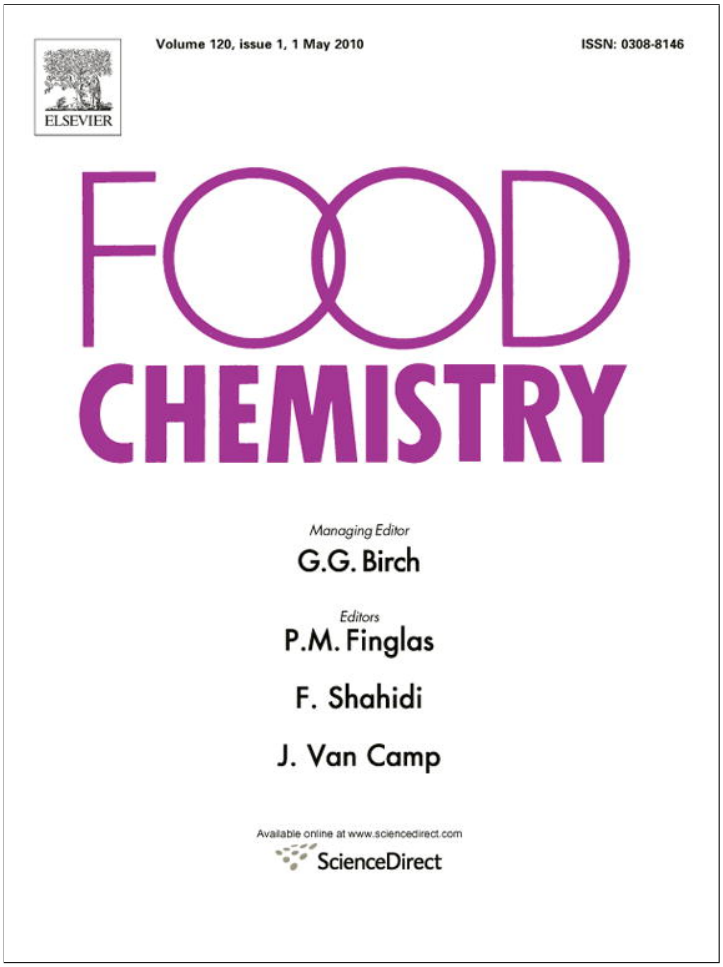

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Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*)

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ABSTRACT

The amount of phenolic acids, flavonoids and betalains in Andean indigenous grains, quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*), was determined. The total amount of phenolic acids varied from 16.8 to 59.7 mg/100 g and the proportion of soluble phenolic acids varied from 7% to 61%. The phenolic acid content in Andean crops was low compared with common cereals like wheat and rye, but was similar to levels found in oat, barley, corn and rice. The flavonoid content of quinoa and kañiwa was exceptionally high, varying from 36.2 to 144.3 mg/100 g. Kiwicha did not contain quantifiable amounts of these compounds. Only one variety of kiwicha contained low amounts of betalains. These compounds were not detected in kañiwa or quinoa. Our study demonstrates that Andean indigenous crops have excellent potential as sources of health-promoting bioactive compounds such as flavonoids.

1. Introduction

Quinoa (*Chenopodium quinoa*), kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*) are nutritious grains that are grown in the Andean highlands. These crops were used by pre-Colombian cultures in South America for centuries. They were very important for the Incas together with corn and potatoes. These plants are cold- and drought-tolerant and can be cultivated in high mountains, particularly kañiwa, which can be grown at over 4000 masl. The genetic variability of quinoa, kañiwa and kiwicha is huge, with cultivars being adapted to growth from sea level to high mountains, and from cold, highland climates to subtropical conditions.

Quinoa, kañiwa and kiwicha are usually referred to as pseudocereals since they are not members of the grass family, but produce seeds that can be milled into flour and used like a cereal crop. Quinoa is mainly used in soups and also instead of rice in main courses. Kañiwa is usually toasted and milled and consumed as meal (*kañiwako*). Kiwicha is toasted to obtain "pop-kiwicha", a puffed product. It is consumed directly or used to make "turrones", a kind of snack bar. All these grains are gluten-free and can be used by persons who suffer from coeliac disease. They are also used in baby foods to make porridge.

