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Research Article

Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (*Chenopodium pallidicaule*): An Andean pseudocereal

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Total antioxidant capacity (TAC), total phenolic compounds (TPH), total flavonoids (TF) and individual phenolic compounds were determined in canihua collected at approx. 3850 m altitude. The TAC values varied among samples from 2.7 to 44.7 by the ferric reducing antioxidant power (FRAP) method and from 1.8 to 41 by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method expressed as μmol of Trolox equivalents/g dw. The content of TPH was 12.4–71.2 μmol gallic acid equivalents/g dw and that of the TF ranged between 2.2 and 11.4 μmol of catechin equivalents/g dw. The data obtained by the four methods showed several significant correlations. Prior to analysis by HPLC, the samples were subjected to acid hydrolysis and in the water-soluble extracts this led to an up to 20-fold increase in the TAC values in comparison with the values of the nonhydrolysed samples. HPLC analysis showed the presence of eight major compounds identified as catechin gallate, catechin, vanillic acid, kaempferol, ferulic acid, quercetin, resorcinol and 4-methylresorcinol. Their estimated contribution to the TAC value (FRAP method) indicated that resorcinols contributed most of the antioxidant capacity of the water-soluble extract. The results show that canihua is a potential source of natural antioxidant compounds and other bioactive compounds which can be important for human health.

Keywords: Antioxidants / Bolivia / Canihua / Flavonoids / Phenolic compounds

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1 Introduction

The vast altiplano at 4000 m above sea level is a main agricultural region of Bolivia. The intense UV radiation, seasonal drought and occasional frost have led to the development of an agricultural system based on crops resistant to these conditions like quinoa, potato, papalisa and oats. Canihua, also named cañihua, kuimi, millmi, and cañahua [1] (*Chenopodium pallidicaule*), is an annual pseudocereal belonging to the *Chenopodiaceae* family, found in semidesert climates at 3600–4400 m altitude. It is usually grown as a secondary spontaneously seeding crop in quinoa fields of the traditional agricultural system and thus requires only minimal care. It is not demanding in terms of fertilization and irrigation and may develop even on depleted soils. However, the crop is laborious to harvest, gives a poor yield and its use is decreasing. It is a starch-rich pseudocereal somewhat similar to quinoa in its use and it is mixed with wheat and used for making breads and cakes or used alone in beverages and gruel-like foods of the local culinary heritage.

The few available studies on the nutritional quality and chemical composition of canihua have shown it to have a crude composition of carbohydrate 63–66%, protein 15–18%, lipids 6–8% and ash 3–4% and a high protein quality with a content of lysine of 5–6% [2, 3]. In other studies,

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Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **CE,** catechin equivalents; **FRAP,** ferric reducing antioxidant power; **GAE,** gallic acid equivalents; **TAC,** total antioxidant capacity; **TF,** total flavonoid

Table 1. Description of the canihua samples collected in Bolivia

Sample	Colour of plant	Altitude	Coordinates	Place	Dry matter (%)
Bel1	Yellow	3838	19532550E8228746N	Belén	66
Bel2	Yellow	3838	19532550E8228746N	Belén	79
Bel3	Yellow	3838	19532550E8228746N	Belén	62
Bel4	Red	3838	19532550E8228746N	Belén	74
Bel5	Green	3838	19532550E8228746N	Belén	90
Bel6	Yellow	3838	19532550E8228746N	Belén	90
SiSi1	Green	3925	19543396E8212681N	Sipa Sipa	41
SiSi2	Purple	3925	19543396E8212681N	Sipa Sipa	39
Tiw1	Yellow	3861	19533943E 8167646N	Tiwanaku	78
Tiw2	Purple	3861	19541046E8165873N	Tiwanaku	91

two new flavonol glycosides and new triterpene saponins from canihua seeds were isolated [4, 5], and preliminary data on the total antioxidant capacity (TAC) of canihua flour were gathered in an initial survey of Bolivian foods [6]. More systematic knowledge of the content of flavonoids and other phenolic compounds is lacking.

Since the canihua can grow under extreme radiation conditions, close to the equator, at high altitude and in a climate dominated by clear skies, the plant has probably developed a good natural protection against oxidation. Thus, canihua can be an interesting potential source of polyphenolic and other antioxidants. Hence, the present work reports measurement of the TAC, phenolic content, flavonoid content and individual phenolic compounds in canihua plants collected at different locations.

2 Materials and methods

2.1 Chemicals

The Folin-Ciocalteu reagent, gallic acid, sodium carbonate, sodium nitrite (99%), aluminium chloride hexahydrate (97%), acetone (p.a.) were purchased from Merck (Darmstadt, Germany), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)], baicalein (98%), catechin (99%), catechin gallate (99%), kaempferol (99%), quercetin (99%), potassium persulphate, resorcinol (99%), 4-methylresorcinol (99%), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%), TPTZ (2,4,6-tripyridyl-*s*-triazine) and vanillic acid (99%) were obtained from Sigma – Aldrich (St. Louis, USA), ferric chloride from ICN Biomedicals (Costa Mesa, CA, USA), acetic acid (glacial p.a.) and sodium acetate from BDH Chemicals (Poole, UK). Ferulic acid was obtained from Extrasynthèse (Genay, France) and methanol HPLC grade from Laboratory Supplies (Poole, UK).

2.2 Plant material

Ten samples of canihua were collected in April 2005 at altitudes ranging from 3839 to 3925 m above sea level from an experimental farm site, Belén, and two regular farms, Sipa Sipa and Tiwanaku, close to Titicaca Lake, Bolivia. Plants at different locations within the farms and with different colours were collected (Table 1). Three samples (sample Bel5, Bel6 and Tiw2) had already been harvested a few days before the collection date, while the rest were collected directly from the field. The samples were stored cool in plastic bags for 24 – 48 h and then transferred to a freezer (−20°C) until extracted.

