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PROXIMATE ANALYSIS, PHENOLICS, BETALAINS, AND ANTIOXIDANT ACTIVITIES OF THREE ECOTYPES OF KAÑIWA (*CHENOPODIUM PALLIDICAULE* AELLEN) FROM PERU

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Abstract

Kañihua (*Chenopodium pallidicaule* Aellen, Chenopodiaceae) is a native plant from the highlands of Peru and Bolivia that is characterized by its high nutritional value. It is considered a species of great genetic diversity and in this study we evaluated the nutritional characteristics, fatty acids profile, antioxidant compounds and antioxidant activities of three kañihua ecotypes from Peru named "Chilliwa" (light grey seeds), "Planta púrpura" (light orange seeds) and "Red kañiwa Condorsaya" (red seeds).

These ecotypes of kañiwa were characterized by their high content of carbohydrates (65.5 - 68%), proteins (14.7 - 15.5%) and fat (7.6 - 8.5%). In relation to fatty acids, linoleic acid was determined as the major component (45.8 - 49.6%), followed by oleic acid; while linolenic acid was found in smaller proportion (5.7 – 6.8%). The content of total phenolics and total flavonoids were found between 1.4 – 1.9 mg-eq gallic acid/g and 1.5 - 2.0 mg-eq catechin/g, respectively. Betalains content was found between 2.3 and 42.0 mg/100 g. Antioxidant activities, measured as the concentration of sample extract (EC_{50}) that decreases by 50% the initial DPPH radical concentration, ranged from 1.3 to 4.2 mg extract/mL.

The kañihua ecotypes "Chilliwa" (light grey seeds), "Planta púrpura" (light orange seeds) and "Red kañiwa Condorsaya" (red seeds) are important sources of protein and polyunsaturated fatty acids. "Red Cañihua" and "Purple Plant" ecotypes also showed a high content of betacyanins and betaxanthines and good antioxidant activity, which supports the use of these Andean grains as functional foods

Keywords: *Antioxidant, Chenopodium pallidicaule, Kañiwa, betalains*

Introduction

Kañiwa (also known as cañihua, cañahua, cañigua, kuimi and millmi) is an annual pseudocereal that belongs to the Chenopodiadeae family (1). It is a crop that grows in the Andes on the border between Peru and Bolivia at altitudes of 3,600 to 4,400 meters above sea level. It adapts well to arid soils with intense UV radiation, with low humidity and very low temperatures (2, 3). It has been cultivated for hundreds of years by the Incas and previous pre-Columbian cultures (3,4).

Kañiwa is a close relative of quinoa (*Chenopodium quinoa*). Both species are well known for their high protein content and good amino acid profile. They are highly demanded by patients with celiac disease because they are a good source of gluten-free proteins (5, 6). Unlike quinoa, kañiwa is less bitter because it contains less saponins. Therefore, it is not necessary to wash kañiwa seeds before consuming them (7). Kañiwa seeds are usually toasted and ground to obtain a flour known as "Kañiwaco" that is consumed mixed with water, broth or milk (3).

Kañihua is considered a species of great genetic diversity, the main classifications of this species are based on differences in size, shape and colors of plant and seeds. It is a branched herbaceous plant with a height between 20 and 70 cm and a vegetative period between 105 and 195 days (8). The diameter of the seeds measures between 1.0 and 1.2 mm and its color varies from yellow, orange and brown to black (9).

Kañiwa is considered one of the strategic components of food security, from which innovative products could be developed in the food industry. In this sense, it is necessary to know the chemical and functional differences between the various ecotypes that are currently cultivated. The objective of this study was to determine the nutritional/chemical profiles and antioxidant activities of three selected ecotypes of kañihua: "Chilliwa" (light grey seeds), "Purple plant" (light orange seeds) and "Red kañihua, Condorsaya" (red seeds). These ecotypes are grown in the region of Puno, Peru, and differ mainly by the color of their seeds.