Several studies have reported the nutritional value of quinoa. This crop contains proteins with a balanced essential amino acid composition that are of high biological value (Koziol, 1992; Ranhotra, Gelroth, Glaser, Lorenz, & Johnson, 1993; Repo-Carrasco, Espinoza, & Jacobsen, 2003). A close relative of quinoa, kañiwa, also has a relatively high protein content with adequate levels of essential amino acids (Repo-Carrasco et al., 2003). The high nutritional value of amaranth proteins was demonstrated by Bressani, De Martell, and Godinez (1993). Among the essential amino acids, the content of lysine in quinoa, kañiwa and kiwicha is notable. The consumption of quinoa and kañiwa has compensated the lack of animal protein, and they are still principal protein sources in many areas (Tapia, 1997). These crops are also very good sources of good quality edible oil (Berganza et al., 2003; Repo-Carrasco et al., 2003) and minerals, such as calcium and iron (Bressani, 1994). Starch is the most abundant component in quinoa, kañiwa and kiwicha seed, as in all cereal crops.

Polyphenols are bioactive secondary plant metabolites that are widely present in commonly consumed foods of plant origin. The three main types of polyphenols are flavonoids, phenolic acids and tannins, which act as powerful anti-oxidants *in vitro*. These compounds are considered to carry many potential beneficial health effects, e.g. in reduction of the risk of cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and osteoporosis. In food, polyphenols may contribute to bitterness,

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astringency, colour, flavour, and oxidative stability of products (Han, Shen, & Lou, 2007; Scalbert, Manach, Morand, & Rémésy, 2005; Shahidi & Naczk, 1995). Very little information exists concerning polyphenols in Andean cereals such as quinoa, kañiwa and kiwicha. A few articles concerning the isolation and characterisation of flavonoids in quinoa and kañiwa seeds have been published (De Simone, Dini, Pizza, Saturnino, & Scettino, 1990; Rastrelli, Saturnino, Schettino, & Dini, 1995; Zhu et al., 2001).

In addition, Peñarrieta, Alvarado, Åkesson, and Bergenståhl (2008) analysed levels of flavonoids and other phenolic compounds in *Chenopodium pallicaule* (edible part of the plant). Repo-Carrasco-Valencia, Peña, Kallio, and Salminen (2009) analysed the content of total phenolic compounds, phytic acid and anti-oxidant activity in two varieties of raw and extruded kiwicha. Anti-oxidant activity with the DPPH method for the raw kiwicha of the two varieties was 410.0 μmol trolox/g sample for Centenario and 398.1 μmol trolox/g sample for Oscar Blanco. With ABTS method those values were 827.6 and 670.1 μmol trolox/g sample for Centenario and Oscar Blanco, respectively. The content of total phenolics, phytic acid and the anti-oxidant activity decreased during the extrusion process.

To our knowledge, there are no previous data on the phenolic acid and flavonoid content in the seeds of *Chenopodium* species. Klimczak, Malecka, and Pacholek (2002) published results concerning the phenolic acid content of amaranth seeds. Another article concerning the phenolic and flavonoid content in amaranth (*Amaranthus hypochondriacus*) has also recently been published (Barba de la Rosa et al., 2009). Pasko et al. (2009) analysed the total polyphenol content and anti-oxidant activity in two amaranth varieties (*Amaranthus cruentus*) and quinoa seeds and sprouts. Anti-oxidant activity of the investigated seeds decreased in the following order: quinoa, amaranth v. Rawa, amaranth v. Aztek for FRAP and quinoa, amaranth v. Aztek, amaranth v. Rawa for both ABTS and DPPH. The data obtained by the three methods showed significant correlation between total polyphenols content in seed and sprouts.

Betalains are yellow and red compounds that are found in few selected plants such as beetroot, cactus pears and amaranthus. Chemically they can be divided into betaxanthins, which are condensation products of betalamic acid and various amino compounds, and betacyanins, which are conjugates of betalamic acid and *cyclo*-Dopa with various substitutions (Stintzing & Carle, 2004). The betalains from red beet have been extensively used as colourants in the modern food industry. Recently, several studies on the antiradical and anti-oxidant activity of betalains (mainly betanin) from beetroot (*Beta vulgaris*) have been published (Kanner, Harel, & Granit, 2001; Pedreno & Escribano, 2000). Cai, Sun, and Corke (2003) studied the anti-oxidant activity of betalains from plants of *Amaranthaceae*. They found that plants of the *Amaranthaceae*, containing betacyanins and betaxanthins, demonstrated very strong anti-oxidant activity.

