{18:0}$	octadecanoic (stearic)	0.6	0.8	25.2
$C_{19:0}$	nonadecanoic		0.6	26.3
$C_{19:1}$	11-nonadecenoic	0.3	0.3	26.1
$C_{20:0}$	eicosanoic (arachidic)	0.9	1.0	27.3
$C_{20:1}$	15-eicosenoic		trace	28.4
$C_{22:0}$	docosanoic (behenic)	0.3	0.7	29.1
$C_{24:0}$	tetracosanoic (lignoceric)		trace	31.5
Fatty acids:	Saturated	28.6	22.7	
	Unsaturated	71.4	72.5	
Saturated/Unsaturated ratio		0.4	0.31	

Column HP-5; 25 m × 0.2 mm; i.d., 0.33 pm film; temperature 180°C for 3 min, then to 290°C at 6°C/rain; injection temperature, 290°C; transfer line temperature 290°C; carrier gas He (6.845 kPa (10 psi)). The compounds were characterized by comparison with retention times of a reference mixture and the MS-spectra. FID area % were corrected to wt % according to total weight. Data are the means of three experiments performed in triplicate. Standard deviations were below 10%.

Table 4. Sterols as steryl acetate derivatives in *Chenopodium pallidicaule* (Cañihua) and *Chenopodium quinoa* (Quinoa) seeds.

Sterol	% of sterol mixture in Cañihua	% of sterol mixture in Quinoa	Rt (min)
Δ^5-Campesterol acetate	4.3	2.3	24.8
$\Delta^{5,22}$-Stigmasterol acetate	5.1	5.5	25.0
Δ^7-Campesterol acetate	3.8	---	28,1
$\Delta^{7,22}$-Stigmastedienol acetate	29.4	---	28.8
β- Sitosterol acetate	10.7	15.0	29.2
Δ^7-stigmasterol	46.6	43.9	30.9
$\Delta^{5,22(28)}$-Avenasterol acetate	---	21.7	

Column: HP-5, 25m x 0-2 mm; i.d., 0.33 /zm film; temperature, 290°C; injection temperature, 290°C; transfer line temperature, 290°C; carrier gas, He (6.845 kPa (10 psi)). FID area percents were corrected to wt % according to total weight. Data are the means of three experiments performed in triplicate. Standard deviations were below 10 %.

Table 5. Mineral composition of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds (mg/Kg dry wt).

Mineral	Quinoa	Cañihua	Wheat	Corn	Rice	Rye
Fe	26.1	24.7	26.4	27.2	10.1	54.3
Mg	39.2	33.6	49.0	-	-	35.1
Cu	2.1	2.6	5.2	-	-	7.2
Zn	27.0	28.3	-	-	-	-
P	4244,1	4189.2	4375.1	2981.1	1136.2	4192.1
Na	313.1	305.3	-	227.0	-	693.1
K	75.6	74.7	368.0	1363.4	897.5	5112.2
Ca	675.5	664.5	431.1	114.3	114.3	693.1

* Data are the means of three experiments performed in triplicate. Standard deviations were below 10 %

Quinoa and cañihua saponins can be divided into three different saponin groups; namely, groups containing either oleanolic acid, hederagenin, or phytolaccagenic acid as the aglycon. Previous studies (Dini et al., 2001; Woldemichael et al., 2001; Zhu et al., 2002) had determined the existence of four monodesmosidic and 22 bidesmosidic triterpene quinoa saponins based on four different aglycones (e.g., oleanolic acid, hederagenin, phytolaccagenic acid and serjanic acid). However, a recent analysis based on nano-HPLC electrospray ionization (ESI) multi-stage mass spectrometry revealed the existence of 87 triterpene saponins, comprising 19 reported and 68 novel components (Madl et al., 2006).

Quinoa and cañihua saponins concentrate in the outer husk of the grain, (8-12% w/w of the grain) which is removed before consumption to reduce the bitter taste of saponins, are considered a by-product with no commercial value. Investigations on the biological and pharmacological activities of *C. quinoa* saponins, inhibition of fungus growth, effects against viral diseases, cholesterol lowering effects, and an enhancing of mucosal drug absorption for this reason, saponin extracts of *C. quinoa* are used in agriculture for treatment, control, and prevention of fungal and viral diseases (San Martin et al., 2008).

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