Methods

Plant material

The ecotypes of kañihua "Chilliwa" (light grey seeds), "Purple plant" (light orange seeds) and "Red kañihua, Condorsaya" (red seeds) used for this study were provided by the specialist Jorge Torres from the Viluyo farm, Ayaviri District, Melgar Province, Puno, Perú. Kañihua samples were identified by Dr. Mario E. Tapia (Universidad Global del Cusco).

Dried kañiwa seeds were ground using a coffee grinder (BOSCH - Model MKM6003).

Proximate analysis

Proximate analysis of samples was carried out according to the standard methods of analysis of the Association of Official Analytical Chemists (10). Moisture contents of the samples were determined by desiccation at 105 °C until constant weight. Total fat was extracted with hexane by Soxhlet method. Nitrogen content estimated by Kjeldahl method was converted to protein content by using the conversion factor 6.25. Crude fiber content was determined by acid base digestion, and the ash content was obtained by incineration in a muffle furnace at 550 °C. Total amount of carbohydrates was calculated by difference. All determinations were carried out in three replicates.

Fatty acid profiles

The fatty acid profiles of kañiwa samples were determined on a gas chromatography-mass spectrometry equipment (Agilent 7890A, 5975C). One hundred miligrams of fat extracted from kañiwa samples were esterified with a 2 N KOH methanolic solution and the methyl esters obtained were separated by means of a gas chromatography column Agilent J&W 122-7063 DB-WAX (60 m x 0.25 mm x 0.50 µm) (11). The operating conditions of the gas chromatograph were: detector temperature 220 °C; injector temperature 250 °C; split ratio: 20:1; furnace temperature 120 °C; 120-175 °C (5 °C/ min), 175-210 °C (2 °C/ min); total run time: 68.5 min. An aliquot of 5 µL of the samples was injected into the apparatus. Helium (1 mL/min) was used as carrier gas. Fatty acid determination was carried out by comparing the peak retention times with the respective standards of fatty acid methyl esters and by comparison of their mass spectra with the NIST MS library.

Total phenolics

Total phenolics content (TPC) was determined by the Folin–Ciocalteu method according to Shotorbani *et al*. (12), with some modifications. The analysis was carried out in three replicates. Two grams of defatted sample were extracted three times for 30 min under sonication with 15 mL 80% ethanol. The extract was centrifugated (4 °C, 5000 rpm for 15 min) and the supernatant was made up to a final volume of 25 mL in a volumetric flask. Fifty-microliter aliquots of sample extract were mixed with 1 mL Folin-Ciocalteu (1/10) reagent. The mixture was incubated for 2 min, followed by the addition of 1 mL of Na_2CO_3 (7.5%) solution and incubated in the dark at room temperature for 15 min. The absorbance of the reaction mixture was measured at 750 nm using a spectrophotometer. TPC of samples were calculated by means of a gallic acid calibration curve. TPC was expressed as g of gallic acid equivalents (GAE) per 100 g of sample.

Total flavonoids

Flavonoid content was determined according to Ivanova *et al*. (13), with some modifications. Two grams of sample was extracted with 20 mL 80% methanol. After filtration, the extract was made up to a final volume of 25 mL in a volumetric flask. A 0.5 mL of sample extract was mixed with 1.5 mL distilled water and 0.15 mL $NaNO_2$ solution (0.05%). After 5 min, 0.15 mL of a 0.1% $AlCl_3$ solution was added and 6 min later, 1 mL of NaOH (1 M) was also added to the mixture. The total volume of the solution was made up to 5 mL with distilled water and the absorbance was measured at 510 nm using a spectrophotometer. Catechin was used for the construction of a calibration curve and the total flavonoids concentrations were expressed as milligrams catechin equivalents per g of sample (mg CAT/g sample).