The aim of this study was to determine the levels of flavonoids, phenolic acids and betalains in the Andean grains quinoa, kañiwa and kiwicha. In addition, the basic composition of these pseudocereals was analysed.

2. Materials and methods

2.1. Samples

Six ecotypes of Quinoa (*C. quinoa*) were obtained from the Agronomical Experimental Station-INIA Salcedo, Puno, Peru (03-21-1181, Witulla, Roja Coporaque, 03-21-0093, Huaripongo, Ccoito) and two varieties (INIA-415 Pasankalla, Salcedo INIA), and two commercial samples from Cusco were purchased for the study.

Table 1
Description of the quinoa, kañiwa and kiwicha samples.

Sample	Colour	Place cultivated
Quinoa		
Ccoito	Grey	Puno
INIA-415 Pasankalla	Grey/red	Puno
Roja de Coporaque	Red	Puno
Witulla	Red	Puno
03-21-0093	Red	Puno
Salcedo INIA	Cream	Puno
Commercial 1.	Red	Cusco
Commercial 2.	Black	Cusco
Huaripongo	Yellow	Puno
03-21-1181	Yellow	Puno
Kañiwa		
Kello	Yellow	Puno
Wila	Brown	Puno
Guinda	Brown	Puno
Ayara	Grey	Puno
Commercial sample	Brown	Cusco
Kiwicha		
1.	Black	Mollepata, Cusco
2.	Black	San Salvador, Cusco
3.	Pink	Mollepata, Cusco
4.	Cream	San Salvador, Cusco

Four ecotypes of kañiwa (*C. pallidicaule*) were obtained from the Agronomical Experimental Station-INIA Salcedo, Puno, Peru (Kello, Wila, Guinda and Ayara) and one commercial sample from Cusco was purchased. Black, pink and white grains of kiwicha (*A. caudatus*) were collected from Mollepata, Cusco and one black sample from San Salvador, Cusco.

See Table 1 for more details. All grain was from 2007 to 2008 growing season.

2.2. Proximate analysis

Water content, proteins, fat, crude fibre and ash were determined according to AOAC methods (1995). The carbohydrates were calculated by difference.

2.3. Flavonoids

Flavonoids were analysed as aglycones according to the method explained by Mattila, Astola, and Kumpulainen (2000). Briefly, a sample (0.3–1 g) was weighed into a 100-ml Erlenmeyer flask and dispersed in 40 ml of 62.5% aqueous methanol containing 2 g/l of 2,3-tert-butyl-4-hydroxyanisole (BHA). To this extract 10 ml of 6 M HCl was added. Hydrolysis was carried out in a shaking water bath at 90 °C for 2 h. After hydrolysis the sample was allowed to cool. Then it was filtered and made up to 100 ml with methanol. Before quantification by HPLC the sample was filtered through a 0.45 μm membrane filter.

The analytical HPLC system consisted of an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector. The HPLC pumps, autosampler, column oven, and diode array system were monitored and controlled using the HP Chem Station computer programme. Wavelengths used for identification and quantification of flavonoids with the diode array detector were 280 nm for eriodictyol, naringenin, and hesperetin, 329 nm for luteolin and apigenin and 370 nm for myricetin, kaempferol, quercetin and isorhamnetin. Flavonoid separation was done by an Inertsil (GL Scienses, Inc., Japan) ODS-3 (4.0 × 150 mm, 3 μm) column with a C-18 guard column. The temperature of the column oven was set at 35 °C. Gradient elution was employed for flavonoids with a mobile phase consisting of 50 mM H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) as follows: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 50%

A, 5–55 min; isocratic elution 50% A, 55–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before next injection. The flow rate of the mobile phase was 0.7 ml/min, and the injection volumes were 10 μl of the standards and sample extracts. All flavonoids were quantified using the external standard method. The samples were analysed in triplicate.