2.3 Sample preparation

The fresh leaves, stems and seeds were homogenized in a mixer with sodium acetate buffer (0.1 M, pH 5.0) in a liquid/sample ratio of 25:1. The samples were centrifuged in a Thermo IEC Multi/RF with an 8850 rotor at 20 000 × *g* for 30 min at 4°C. The supernatant was aspirated and stored at −80°C before being analysed. One gram of the remaining pulp was homogenized with 8 mL of acetone and was stirred for 30 min at room temperature. Then the mixture was centrifuged for 10 min at 1200 × *g* and room temperature and the supernatant solution was separated and stored at −80°C [7].

2.4 Measurement of TAC

TAC was measured by the ABTS method [8] and a modification of the ferric reducing antioxidant power (FRAP) method [9] by use of spectrophotometry performed using an Ultrospec 3000 (Pharmacia Biotech, Uppsala, Sweden) at 25°C. As a standard Trolox was used, a water-soluble analogue of alpha-tocopherol. A stock solution containing 5 mmol/L of Trolox in ethanol was stored at −20°C.

2.4.1 The ABTS method

The colourless ABTS (7 mmol/L) was oxidized to the green $ABTS^{\bullet+}$ radical cation by the addition of potassium persulphate (2.42 mmol/L) and kept for 12 – 16 h at room temperature in the dark. The reagent was stable for 2 – 3 days when stored in the dark. On the day of analysis the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70

(±0.02) at 734 nm. After the addition of 1.0 mL of $ABTS^{\bullet+}$ solution to 100 μL of sample the mixture was stirred for 30 s and the absorbance at 734 nm and 25°C was recorded for 6 min. The decrease in absorbance caused by the addition of sample was compared with that of a standard curve made by use of Trolox (20–200 μmol/L).

2.4.2 The FRAP method

The yellow Fe^{3+}-TPTZ complex is reduced to the blue Fe^{2+}-TPTZ complex by electron donating substances under acidic conditions. Any electron donating substance with a half reaction of lower redox potential than Fe^{3+}/Fe^{2+} TPTZ will drive the formation of the blue complex forward. The FRAP reagent was a mixture of 0.1 mol/L sodium acetate buffer (pH 3.6), 10 mmol/L TPTZ and 20 mmol/L ferric chloride (10:1:1 v/v/v). To 900 μL of reagent 90 μL of water and 30 μL of sample were added. The absorbance readings were performed at 593 nm for 10 min. The blank consisted of 120 μL of water and 900 μL of reagent. The final absorbance of each sample was compared with that of a standard curve made using Trolox (100–1000 μmol/L). The data were expressed as μmol Trolox equivalents *per* gram of dry matter. To assess the TAC of reference compounds these compounds were dissolved in ethanol at 25–180 μmol/L.

2.5 Measurement of total phenolic compounds

The total phenolic compounds (TPH) were determined using the Folin-Ciocalteu reagent which oxidizes the phenolic compounds to phenolates at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum–tungsten complex [10]. The Folin-Ciocalteu reagent, diluted ten times (2.5 mL) and 2 mL of saturated sodium carbonate (75 g/L) and 50 μL of sample (diluted ten times) were mixed for 10 s and heated for 30 min at 45°C. The absorbance at 765 nm was read after cooling to room temperature. The absorbance of each sample was compared with those obtained from the standard curve made from gallic acid (235–1176 μmol/L). The data were expressed as mmol gallic acid equivalents (GAE) *per* gram of dry matter.

2.6 Measurement of total flavonoids (TF)

The TF content was determined using a reagent containing aluminium chloride and sodium nitrite, giving rise to a pink-coloured flavonoid–aluminium complex in the alkaline medium [11]. A solution corresponding to 30 μL of sodium nitrite (10%), 60 μL of aluminium chloride hexahydrate (20%), 200 μL of NaOH (1 M) and 400 μL of water was added to 100 μL sample. The absorbance readings at 510 nm were started 5 min after the addition of the sample, and were performed every 20 s for 1 min. A reagent blank containing water instead of sample was used. The final absorbance of each sample was compared with a standard curve made from catechin (69–689 μmol/L). The data were expressed as μmol catechin equivalents (CE) *per* gram of dry matter.

2.7 HPLC

Before the HPLC analysis the water-soluble extract was refluxed in 1.5 M HCl for 90 min, while the water-insoluble extracts were evaporated in a nitrogen stream and reconstituted in methanol before the reflux. Baicalein was added as an internal standard before the hydrolysis. Phenolic compounds were separated using a Shimadzu liquid chromatograph system (LC 10ADVP), comprising a vacuum degasser (DGU 14-A), a solvent delivery module (FCV-10ALVP), an auto-injector (SIL-10ADVP), a column oven (CTO-10ASVP) and a diode-array detector (SPD-M10AVP). The column was a 3.5 μm Kromasil RP column 150 mm × 4 mm protected by a Kromasil C18 10 mm precolumn (Scantec Lab, Sävedalen, Sweden). The flow rate was 0.8 mL/min and the injection volume was 20 μL. The mobile phase was a binary solvent system consisting of (A) 1% acetic acid/water and (B) methanol and the gradient used was 0 min 40% B, 5 min 65% B, 10 min 90% B, 15 min 40% B until 17 min as modified from ref. [12]. The UV absorbance at 260, 280 and 330 nm was recorded in the eluate. The compounds were identified by comparing with standards of each identified compound using retention time, the absorbance spectrum profile and also by running the samples after the addition of pure standards. The chromatographic and spectral features of the standards are shown in Table 2. The concentrations were calculated from the peak heights of the internal standard and each compound in the samples and in reference solutions.