Betalain content

The spectrophotometric method described by von Elbe (14) was used to determine the betalain content in kañiwa samples. Total content of pigmented components (betalains) was expressed as the sum of betacyanins and betaxanthins. Concentrations of betacyanins and betaxanthins were calculated in terms of betanin and vulgaxanthin-I, respectively.

0.2 g of sample was weighed in a Falcon tube, 12 mL of pH 6.5 phosphate buffer was added and the mixture stirred in the ultrasound equipment for 30 min in the dark. Then, the Falcon tube was centrifuged at 5000 rpm (4 °C, 15 min). The supernatant was filtered through a 0.45 µm Phenomenex filter. Finally, the absorbance of the solution was measured in a UV-Vis spectrophotometer (Spectroquant® Pharo 300) at a wavelength of 538, 476 and 600 nm.

The following equations were used to calculate the corrected light absorption of betanin and vulgaxanthin-I: x= 1.095 * (a - c); y= b – z – x/3.1; z= a – x. Where: a= light absorption of the sample at 538 nm; b= light absorption of the sample at 476 nm; c= light absorption of the sample at 600 nm; x= light absorption of betanin minus the colored impurities; y= light absorption of vulgaxanthin-I corrected for the contribution of betanin and colored impurities; z= light absorption of the impurities.

The concentrations (mg/100 g) of betanin and vulgaxanthin-I were calculated according to the following formula: C= (p * F * 12000)/ ($A^{1\%}$ * M); where p is "x" for betanin or "y" for vulgaxanthin-I; F is the dilution factor and $A^{1\%}$ is the absorptivity value (1120 for betanin and 750 for vulgaxanthin-I).

The content of betacyanins and betaxanthins were reported as mg betanin/100 g sample and mg vulgaxanthin-I/100 g sample, respectively.

Antioxidant activity

The antioxidant activity was determined according to the method of Othman *et al*. (15), with some modifications. One gram of defatted sample was extracted three times with 5 mL 80% ethanol. After filtration, the extract was made up to a final volume of 25 mL in a volumetric flask. An aliquot of kañiwa extract (50 µL, 0.063–0.63 mg/mL in 80% ethanol) was mixed with 0.1 mM DPPH (2,2-Diphenyl-1-picrylhydrazyl). The mixture was left to stand for 30 min in the dark at room temperature. Afterwards, the absorbance was read in a spectrophotometer at 517 nm using ethanol (80%) as a blank control. DPPH radical scavenging activity of each sample was calculated according to the following formula: DPPH inhibition (%) = 100*($Abs_{control}$ - Abs_{sample})/$Abs_{control}$. EC_{50} value (concentration of sample extract that decreases by 50% the initial DPPH radical concentration) was

calculated by plotting the curve of DPPH inhibition (%) against the concentration of sample extracts.

Results

The proximate chemical analysis (dry basis) of the three kañihua ecotypes shows a composition rich in carbohydrates (65.5 - 68.0%), followed by proteins (14.7 - 15.5%) and lipids (7.6 - 8.5%) (Table 1).

The main fatty acids in the three kañihua ecotypes are linoleic acid (45.8-49.6%), oleic acid (25.8-27.9%), palmitic acid (12.9-13.5%) and linolenic acid (5.7-6.8%) (Table 2).

Table 3 summarizes the content of total phenolic compounds expressed in mg-eq of gallic acid per gram of sample (mg-eq AG/g) and the total flavonoid content expressed in mg-eq of catechin per gram of sample (mg-eq CAT/g). The levels of total phenolic compounds in the three ecotypes of kañihua vary between 1.4 and 1.9 mg-eq gallic acid/g, while the total flavonoid content fluctuates between 1.5 and 2.0 mg-eq catechin/g.

Total values of betalains of our three kañiwa ecotypes ranged between 2.3 and 42.0 mg/100 g. The ecotype "Purple plant" showed the highest content of betaxanthins (36.3 mg/100 g), which explains the orange color of its seeds. In turn, "Red Kañiwa" ecotype, which has a red-violet color, was rich in betacyanins (14.8 mg/100 g) and betaxanthins (27.1 mg/100 g) (Table 3).