2.4. Phenolic acids

Phenolic acids were analysed according to the method of Mattila and Hellström (2007). Briefly, a 0.5 g sample was homogenised in 7 ml of a mixture of methanol, containing 2 g/l of butylated hydroxyanisole (BHA) and 10% acetic acid (85:15) using a Heidolph Diax 900 homogenizer. The homogenised extract was ultrasonicated for 30 min and made up to a volume of 10 ml with distilled water. After mixing, 1 ml was filtered for HPLC analysis of soluble phenolic acids. Next, 12 ml of distilled water containing 1% ascorbic acid and 0.415% EDTA and 5 ml of 10 M NaOH were added into the test tube, sealed, and stirred overnight (about 16 h) at 20 °C using a magnetic stirrer. The solution was then adjusted to pH 2 with concentrated HCl, and the liberated phenolic acids were extracted with 15 ml of a mixture of cold diethyl ether and ethyl acetate (1:1), centrifuged at 620*g* (Rotofix 32, Hettich Zentrifugen, Germany) and the organic layer was recovered. The extraction was repeated twice and the organic layers were combined. After alkaline hydrolysis, an acid hydrolysis was performed by adding 2.5 ml of concentrated HCl into the test tube and incubating in a water bath at 85 °C for 30 min. The sample was then cooled, and further sample handling was performed in the same manner following alkaline hydrolysis.

The organic layers from alkaline and acid hydrolyses were combined, evaporated to dryness, dissolved into 2 ml of methanol, filtered and analysed for total phenolic acids by HPLC.

The analytical HPLC system was the same for phenolic acids as for flavonoids except for a modification in gradient elution: isocratic elution 95% A, 0–5 min; linear gradient from 95% A to 85% A, 5–17 min; linear gradient from 85% A to 80% A, 17–40 min; linear gradient from 80% A to 50% A, 40–60 min; isocratic elution 50% A, 60–65 min; linear gradient from 50% A to 95% A, 65–67 min; post-time 6 min before the next injection. The wavelengths used for the quantification of phenolic acids with the diode array detector were: 254 nm for protocatechuic acid, *p*-hydroxybenzoic acid and vanillic acid; 280 nm for syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, and *E*-cinnamic acid; and 329 nm for caffeic acid, ferulic acid, sinapic acid and chlorogenic acid. The samples were analysed in triplicate. Both total and soluble forms were quantified as aglycones. Phenolic acids obtained after hydrolysis were identified according to their retention times and UV spectra (190–600 nm) consistent with commercial reference compounds while soluble forms were identified solely by their UV spectra.

2.5. Betalains in kiwicha species

Finely ground material (0.5 g) was weighed in a test tube and made up to 5 ml with acidified water (pH 3–4). The test tube was carefully flushed with argon, sealed and extracted overnight in a magnetic stirrer. After extraction 5 ml of methanol was added and the sample was centrifuged (10 min at 1500 rpm). The supernatant was transferred to another test tube. To the solid residue 2 ml of acetone was added and after vortexing the sample was centrifuged again. Acetone supernatants were combined and the sample was evaporated to near dryness with a stream of nitrogen. After evaporation the volume was adjusted to 1 ml with methanol, filtered through a 0.45 μm membrane filter and analysed by HPLC-DAD (Hewlett–Packard 1100 series). Nova Pak C18 (3.9 × 150 mm, 4 μm, Waters, Milford, USA) was used as an analytical column protected with the same manufacturer's precolumn. The mobile phase consisted of 0.05 M phosphate buffer (A) pH 2.4 and methanol (B). Gradient elution was used: 5–60% B in 50 min followed by 60–90% B in 6 min, hold at 90% B for 12 min, and finally to 100% B within 32 min. The HPLC method was basically the same as described by Mattila, Pihlava, and Hellström (2005) for avenanthramides except that the quantification of betalains was done at 535 nm. For identification purposes UV spectra were recorded at 190–600 nm. Three compounds were detected in one kiwicha sample at 535 nm and tentatively identified as betacyanin amaranthine, iso-amaranthine and betanin according to the elution order and UV spectra presented in the literature (Cai, Sun, & Corke, 2001; Cai, Sun, Wu, Huang, & Corke, 1998). Amaranthine, previously isolated at MTT, was used as a reference compound for quantification.

2.6. Statistical analysis

Each analysis was done at least in duplicate and the results are expressed as mean and standard deviation (SD). The data were analysed by analysis of variance, and Tukey's test (significance of differences $p < 0.05$) was used to find significant differences between the species.

3. Results and discussion

The results of the proximate analysis of the Andean grains are presented in Table 2. The protein content of quinoa varieties was 12.61% on average. This value is similar to that reported by Guzman-Maldonado and Paredes-Lopez (1998), at between 11.0% and 15.0%. The protein content of kañiwa grains was significantly higher than that of quinoa and kiwicha. There were no statistically significant differences in the fat content of quinoa, kiwicha and kañiwa. In general, quinoa, kañiwa and kiwicha seeds are good sources of protein and fat. The main component of all three grain species was carbohydrates.