Table 2. Molar TAC values, and chromatographic and spectral features of substances tentatively identified in canihua

Substance	R_t (min)	Maxima in UV–Vis spectrum (nm)	Molar TAC (μmol/μmol Trolox)	
			ABTS	FRAP
Catechin	2.4	278	3.2	1.4
Resorcinol	3.3	275	1	0.0
Catechin gallate	3.9	278	2.6	3.1
Vanillic acid	4.3	260, 292	0.09	0.9
4-Methylresorcinol	5.2	279	1.7	1.04
Ferulic acid	5.7	295, 330	1.6	1.37
Quercetin	8.9	255, 370	3.1	3.7
Kaempferol	10.1	265, 365	0.45	0.95

The molar TAC of the reference compounds was measured in solutions in ethanol at 25–180 μmol/L.
R_t, retention time.

2.8 Dry matter content

The dry matter content was determined in triplicate after drying approximately 2 g of canihua sample at 100°C to constant weight.

2.9 Statistical analysis

The results were expressed as mean values (SD) of six replicates measured over three days for TAC, FRAP, TPH and TF. Linear correlation coefficients were calculated according to the Pearson method. All calculations were done using Excel software.

3 Results

3.1 TAC in canihua

The highest TAC value by the ABTS method was observed in sample Tiw2 both in the water-soluble and water-insoluble extracts, 37 and 3.9 μmol of Trolox/g dw, respectively (Table 3). Several intermediary values of 1.4 – 5.5 μmol/g dw were found in the water-soluble extracts and of 0.9 – 2.3 μmol/g dw in the water-insoluble extracts. The lowest values in the water-soluble and water-insoluble extracts were observed in sample Bel2, 1.3 and 0.5 μmol/g dw, respectively.

Also by the FRAP method the highest TAC value was observed in sample Tiw2 in both extracts (38 and 6.7 μmol of Trolox/g dw, respectively). The intermediary values were between 2.3 and 15 μmol/g dw in the water-soluble extracts and from 0.7 to 3.4 μmol/g dw in water-insoluble extracts while the lowest values were determined in the water-soluble extract of sample Bel2 (2 μmol/g dw) and in the water-insoluble extract of sample Bel3 (0.6 μmol/g dw). It is apparent that the Tiw2 sample had a very high content of antioxidants and it was excluded from the ranges and summary measures calculated in this report.

3.2 Total phenolic substances and TF

The highest TPH values were observed in the sample Tiw2 in both extracts (Table 4). Intermediary values were found between 12.3 and 43.5 μmol GAE/g dw in the water-soluble extracts, and 1.8 and 3.5 in the water-insoluble extracts. The lowest values were determined in the water-soluble extract of sample Bel2 (10 μmol GAE/g dw), and in the water-insoluble extract of sample Bel3 (1.2 μmol mg GAE/g dw).

The highest value assessed with the TF method was observed in the water-soluble extract of sample Tiw2 (11 μmol CE/g dw) and in the water-insoluble extract of the sample SiSi1 (8.3 μmol CE/g dw). Values between 2 and 5.2 μmol CE/g dw in the water-soluble extract and from 1.4 to 5.9 in the water-insoluble extract were found as intermediary values and the lowest values were determined in the water-soluble extracts of samples Bel2 and Tiw1 (1.8 μmol CE/g dw) and in the water-insoluble extracts of samples Bel2 and Tiw2 (0.4 μmol CE/g dw).

3.3 Content of individual phenolic substances

Eight phenolic compounds in the canihua extracts were found by the HPLC method. Resorcinol, 4-methylresorcinol, ferulic acid, kaempferol and quercetin were found both in the water-soluble and water-insoluble extracts while catechin and vanillic acid were only found in the water-soluble extract and catechin gallate only in the water-insoluble extract (Table 5). The content of each compound expressed

Table 3. TAC in water-soluble and water-insoluble extracts of canihua as measured by the ABTS and FRAP methods before and after acid hydrolysis

Sample code	ABTS				FRAP			
	Water-soluble		Water-insoluble	Water-soluble + water-insoluble	Water-soluble		Water-insoluble	Water-soluble + water-insoluble
	Before[a)]	After[a)]			Before[a)]	After[a)]		
Bel1	5.5 (0.8)	23.3 (1.2)	2.3 (0.3)	7.8	15 (2.5)	34.4 (1.2)	3.1 (0.77)	18.1
Bel2	1.3 (0.4)	11.0 (0.4)	0.5 (0.1)	1.8	2.0 (0.2)	13.2 (0.9)	0.7 (0.08)	2.7
Bel3	3.0 (0.6)	17.6 (0.8)	0.9 (0.1)	3.9	2.3 (0.5)	20.8 (0.8)	0.6 (0.06)	2.9
Bel4	4.4 (0.4)	21.6 (2.7)	1.3 (0.2)	5.7	13 (0.9)	32.3 (1.9)	1.9 (0.24)	14.9
Bel5	4.6 (0.2)	18.7 (1.9)	0.9 (0.2)	5.5	12 (0.4)	9.4 (4.2)	1.8 (0.28)	13.8
Bel6	4.2 (0.1)	20.8 (1.2)	1.4 (0.2)	5.6	15 (2.0)	30 (2.4)	2.2 (0.09)	17.2
SiSi1	2.0 (0.3)	11.7 (1.5)	1.7 (0.3)	3.7	4.5 (0.3)	21.8 (4.1)	3.4 (0.17)	7.9
SiSi2	1.4 (0.4)	26.7 (3.6)	1.4 (0.3)	2.8	5.5 (1.3)	33.6 (3.8)	1.8 (0.20)	7.3
Tiw1	4.4 (0.6)	14.6 (1.7)	0.9 (0.2)	5.3	9.0 (1.6)	19.1 (1.4)	1.3 (0.06)	10.3
Tiw2	37 (1.3)	67 (4.4)	3.9 (0.4)	41	38 (6.4)	88.6 (4.4)	6.7 (0.37)	44.7
Median (range)	4.2 (1.3 – 5.5)	18.7 (11.0 – 26.7)	1.3 (0.5 – 2.3)	5.3 (1.8 – 7.8)	9.0 (2.0 – 15)	21.8 (9.4 – 34.4)	1.8 (0.6 – 3.4)	10.3 (2.7 – 18.1)