The antioxidant capacity was determined by the DPPH test (2,2-diphenyl-1-picrylhydrazyl), expressing the results as the EC_{50} value (concentration of the test sample that produces a 50% inhibition of the DPPH free radical). Kañihua samples showed EC_{50} values between 1.3 and 4.2 mg extract/ml (Table 3).

Discussion

The proximate chemical analysis results of the three kañihua ecotypes were compared with a study conducted by De Bruin (16), which reports for kañiwa similar values of carbohydrates (67.6%), proteins (16.9%) and lipids (8.8%). A similar protein content (15.2%) was found by Repo-Carrasco *et al.* (17), which was obtained as an average of five kañihua ecotypes from Puno and Cusco.

The three kañihua ecotypes are a good source of proteins and unsaturated fatty acids. A study conducted by Salas *et al.* (18) to other two ecotypes of kañihua from Puno showed very similar values for linoleic acid (46.9-48.5%), oleic acid (24.8-25.9%) and linolenic acid (5.3-5.7%). Gallego *et al.* (19) reported for kañiwa a lower value of total unsaturated fatty acids (71.4%) compared to the ones we obtained in the three ecotypes studied (>83%) (Table 2).

A study conducted by Wood *et al.* (20) to three quinoa (*Chenopodium quinoa*) cultivars indicates, on average, a lower content of saturated fatty acids (11.3%) than our kañihua samples (16.5%). Several studies argue that a reduction in saturated fatty acid consumption decreases the risk of cardiovascular disease (21,22). In this context, both species (*Chenopodium quinoa* and *Chenopodium pallidicaule*) could be added to a balanced diet regimen due to their low saturated fatty acid content.

In a study of ten kañihua ecotypes from Bolivia, Peñarrieta *et al.* (1) report a higher content of total phenolics (2.1 - 8.0 mg gallic acid/g) and a similar content of total flavonoids (0.6 - 3.1 mg catechin/g) compared to the values found in the three ecotypes of this study. Repo-Carrasco *et al.* (2010) report total flavonoids values which fluctuates between 0.6 and 1.4 mg catechin/g. Quercetin is indicated as the flavonoid with the highest concentration (0.4-0.6 mg/g) for both species (quinoa and kañiwa) (17).

Betalains are water-soluble natural pigments derived from the amino acid tyrosine. They can be subdivided as betacyanins, which provide red-violet tones; or as betaxanthins that provide orange-yellowish color (23). Compared to quinoa, red kañiwa ecotype has a higher concentration of betalains. For example, a study carried out by Abderrahim *et al.* (24) on thirteen colored ecotypes of quinoa (*Chenopodium quinoa*) from Puno revealed lower values of betacyanins (0.2 - 5.2 mg/100 g) and betaxanthines (0.0 - 1.6 mg/100 g) than the ones reported here for the red kañiwa ecotype.

The antioxidant activity was superior in the ecotypes that presented the red and orange color ("Red Cañihua" and "Purple Plant", respectively), which in turn were the ones with the highest concentration of betalains, flavonoids and total phenolics. The antioxidant activity in the DPPH test is proportional to the concentration of hydrogen donor species, which may be present in the three chemical groups mentioned (25).

The kañiwa ecotypes of the present study are important sources of protein and polyunsaturated fatty acids. Moreover, "Red Cañihua" and "Purple Plant" ecotypes stand out for their high content of betacyanins and betaxanthines and good antioxidant activity, which supports the use of these Andean grains as functional foods.