Both the soluble and total phenolic acid contents in the Andean cereals were quantified as aglycones (Table 3). Soluble phenolic acids (free and bound soluble forms) were extracted with methanolic acetic acid whereas the total phenolic acid content (the sum of bound soluble, insoluble and free phenolic acids) was obtained after alkaline and acid hydrolyses. Due to a lack of reference standards for soluble bound phenolic acids, the results are to be considered tentative and are reported only as percentage shares of total phenolic acids in Table 3. However, this information may be of interest because the bioavailability of soluble phenolic acids may differ from that of insoluble ones.

The total content of phenolic acids varied from 16.8 to 59.7 mg/100 g in the samples analysed and the percentage share of soluble phenolic acids varied from 7% to 61%.

There were several differences in the phenolic acid composition of the three different grains (quinoa, kañiwa and kiwicha) (Table 3). The samples of *Chenopodium* species contained caffeic acid, ferulic acid, *p*-coumaric acid, *p*-OH-benzoic acid and vanillic acid. In addition to these sinapinic acid and protocatechuic acid were detected in *Amaranthus* samples (Table 3). There was a statistically significant difference in the content of ferulic acid in quinoa, kañiwa and kiwicha, kañiwa having the highest and kiwicha the lowest. Of the *Chenopodium* species kañiwa samples contained less vanillic acid but more caffeic and ferulic acids than quinoa samples. The content of total phenolic acids was higher in quinoa than in kiwicha but much variation existed between samples. In quinoa varieties the proportion of soluble phenolic acids was high (mean 39 ± 11%). In kañiwa and amaranthus varieties these mean values were 21 ± 9% and 10 ± 3%, respectively.

To our knowledge, very little information has been published concerning the phenolic acid content of *Chenopodium* and

Table 2
Proximate composition of Andean grains (g/100 g).

Sample	Moisture	Protein	Fat	Crude fiber	Ash	Carbohydrates
Quinoa samples						
Ccoito	8.47 ± 0.08	14.72 ± 0.11	5.33 ± 0.06	1.81 ± 0.02	2.83 ± 0.00	68.1
INIA-415 Pasankalla	9.76 ± 0.07	12.69 ± 0.06	6.85 ± 0.10	2.20 ± 0.02	2.49 ± 0.03	67.0
Roja de Coporaque	8.30 ± 0.07	11.51 ± 0.10	5.22 ± 0.08	2.26 ± 0.02	2.93 ± 0.05	70.8
Witulla	8.81 ± 0.08	12.28 ± 0.00	5.32 ± 0.01	2.62 ± 0.02	2.57 ± 0.04	69.5
03-21-0093	8.47 ± 0.07	11.79 ± 0.11	nd	nd	2.76 ± 0.02	nd
Salcedo INIA	8.26 ± 0.05	13.23 ± 0.01	5.30 ± 0.09	1.84 ± 0.20	2.37 ± 0.05	70.0
Commercial 1	10.13 ± 0.05	13.18 ± 0.01	6.51 ± 0.04	4.23 ± 0.03	2.34 ± 0.10	63.6
Commercial 2	11.51 ± 0.04	13.48 ± 0.06	6.34 ± 0.07	7.04 ± 0.03	2.27 ± 0.10	59.4
Huaripongo	10.34 ± 0.02	11.32 ± 0.01	6.14 ± 0.01	2.51 ± 0.01	2.92 ± 0.04	67.8
03-21-1181	9.37 ± 0.06	11.89 ± 0.02	3.95 ± 0.03	2.88 ± 0.01	3.12 ± 0.02	69.8
Mean ± SD	*9.34 ± 1.1*^{a}	*12.61 ± 1.1*^{a}	*5.66 ± 0.9*^{a}	*3.04 ± 1.7*^{a}	*2.66 ± 0.3*^{a}	*67.3 ± 3.7*^{a}
Kañiwa samples						
Kello	10.37 ± 0.04	15.38 ± 0.03	7.36 ± 0.08	5.33 ± 0.04	3.56 ± 0.19	59.4
Wila	9.61 ± 0.07	13.29 ± 0.09	6.87 ± 0.08	7.52 ± 0.01	3.67 ± 0.08	60.3
Guinda	9.79 ± 0.07	14.72 ± 0.11	4.46 ± 0.00	7.46 ± 0.05	3.38 ± 0.04	61.5
Ayara	10.39 ± 0.09	14.38 ± 0.01	6.66 ± 0.07	14.37 ± 0.20	3.13±	52.4
Commercial sample	9.38 ± 0.09	18.28 ± 0.12	7.92 ± 0.03	4.79 ± 0.06	2.73 ± 1.16	58.5
Mean ± SD	*9.91 ± 0.5*^{a}	*15.21 ± 1.9*^{b}	*6.65 ± 1.3*^{a}	*7.89 ± 3.8*^{b}	*3.31 ± 0.4*^{b}	*58.39 ± 3.5*^{b}
Kiwicha samples						
1	12.07 ± 0.17	15.88 ± 0.10	6.54 ± 0.02	7.49 ± 0.09	2.50 ± 0.06	55.5
2	11.52 ± 0.04	12.80 ± 0.11	6.74 ± 0.10	3.07 ± 0.07	2.23 ± 0.13	63.7
3	11.40 ± 0.08	14.54 ± 0.10	7.56 ± 0.20	2.68 ± 0.03	2.16 ± 0.09	61.7
4	11.09 ± 0.08	13.69 ± 0.11	6.31 ± 0.02	6.73 ± 0.05	2.77 ± 0.08	59.4
Mean ± SD	*11.52 ± 0.4*^{b}	*14.23 ± 1.3*^{a,b}	*6.79 ± 0.5*^{a}	*4.99 ± 2.5*^{a,b}	*2.42 ± 0.3*^{a}	*60.06±3.5*$^{b\pm}$