The TAC data are expressed as μmol Trolox equivalents *per* gram of dry matter and are means (SD) from six measurements.
a) Data obtained in samples before or after acid hydrolysis.

Table 4. The content of total phenolic compounds (TPH) and total flavonoids (TF) in canihua

Sample	TPH water-soluble extract (μmol GAE/g dw)	TPH water-insoluble extract (μmol GAE/g dw)	Total TPH[a)] μmol GAE/g dw)	TF water-soluble extract (μmol CE/g dw)	TF water-insoluble extract (μmol CE/g dw)	Total TF[a)] (μmol CE/g dw)
Bel1	39.4 (1.3)	2.9 (0.3)	42.3	2.7 (0.1)	5.9 (0.4)	8.6
Bel2	10.0 (0.2)	2.4 (0.4)	12.4	1.8 (0.4)	0.4 (0.1)	2.2
Bel3	12.3 (0.5)	1.2 (0.2)	13.5	2 (0.4)	2.8 (0.3)	4.8
Bel4	23.5 (1.2)	1.8 (0.3)	25.3	2.9 (0.4)	1.4 (0.1)	4.3
Bel5	27.6 (1.2)	2 (0.8)	29.6	3.1 (0.6)	1.5 (0.2)	4.6
Bel6	43.5 (2.4)	3.5 (0.4)	47	4 (0.8)	4.1 (0.3)	8.1
SiSi1	19.4 (0.4)	3.4 (0.6)	22.8	2.3 (0.6)	8.3 (0.6)	10.6
SiSi2	35.3 (1.2)	2.9 (0.6)	38.2	5.2 (0.1)	1.7 (0.2)	6.9
Tiw1	15.9 (0.8)	1.8 (1.5)	17.7	1.8 (0.3)	1.4 (0.1)	3.2
Tiw2	64.7 (3.2)	6.5 (1.1)	71.2	11 (0.2)	0.4 (0.1)	11.4
Median	23.5	2.4	25.3	2.7	1.7	4.8
Range	10.0–43.5	1.2–3.5	12.4–47	1.8–5.2	0.4–8.3	2.2–10.6
Range (mg/g dw)	1.7–7.4	0.2–0.6	12.1–18	0.52–1.51	0.11–2.41	0.63–3.08

Data are expressed as mean (SD) of six measurements. GAE, gallic acid equivalents; CE, catechin equivalents.
a) Sum of the content in water-soluble extract and water-insoluble extract.

Table 5. Content of individual phenolic compounds in canihua in the (A) water-soluble and (B) water-insoluble extracts expressed as μmol per gram of dry weight

Sample	Quercetin	Kaempferol	Ferulic acid	Vanillic acid	Catechin	Resorcinol	4-Methylresorcinol
(A) Water-soluble extract							
Bel1	0.02	0.08	0.03	1.55	3.6	11.1	14.4
Bel2	0.0007	0.01	0.01	0.02	1.6	5.9	7.7
Bel3	0.007	0.01	0.02	0.07	2.3	11.5	10.4
Bel4	0.04	0.1	0.09	1.64	3.6	7.6	9.5
Bel5	0.04	0.09	0.08	1.68	2.0	6.8	8.1
Bel6	0.05	0.28	0.05	0.58	1.9	5.6	6.6
SiSi1	0.01	0.03	0.04	0.64	0.1	2.4	2.9
SiSi2	0.003	0.02	0.03	0.23	4.0	15.5	4.9
Tiw1	0.004	0.01	0.02	0.76	1.6	3.2	2.8
Tiw2	0.4	0.43	0.60	3.39	3.6	12.8	37.7
Median	0.01	0.03	0.03	0.64	2.0	6.8	7.7

Sample	Quercetin	Kaempferol	Ferulic acid	Catechin gallate	Resorcinol	4-Methyl-resorcinol
(B) Water-insoluble extract						
Bel1	0.05	0.1	0.02	0.2	3.1	0.4
Bel2	0.01	0.02	0.01	0.4	0.7	0.6
Bel3	0.1	0.03	0.004	0.5	0.5	0.9
Bel4	0.01	0.1	0.01	0.6	0.6	0.9
Bel5	0.01	0.1	0.02	0.4	0.8	0.7
Bel6	0.04	0.1	0.01	0.5	0.6	0.9
SiSi1	0.02	0.03	0.02	0.3	2.1	0.4
SiSi2	0.02	0.02	0.02	0.06	2.4	5.7
Tiw1	0.03	0.1	0.01	0.05	0.2	0.2
Tiw2	0.1	0.2	0.05	4.0	1.2	4.7
Median	0.03	0.1	0.02	0.3	0.8	0.7

as the sum of that in the water-soluble and water-insoluble extracts showed considerable variation. Among flavonols the variation was from 0.01 to 0.5 μmol/g dw of quercetin and from 0.03 to 0.63 μmol/g dw of kaempferol. Vanillic acid was the main phenolic acid present in canihua (0.02–3.4 μmol/g dw) and it was only observed in the water-soluble extract while ferulic acid was found in both extracts from 0.02 to 0.60 μmol/g dw. The content of catechin gallate in the water-insoluble extract was usually 0.2–4 μmol/g dw and that of catechin in the water-soluble extract was 0.1–4 μmol/g dw. The amount of resorcinol was 3.4–14.5 μmol/g dw and that of 4-methylresorcinol was 3–42.4 μmol/g dw.