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References

1. Peñarrieta JM, Alvarado JA, Åkesson B, Bergenståhl. Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (*Chenopodium pallidicaule*): An Andean pseudocereal. Mol. Nutr. Food Res. 2008; 52: 708-717.
2. Bartolo D. Propiedades nutricionales y antioxidantes de la Cañihua (*Chenopodium pallidicaule* Aellen). Rev. Inv. Univ. 2013; 2(1): 47-53.
3. Gade DW. Ethnobotany of Cañihua (*Chenopodium pallidicaule*), rustic seed crop of the Altiplano. Econ. Bot. 1970; 24(1): 55-61.
4. IPGRI, PROINPA, IFAD. 2005. Descriptores para cañahua (*Chenopodium pallidicaule* Aellen). Instituto Internacional de Recursos Fitogenéticos, Roma, Italia; Fundación PROINPA, La Paz, Bolivia; International Fund for Agricultural Development, Roma, Italia..
5. Ramos Diaz JM, Kirjoranta S, Tenitz S, *et al*. Use of amaranth, quinoa, and kañiwa in extruded corn-based snacks. J. Cereal Sci. 2013; 58(1): 59-67.
6. Zegarra S, Muñoz AM, Ramos-Escudero F. Elaboration of a gluten-free bread based on cañihua (*Chenopodium pallidicaule* Aellen) flour and sensory acceptability evaluation. Rev. Chil. Nutr. 2019; 46(5): 561-570.
7. Betalleluz-Pallardel I, Inga M, Mera L, Pedreschi R, Campos D, Chirinos R. Optimisation of extraction conditions and thermal properties of protein from the Andean pseudocereal cañihua (*Chenopodium pallidicaule* Aellen). Int. J. Food Sci. 2017; 52(4): 1026-1034.
8. Rojas W, Soto JL, Pinto M, Jager M., Padulosi S. 2010. Granos Andinos. Avances, logros y experiencias desarrolladas en quinua, cañahua y amaranto en Bolivia. Bioversity International, Roma, Italia.
9. Rodríguez JP, Aro M, Coarite M, *et al*. Seed shattering of cañahua (*Chenopodium pallidicaule* Aellen). J. Agro. Crop. Sci. 2017; 203: 254-267.
10. AOAC. (2010). Official Methods of Analysis of the AOAC. Association of Official Analytical Chemists. AOAC International. 2010. Official method of analysis of A.O.A.C International. 18th Edition, Revision 3.
11. Ichihara K, Shibahara A, Yamamoto K, Nakayama T. An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 1996; 31(5): 535-539.
12. Shotorbani N, Jamei R, Heidari R. Antioxidant activities of two sweet pepper *Capsicum annuum* L. varieties phenolic extracts and the effects of thermal treatment. Avicenna J. Phytomed. 2013; 3(1): 25-34.
13. Ivanova V, Stefova M, Chinnici F. Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. J. Serb. Chem. Soc. 2010; 75(1): 45-59.
14. Von Elbe J. (2001). Miscellaneous Colorants. F3.1. Betalains. In: Handbook of Food Analytical Chemistry: Pigments, Colorants, Flavors, Texture, and Bioactive Food Components Edited by Ronald E. Wrolstad, Terry E. Acree, Eric A. Decker, Michael H. Penner, David S. Reid, Steven J. Schwartz, Charles F. Shoemaker, Denise Smith, Peter Sporns. 2005 John Wiley & Sons, Inc. 1-7.