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).
nd = not determined.

Amaranthus seeds. Peñarrieta, Alvarado, Åkesson, and Bergenståhl (2008) identified vanillic and ferulic acids in whole plants of *C. pallicaule*. Their result for vanillic acid was of the same magnitude, whereas a lower level of ferulic acid was found compared with the present study. This discrepancy probably arises from the sample differences (seeds vs. whole plants) as well as different methodology. Peñarrieta et al. (2008) only analysed extractable phenolic acids, and according to our study a large proportion of vanillic acid, unlike ferulic acid, is present in soluble forms in *C. pallicaule* samples. However, Peñarrieta et al. (2008) also found much variation between samples. Klimczak et al. (2002) analysed the free phenolic acid content of *A. caudatus* seeds and found the same phenolic acids as in the present study except for sinapinic and vanillic acids. However, in our study soluble (or free) caffeic, ferulic, *p*-coumaric and protocatechuic acids were not found. Recently, Barba de la Rosa et al. (2009) published information concerning the phenolic acid content of a different amaranth species (*A. hypochondriacus*). According to their data amaranth seed flour contained soluble 4-hydroxybenzoic acid 0.17–0.22 mg/100 g, vanillic acid 0.15–0.18 mg/100 g and syringic acid 0–0.08 mg/100 g. These figures are much lower than those obtained in our study. This is probably due to the different methodology as well as the different species studied.

The Andean cereals contained lower levels of phenolic acids compared with common cereals like wheat (*Triticum* spp.) and rye (*Hordeum vulgare*). In these cereals the phenolic acids accumulate in bran where their levels are as high as 419 and 453 mg/100 g in rye and wheat bran while whole grain flours of these grains contain 137 and 134 mg/100 g, respectively (Mattila et al., 2005). However, according to Mattila et al. (2005) the phenolic acid content of other cereals like oat (*Avena sativa*), barley (*H. vulgare*), corn (*Zea mays*), rice (*Oryza sativa*), millet (*Panicum miliaceum*) and buckwheat (*Fagopyrum esculentum*) is of the same magnitude (25–60 mg/100 g) as in the Andean grains studied here.

The flavonoid content of *Chenopodium* species was exceptionally high, varying from 36.2 to 144.3 mg/100 g (Table 4). The predominant flavonoids in quinoa samples were quercetin and kaempferol while in some varieties myricetin and isorhamnetin were also found. Kañiwa samples contained mostly quercetin and isorhamnetin with smaller amounts of myricetin, kaempferol and rhamnetin in some varieties. As in the case of phenolic acids much variation was found between different samples. There were no statistically significant differences in the content of quercetin, rhamnetin and total flavonoids in quinoa and kañiwa. The content of isorhamnetin was significantly higher in kañiwa compared with quinoa. In the case of kaempferol, the content in kañiwa was significantly lower than in quinoa.