Table 6. Correlation coefficients between different measurements performed in the water-soluble and the water-insoluble extracts of canihua

Water-soluble extract	Correlation coefficient (*r*)
ABTS – FRAP	0.86**
TPH – ABTS	0.41
TPH – FRAP	0.28
TPH – TF	0.77**
TF – ABTS	0.11
TF – FRAP	0.74*
Water-insoluble extract	
ABTS – FRAP	0.77**
TPH – ABTS	0.59
TPH – FRAP	0.70*
TPH – TF	0.65
TF – ABTS	0.76*
TF – FRAP	0.82**

The correlation coefficient was calculated according to the Pearson method. Sample Tiw2 was omitted from these calculations.
*, $p < 0.05$; **, $0.001 < p < 0.01$.

3.4 Contribution of the phenolic substances to TAC

After acid hydrolysis of water-soluble extracts the TAC values increased by 1.8 – 19-fold (ABTS method) and up to 9-fold (FRAP method) (Table 3). The TAC data of the hydrolysed samples were used to assess the contribution of the individual eight compounds to the total TAC value. For this purpose the molar TAC values were measured of the individual reference compounds (Table 2). To assess the contribution of each substance to the TAC value after hydrolysis, the amount of each compound was multiplied by its molar TAC value. In this calculation, 20 – 80% of the total water-soluble TAC was explained by the eight identified compounds and resorcinols were the principal contributors to TAC of the water-soluble extracts (15 – 62%), followed by catechins (1 – 16%), phenolic acids (0.2 – 5.7%) and flavonols (0.1 – 2.4%). The contribution of flavonols to the TAC values was small since they occurred mainly in the water-insoluble extract.

3.5 Correlation among measurements

Several data sets obtained by the ABTS, FRAP, TPH and TF methods were correlated to each other (Table 6) and in these calculations the outlier Tiw2 was omitted. Statistically significant correlations were observed between data obtained by the ABTS method *versus* those of the FRAP method (both for the water-soluble extracts and water-insoluble extracts, TPH *versus* FRAP (only in the water-insoluble extracts) and TF *versus* TPH (only in the water-soluble extracts), TF *versus* FRAP (both extracts) and TF *versus* ABTS (in the water-insoluble extracts).

4 Discussion

4.1 Antioxidants in pseudocereals and cereals

This study shows that canihua contains a number of antioxidant and polyphenolic compounds in appreciable concentrations which may have considerable impact on its use as a food ingredient and for human nutrition. In comparison with literature data on pseudocereals and cereals (Table 7), the present data using the ABTS method (1.8 – 7.8 μmol/g dw) were lower than the higher TAC values found in black sorghum, wheat bran, buckwheat and barley [13 – 15], our intermediary values were close to data obtained for rye and barley [14, 15] and the lower values were close to those of wheat [16]. The TAC (FRAP) values obtained in canihua (2.7 – 18.1 μmol/g dw) were comparable to or higher than data obtained in buckwheat (6 – 10 μmol/g fw) and in 14 different cereals [17]. The data previously obtained on canihua flour by the FRAP method (14.1 μmol/g dw) and the ABTS method (7.2 μmol/g dw) [6] are similar to the intermediary TAC values found in the present investigation. The increase in the TAC values after acid hydrolysis suggested that antioxidants were released by glycoside hydrolysis as also reported by others [18]. This probably is similar to the digestion conditions in the human intestine suggesting that the antioxidant capacity exerted by pseudocereals and cereals in the gut is higher than the effect indicated by data reported on unhydrolysed samples.

4.2 Total phenolic substances in cereals

Compared to the few data on TPH in cereals and pseudocereals available in the literature our TPH values were higher (the sum of the water-soluble and water-insoluble extracts was used) (Table 7). For instance, the canihua samples Tiw2, Bel1, Bel4, Bel5 and Bel6 showed higher values than those obtained for sorghum [19] and buckwheat [20]. The low value of the canihua sample Bel2 was still higher than that found for millet [19], a mean value for cereals [21], and values for sweet corn [22], oat and amaranth [23].

4.3 TF in cereals

Only two publications were found about the content of TF in cereals determined with the same method as used in the present report (Table 7) [22, 23]. The TF content of the ten canihua samples expressed as the sum of water-soluble and water-insoluble values were higher than in oats (0.6 μmol CE/g dw) and amaranth (0.5 μmol CE/g dw) [23] and in sweet corn (0.2 μmol CE/g fw) [22]. The high values of TF found in the canihua samples were probably accounted for by catechins.