15. Othman A, Ismail A, Ghani N, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem. 2007; 100: 1523-1530.
16. De Bruin A. Investigation of the food value of quinua and cañihua seed. J. Food Sci. 1964; 29(6): 872-876.
17. Repo-Carrasco R, Hellstrom J, Pihlava JM, Mattila P. Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*). Food Chem. 2010; 120: 128-133.
18. Salas L, Tapia D. Menegalli F. Biofilms based on canihua flour (*Chenopodium pallidicaule*): Design and characterization. Quim. Nova. 2015; 38(1): 14-21.
19. Gallego D, Russo L, Kerbab K, Landi M, Rastrelli L. Chemical and nutritional characterization of *Chenopodium pallidicaule* (cañihua) and *Chenopodium quinoa* (quinoa) seeds. Emir. J. Food Agric. 2014; 26(7): 609-615.
20. Wood S, Lawson L, Fairbanks D, Robison L, Andersen W. Seed lipid content and fatty acid composition of three quinoa cultivars. J. Food Compos. Anal. 1993; 6: 41-44.
21. Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids 2010; 45: 893-905.
22. Lichtenstein A, Appel L, Brands M, *et al*. Diet and Lifestyle Recommendations Revision 2006: A Scientific Statement from the American Heart Association Nutrition Committee. Circulation 2006; 114: 82-96.
23. García L, Salinas Y, Valle S. Betalaínas, compuestos fenólicos y actividad antioxidante en Pitaya de Mayo (*Stenocereus griseus* H.). Rev. Fitotec. Mex. 2012; 35(5): 1-5.
24. Abderrahim F, Huanatico E, Segura R, Arribas S, Gonzalez MC, Condezo-Hoyos L. Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (*Chenopodium quinoa* Willd.) from Peruvian Altiplano. Food Chem. 2015; 183: 83-90.
25. Kedare S, Singh R. Genesis and development of DPPH method of antioxidant assay. J. Food Sci. Technol. 2011; 48(4): 412-422

Table 1. Proximate analysis of three kañiwa ecotypes

Component	Content (%)		
	"Chilliwa" (light grey seeds)	"Purple plant" (light orange seeds)	"Red kañiwa Condorsaya" (red seeds)
Protein	15.3 ± 0.3	15.5 ± 0.4	14.7 ± 0.3
Fat	8.5 ± 0.3	8.0 ± 0.1	7.6 ± 0.1
Fiber	5.6 ± 0.2	7.0 ± 0.4	6.0 ± 0.5
Ash	4.6 ± 0.1	4.0 ± 0.1	3.7 ± 0.1
Carbohydrates	66.0 ± 0.4	65.5 ± 0.6	68.0 ± 0.4

Table 2. Fatty acid profiles of three kañiwa ecotypes

Fatty acid	**Content (%)**		
	"Chilliwa" (light grey seeds)	**"Purple plant" (light orange seeds)**	**"Red kañiwa Condorsaya" (red seeds)**
Myristic (C 14:0)	0.2	0.2	0.2
Palmitic (C 16:0)	13.0	12.9	13.5
16-methyl-heptadecanoic (C 17:0)	0.4	0.4	0.4
Stearic (C 18:0)	1.9	2.0	1.6
Oleic (C 18:1)	27.9	27.8	25.8
Elaidic (C 18:1)	1.6	1.4	1.3
Linoleic (C 18:2)	46.6	45.8	49.5
Linolenic (C 18:3)	5.9	6.8	5.7
Araquidic (C 20:0)	1.0	1.0	0.7
cis-11-eicosenoic (C 20:1)	1.5	1.7	1.3
Saturated	16.5	16.5	16.4
Monounsaturated	31.0	30.9	28.4
Polyunsaturated	52.5	52.6	55.2

Table 3. Total phenolics, total flavonoids, betalains and antioxidant activities of three kañiwa ecotypes

Component	Content (%)		
	"Chilliwa" (light grey seeds)	"Purple plant" (light orange seeds)	"Red kañiwa Condorsaya" (red seeds)
Total phenolics (mg-eq AG/g)	1.4 ± 0.0	1.7 ± 0.1	1.9 ± 0.1
Total flavonoids (mg-eq CAT/g)	1.5 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Betacyanins (mg Betanin/100 g)	0.7 ± 0.1	3.3 ± 0.0	14.8 ± 0.3
Betaxanthins (mg Vulgaxanthin-I/100 g)	1.6 ± 0.2	36.3 ± 0.7	27.1 ± 0.4
Antioxidant activity (EC_{50}, mg extract/mL)	4.2 ± 0.0	1.4 ± 0.0	1.3 ± 0.0