Berries have been considered as an excellent source of flavonols, especially quercetin and myricetin. For example, lingonberry contains 10 mg/100 g fw of quercetin and cranberry contains 10.4 and 6.9 mg/100 g fw quercetin and myricetin, respectively (Mattila et al., 2000). The levels in these flavonoid-rich berries are 5–10-fold lower than those found in *Chenopodium* seed samples. When compared on a dry weight basis the flavonoid contents in berries and *Chenopodium* samples are of the same magnitude. Quinoa and kañiwa seeds can thus be considered very good source of flavonoids. Common cereals (wheat, rye, oat, barley, etc.) do not contain any flavonols (Shahidi & Naczk, 1995).

To our knowledge, this is the first paper reporting the total content of flavonoids in quinoa and kañiwa seeds. Peñarrieta et al. (2008) analysed extractable flavonoids in the whole plant of *C. pallicaule* and found quercetin and kaempferol. The levels of quercetin were much lower than those obtained in the present study. De Simone, Dini, Pizza, Saturnino, and Scettino (1990) and Zhu et al. (2001) characterised flavonol glycosides in quinoa (*C. quinoa* Willd) seeds. Zhu et al. (2001) isolated and characterised six flavonol glycosides: four kaempferol glycosides and two quercetin glycosides. Among them kaempferol 3-O-[β-D-apiofuranosyl(1‴-2″)]-β-D-galactopyranoside, kaempferol 3-O-[2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside and quercetin 3-O-[2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside were the main flavonoid glycosides found in quinoa seeds. Kaempferol and quercetin were also the main flavonoid aglycones found in the present study.

There were no quantifiable amounts of flavonoids in amaranth samples: only traces of quercetin were found. Barba de la Rosa et al. (2009) also detected low levels of quercetin glycoside, rutin (4.0–10.2 μg/g) in *A. hypochondriacus* seeds.

Table 3
Total contents (mg/100 g) and percentual shares of soluble phenolic acids in quinoa, kaniwa and kiwicha grains.