Table 7. Comparison of the levels of antioxidant capacity, total phenolic compounds and total flavonoids in canihua to the corresponding published data obtained in pseudo-cereals and cereals

Sample	ABTS (μmol TE/g dw)	FRAP (μmol TE/g dw)	TPH (μmol GAE/g dw)	TF (μmol CE/g dw)
Range in the current work[a)]	1.8-7.8	2.7-18.1	12-47	2.2-11
Canihua flour	7.2[b)]	14[b)]		
Black sorghum	52[c)]-78[d)]	1.5[e)]	24[c)]	
Wheat bran	16[f)]	1.6[e)]		
Buckwheat	58[f)]	10[e)]	19[g)]	
Barley	2.4[h)]-15[c)]	5.4[e)]	5.1[c)]	
Rye	0.3-13[c)f)h)]	2.4[e)]	6[c)]	
Oats		3.0[e)]	1.2[i)]	0.6[i)]
Wheat	1.1-1.9[j)]	0.7[e)]	2.9-5.4[j)]	
Sweet corn			3.0[k)]	0.2[k)]
Millet	21[c)]	1.2[e)]	8[c)]	
Amaranth			0.9[i)]	0.5[i)]
Cereals	0.2[l)]	2.2[l)]-4.4[m)]	6.3[l)]	

The values are expressed as the sum of water-soluble and water-insoluble extracts.
a) Data on sample Tiw2 was omitted
b) Peñarrieta *et al.* [6]
c) Ragaee *et al.* [19]
d) Awicka *et al.* [13]
e) Halvorsen *et al.* [17] (These data refer to fresh weight. Since Trolox had double the activity compared with Fe^{2+} in the FRAP method the results in this reference were divided by two for the comparison)
f) Gallardo *et al.* [14]
g) Quetier-Delau *et al.* [20]
h) Zielinsky and Kozlowska [15]
i) Czerwinski *et al.* [23]
j) Yu *et al.* [16]
k) Chun *et al.* [22]
l) Saura-Calixto and Goñi [21]
m) Perez-Jiménez and Saura-Calixto [18]

4.4 Content of individual phenolic substances in canihua

4.4.1 Flavonols

The presence of quercetin and kaempferol in canihua was in accordance with results of a previous study [4]. Similar results were obtained in kancolla, another *Chenopodiaceae* species [24]. In the present study the content of kaempferol was at least two times higher than that of quercetin in most hydrolysed samples. Moreover, the amounts of kaempferol and quercetin in canihua were considerably higher than those in many other plant foods, and for instance the lowest value of kaempferol content found was comparable to that found in beans [25]. On the other hand, the highest values obtained were comparable with the values of kaemperol found in kale, and the highest value on quercetin content was comparable with the values obtained in beans and fruits [25–27]. The intermediary values of kaempferol and quercetin in canihua were close to the flavonol content found in cabbage, leek and brussels sprouts [28] and in buckwheat (0.08 μmol/g dw of quercetin) [29].

4.4.2 Phenolic acids

Vanillic and ferulic acids were identified in canihua, and previously vanillic acid was found in other *Chenopodiaceae* species as well [24]. The amount of vanillic acid in canihua was higher than that in oats [30], sorghum, barley, wheat [31] and purple corn [32], suggesting that canihua is an important source of this phenolic acid. The comparatively high content of vanillic acid could explain why the consumers in Bolivia use canihua flour for the preparation of a fresh beverage with an attractive taste. The content of ferulic acid in canihua was lower than that reported for wheat bran and corn and close to that in oats and sorghum [31].

4.4.3 Catechins

The presence of catechin in the water-soluble extracts and of catechin gallate in the water-insoluble extracts of canihua may at least partly be accounted for by the occurrence of oligomeric flavonolic proanthocyanidins. They consist of catechin, gallocatechin structures and their epimers, *e.g.*, as 2-*O*-gallates [31, 33]. In buckwheat, sorghum and barley several catechin derivates have been characterized [33, 34].

4.4.4 Resorcinols

Resorcinol and 4-methylresorcinol were tentatively identified as being the principal phenolic compounds of canihua. They are not common in nature but several plant families contain 5-alkyl-resorcinols, for instance *Graminae*, *Gink-*

goaceae and *Anacardiaceae* [35]. However, the 5-alkyl resorcinols had different retention times at HPLC compared with the substances found in the present study, are primarily acetone-soluble and have two UV maxima at 275 and 282 nm [36], and thus it seems unlikely that the compounds observed here are 5-alkylresorcinols. Resorcinol and 4-methylresorcinol have been reported to be released when maize is infected by fungi [37] and furthermore, 4-methylresorcinol has been reported to be formed in during roasting of coffee [38]. Although the derivatives of 4-methylresorcinol seem to be unusual in nature, some of them have been isolated, for instance 5-methoxy-4-methylresorcinol from ferns [39]. Thus, we may speculate whether 4-methylresorcinol is present under optimal growing conditions or if it is a result of fungal growth, high altitude or another stress on the plant. The accumulation of resorcinol and some of its derivatives is considered to be a defensive response of the plants against bacterial infection [40, 41]. In addition, resorcinol could be a degradation product of flavonoids such as quercetin and morin [42].

4.5 Contribution of the phenolic substances to TAC

The molar TAC values found for pure phenolic compounds by the ABTS and FRAP methods were in accordance with data from the literature [7]. The sum of the TAC values for individual compounds calculated by multiplying the molar TAC by the values assessed by ABTS were three times higher than the experimental TAC values after hydrolysis while the calculated values obtained using the FRAP method were lower than the experimental values (see Section 3). One possible explanation for the higher calculated TAC values obtained by the ABTS method could be the presence of resorcinols and catechin, which had high molar TAC values. In addition, unknown interactions among antioxidant and other compounds might have contributed. The total calculated contribution of flavonoids and other polyphenolic compounds to the total TAC was 20 – 80% using the FRAP method and their large contribution was also shown by the many significant correlations found among the different measurements (Table 6). The data also indicated that a number of other compounds would contribute to total TAC. It also possible that synergistic or antagonistic effects or other interactions contribute to the differences obtained between the data obtained by the ABTS and FRAP methods [43].

4.6 Health aspects of canihua consumption

Several studies have shown that cereal grain contain constituents with health benefits for humans [44 – 46]. For example, alkylresorcinols occurring in cereals bran fractions [47, 48] show antitumoural, antibacterial, antifungal and antiparasitic activities [49, 50]. Although the detailed mechanisms of action of the antioxidants found in canihua are not known, it is possible that consumption of canihua would be associated with such health benefits. It could be calculated that consumption of 200 g canihua with a dry weight of 40%, would correspond to a median intake of water-soluble antioxidants of approx. 0.16 mmol. This is very close to the TAC intake calculated for 200 g of cauliflower [51]. This may stimulate a renewed interest in the canihua crop for the production of breads, cakes, beverages, and other foods in the Andean region.

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5 References

[1] USDA, ARS, National Genetic Resources Program, Germplasm Resources Information Network (GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/tax_search.pl.

[2] Gross, R., Koch, F., Malaga, I., Miranda, A. F., *et al.*, Chemical composition and protein quality of some local Andean food sources, *Food Chem.* 1989, *34*, 25 – 13.

[3] Repo-Carrasco, R., Espinoza, C., Jacobsen, S. E., Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kaniwa (*Chenopodium pallidicaule*), *Food Rev. Int.* 2003, *19*, 179 – 189.

[4] Rastrelli, L., Saturnino, P., Schettino, O., Dini, A., Studies on the constituents of *Chenopodium pallidicaule* (Canihua) seeds. Isolation and characterization of two new flavonol glycosides, *J. Agric. Food Chem.* 1995, *43*, 2020 – 2024.

[5] Rastrelli, L., De Simone, F., Schettino, O., Dini, A., Constituents of *Chenopodium pallidicaule* (canihua) seeds. Isolation and characterization of new triterpene saponins, *J. Agric. Food Chem.* 1996, *44*, 3528 – 3553.

[6] Peñarrieta, M., Alvarado, J. A., Åkesson, B., Bergenståhl, B., Antioxidant capacity in Andean foods species from Bolivia, *Rev. Bol. Quim*. 2005, *22*, 89 – 93.

[7] Nilsson, J., Pillai, D., Önning, G., Persson, C., *et al.*, Comparison of the ABTS and FRAP methods to assess the total antioxidant capacity in extracts of fruit and vegetables, *Mol. Nutr. Food Res.* 2005, *49*, 239 – 246.

[8] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., *et al.*, Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic. Biol. Med.* 1999, *26*, 1231 – 1237.

[9] Benzie, I. F., Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Anal. Biochem.* 1996, *239*, 70 – 76.

[10] Singleton, V. L., Rossi, J. A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent, *Am. J. Enol. Vitic.* 1965, *16*, 144 – 158.

[11] Zhishen, J., Mengcheng, T., Jianming, W., Determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem.* 1999, *64*, 555 – 559.

[12] Cristea, D., Bereau, I.,Vilarem, G., Identification and quantitative HPLC analysis of the main flavonoids present in weld (*Reseda luteola L.*), *Dyes Pigm.* 2003, *57*, 267 – 272.

[13] Awicka, J. M., Rooney, L. W., Waniska, R. D., Anthocyanins from black sorghum and their antioxidant properties, *Food Chem.* 2004, *90*, 293 – 301.

[14] Gallardo, C., Jiménez, L., García-Conesa, M. T., Hydroxycinnamic acid composition and in vitro antioxidant activity of selected grain fractions, *Food Chem.* 2006, *99*, 455 – 463.

[15] Zielinski, H., Kozlowska, H., Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions, *J. Agric. Food Chem.* 2000, *48*, 2008 – 2016.

[16] Yu, L., Haley, S., Perret, J., Harris, M., *et al.*, Free radical scavenging properties of wheat extracts, *J. Agric. Food Chem.* 2002, *50*, 1619 – 1624.

[17] Halvorsen, B. L., Holte, K., Myhrstad, M. C. W., Barikmo, I., *et al.*, A systematic screening of total antioxidants in dietary plants, *J. Nutr.* 2002, *132*, 461 – 471.

[18] Perez-Jiménez, J., Saura-Calixto, F., Literature data may underestimate the actual antioxidant capacity of cereals, *J. Agric. Food Chem.* 2005, *53*, 5036 – 5040.

[19] Ragaee, S., El-Sayed, M., Abdel, A., Noaman, M., Antioxidant activity and nutrient composition of selected cereals for food use, *Food Chem.* 2006, *98*, 32 – 38.

[20] Quettier-Delau, C., Gressier, B., Vasseur, J., Dine, T., *et al.*, Phenolic compounds and antioxidant activities of buchwheat (*Fagopyrum esculentum* Moench) hulls and flour, *J. Ethnopharmacol.* 2000, *72*, 35 – 42.

[21] Saura-Calixto, F., Goñi, I., Antioxidant capacity of the spanish mediterranean diet, *Food Chem.* 2006, *94*, 442 – 447.

[22] Chun, O. K., Kim, D. O., Smith, N., Schroeder, D., *et al.*, Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet, *J. Sci. Food Agric.* 2005, *85*, 1715 – 1724.

[23] Czerwinski, J., Bartnikowska, E., Leontowicz, H., Lange, E., *et al.*, Oat (*Avena sativa L.*) and amaranth (*Amaranthus hypochondriacus*) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets, *J. Nutr. Biochem.* 2004, *15*, 622 – 629.

[24] Dini, I., Tenore, G. C., Dini, A., Phenolics constituents of kancolla seed, *Food Chem.* 2004, *84*, 163 – 168.

[25] Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjöholm, I., *et al.*, Effect of processing on major flavonoids in processed onions, green beans, and peas, *Food Chem.* 1999, *64*, 231 – 235.

[26] Hertog, M. G. L., Hollman, P. C. H., Venema, D. P., Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits, *J. Agric. Food Chem*. 1992, *40*, 1591 – 1598.

[27] Crozier, A., Lean, M. E. J., McDonald, M. S., Black, C., Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery, *J. Agric. Food Chem.* 1997, *45*, 590 – 595.

[28] Hollman, P. C. H., Arts, L. C. W., Flavonols, flavone and flavanol – nature, occurrence and dietary burden, *J. Sci. Food Agric.* 2000, *80*, 1081 – 1093.

[29] Watanabe, M., Ohshita, Y., Antioxidant compounds from buckwheat (*Fagopyrum esculentum*) hulls, *J. Agric. Food Chem.* 1997, *45*, 1039 – 1044.

[30] Dimberg, L. H., Molteberg, E. L., Solheim, R., Frolich, W., Variation in oat groats due to variety, storage and heat treatment. I: Phenolic compounds, *J. Cereal Sci.* 1996, *24*, 263 – 272.

[31] Shahidi, F., Naczk, M., *Phenolics in Food and Nutraceuticals. Chap. 2: Cereals, Legumes and Nuts*, CRC Press LLC, Boca Raton, FL 2004.

[32] Pedestri, R., Cisneros-Cevallos, L., Phenolic profiles of Andean purple corn (*Zea mays* L.), *Food Chem.* 2007, *100*, 956 – 963.

[33] Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., *et al.*, Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation, *J. Agric. Food Chem.* 2003, *51*, 7513 – 7521.

[34] Watanabe, M., Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats, *J. Agric. Food Chem.* 1998, *46*, 839 – 845.

[35] Alonso, E., Ramón, D. J., Yus, M., Simple synthesis of 5-substituted resorcinols: A revisited family of interesting bioactive molecules, *J. Org. Chem.* 1997, *62*, 417 – 421.

[36] Ross, A. B., Åman, P., Andersson, R., Kamal-Eldin, A., Alkylresorcinols in cereals and cereal products, *J. Chromatogr. A*. 2004, *1054*, 157 – 164.

[37] Angra-Sharma, R., Singh, D., Sharma, D. K., Identification of phenolics accumulating as an initial response to infection of maize by *Dreschslera maydis* and of barley by *Pyrenophora teres*, *Res. Bull. Panjab Univ. Sci.* 2000, *50*, 53 – 62.

[38] Heinrich, L., Baltes, W., Über die Bestimmung von Phenolen im Kaffeegetränk, *Z. Lebensm. Unters. Forsch.* 1987, *185*, 362 – 365.

[39] Aebi, A., Beitrag zur Konstitution der Inhaltsstoffe von Farnwurzeln, *Helv. Chim. Acta.* 1956, *39*, 153 – 158.

[40] Harborne, J. B., Simmond, N. W., *The Natural Distribution of Phenolic Aglycones*, Academic Press, London 1964.

[41] Sutfeld, R., Petereit, F., Nahrstedt, A. J., Resorcinol in exudates of *Nuphar lutea*, *Chem. Ecol.* 1996, *22*, 2221 – 2231.

[42] Makris, D. P., Rossiter, J. T., Hydroxyl free radical-mediated oxidative degradation of quercetin and morin: A preliminary investigation, *J. Food Comp. Anal.* 2002, *15*, 103 – 113.

[43] Arts, J. T. J., Dallinga, S. J., Voss, H. P., Haenen, G. R. M., *et al.*, A critical appraisal of the use of the antioxidant capacity (TEAC) assay in defining optimal antioxidant structures, *Food Chem.* 2003, *80*, 409 – 414.

[44] Juntunen, K. S., Mazur, W. M., Liukkonen, K. H., Uehara, M., *et al.*, Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women, *Br. J. Nutr.* 2000, *84*, 839 – 846.

[45] Rieckhoff, D., Trautwein, E. A., Malkki, Y., Erbersdobler, H. F., Effects of different cereal fibers on cholesterol and bile acid metabolism in the Syrian golden hamster, *Cereal Chem.* 1999, *76*, 788 – 795.

[46] Biörklund, M., Van Rees, A., Mensink, R. P., Önning, G., Changes in serum lipids and postprandial glucose and insulin concentrations after consumption of beverages with β-glucans from oats or barley: A randomised dose-controlled trial, *Eur. J. Clin. Nutr.* 2005, *59*, 1272 – 1281.

[47] Kamal-Eldin, A., Pouru, A., Eliasson, C., Åman, P., Alkylresorcinols as antioxidants: hydrogen donation and peroxyl radical-scavenging effects, *J. Sci. Food Agric.* 2000, *81*, 353 – 356.

[48] Ross, A. B., Shepherd, M. J., Schüpphaus, M., Sinclair, V., *et al.*, Alkylresorcinols in cereals and cereal products, *J. Agric. Food Chem.* 2003, *51*, 4111 – 4118.

[49] Kozubek, A., Tyman, J. H. P., Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity, *Chem. Rev.* 1999, *99*, 1 – 26.

[50] Hertog, M. G. L., Hollman, P. C. H., Katan, M. B., Content of potencially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands, *J. Agric. Food Chem.* 1992, *40*, 2379 – 2383.

[51] Nilsson, J., Olsson, K., Engqvist, G., Ekvall, J., *et al.*, Variation in the content of glucosinolates, hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular-weight carbohydrates in Brassica vegetables, *J. Sci. Food Agric.* 2006, *86*, 528 – 538.