Sample	Caffeic acid	Ferulic acid	*p*-Coumaric acid	*p*-OH-benzoic acid	Vanillic acid	Sinapic acid	Protocatechuic acid	Total
Quinoa samples								
Ccoito	0.95 ± 0.04 (63%)	15.3 ± 0.5 (3%)	6.46 ± 0.18 (48%)	3.87 ± 0.07 (66%)	8.97 ± 0.01 (53%)			35.6 ± 0.4 (40%)
INIA-415 Pasankalla	0.61 ± 0.03 (84%)	20.0 ± 0.2 (36%)	27.5 ± 0.4 (72%)	2.44 ± 0.02 (71%)	9.19 ± 0.36 (56%)			59.7 ± 0.5 (61%)
Roja de Coporaque	0.50 ± 0.03 (98%)	13.9 ± 0.6 (50%)	4.07 ± 0.01 (49%)	2.60 ± 0.08 (60%)	11.0 ± 0.3 (42%)			32.1 ± 1.0 (49%)
Witulla	1.47 ± 0.21 (33%)	14.9 ± 0.7 (21%)	2.26 ± 0.08 (39%)	2.46 ± 0.09 (68%)	9.20 ± 0.28 (57%)			30.3 ± 0.6 (38%)
03-21-0093	0.86 ± 0.02 (61%)	16.6 ± 0.5 (39%)	8.72 ± 0.02 (46%)	2.80 ± 0.13 (92%)	10.7 ± 0.5 (44%)			39.7 ± 1.1 (46%)
Salcedo INIA	0.25 ± 0.01 (92%)	12.3 ± 0.9 (17%)	8.02 ± 0.36 (46%)	3.17 ± 0.02 (83%)	14.6 ± 0.2 (39%)			38.4 ± 1.5 (37%)
Commercial 1	0.57 ± 0.02 (39%)	18.6 ± 1.7 (19%)	2.84 ± 0.14 (18%)	3.38 ± 0.24 (63%)	11.9 ± 0.3 (41%)			37.2 ± 1.9 (30%)
Commercial 2	0.87 ± 0.03 (16%)	14.3 ± 0.1 (15%)	2.60 ± 0.03 (27%)	3.88 ± 0.04 (55%)	10.3 ± 0.1 (39%)			32.0 ± 0.1 (29%)
Huaripongo	0.37 ± 0.04 (95%)	12.0 ± 0.1 (19%)	4.01 ± 0.06 (0%)	2.65 ± 0.02 (98%)	12.4 ± 0.1 (10%)			31.4 ± 0.2 (21%)
03-21-1181	0.59 ± 0.07 (0%)	13.7 ± 0.7 (52%)	9.50 ± 0.36 (0%)	1.92 ± 0.08 (56%)	10.7 ± 0.5 (60%)			36.3 ± 1.2 (40%)
Mean ± SD	*0.7 ± 0.4*^{a}	*15 ± 3*^{a}	*8 ± 7*^{a}	*2.9 ± 0.6*^{a}	*11 ± 2*^{a}	*0 ± 0*^{a}	*0 ± 0*^{a}	*37 ± 9*^{a}
Kañiwa samples								
Kello	1.10 ± 0.01 (63%)	26.1 ± 1.9 (4%)	1.34 ± 0.12 (0%)	1.77 ± 0.09 (37%)	4.34 ± 0.30 (20%)			34.7 ± 2.4 (10%)
Wila	2.16 ± 0.02 (69%)	29.8 ± 0.2 (18%)	1.00 ± 0.01 (0%)	1.77 ± 0.04 (30%)	3.61 ± 0.08 (92%)			38.3 ± 0.3 (28%)
Guinda	2.37 ± 0.12 (8%)	26.0 ± 0.8 (10%)	1.74 ± 0.19 (0%)	1.55 ± 0.08 (18%)	3.04 ± 0.18 (89%)			34.7 ± 1.3 (17%)
Ayara	7.04 ± 0.11 (15%)	23.4 ± 1.2 (10%)	0.70 ± 0.04 (0%)	1.97 ± 0.19 (25%)	6.95 ± 0.21 (56%)			40.1 ± 1.7 (19%)
Commercial sample	1.10 ± 0.09 (83%)	12.0 ± 0.4 (6%)	0.37 ± 0.02 (0%)	1.54 ± 0.13 (72%)	3.23 ± 0.38 (97%)			18.3 ± 0.8 (32%)
Mean ± SD	*3 ± 2*^{b}	*23 ± 7*^{b}	*1.0 ± 0.5*^{a}	*1.7 ± 0.2*^{b}	*4 ± 2*^{b}	*0 ± 0*^{a}	*0 ± 0*^{a}	*33 ± 9*^{a,b}
Kiwicha samples								
1	0.85 ± 0.01 (0%)	8.32 ± 0.70 (0%)	0.81 ± 0.04 (0%)	3.16 ± 0.02 (71%)	6.67 ± 0.03 (9%)	0.32 ± 0.04 (0%)	12.8 ± 0.4 (0%)	32.9 ± 1.3 (9%)
2	0.87 ± 0.02 (0%)	6.46 ± 0.64 (0%)	0.99 ± 0.09 (0%)	1.97 ± 0.15 (65%)	4.28 ± 0.42 (5%)	0.09 ± 0.01 (0%)	6.28 ± 0.42 (0%)	20.9 ± 1.4 (7%)
3	0.70 ± 0.07 (0%)	6.21 ± 0.09 (0%)	0.80 ± 0.05 (0%)	3.19 ± 0.02 (47%)	6.38 ± 0.40 (15%)	0.09 ± 0.01 (0%)		17.4 ± 0.6 (14%)
4	1.13 ± 0.04 (0%)	6.57 ± 0.01 (0%)	0.98 ± 0.02 (0%)	3.68 ± 0.10 (15%)	4.35 ± 0.26 (25%)	0.09 ± 0.01 (0%)		16.8 ± 0.4 (10%)
Mean ± SD	*0.9 ± 0.2*^{a,b}	*6.9 ± 1.0*^{c}	*0.89 ± 0.11*^{a}	*3.0 ± 0.7*^{a}	*5.4 ± 1.3*^{b}	*0.15 ± 0.11*^{a}	*5 ± 6*^{b}	*22 ± 7*^{b}

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).

Table 4
Contents of flavonoids in quinoa and kaniwa grains (mg/100 g).

Sample	Myricetin	Quercetin	Kaempferol	Isorhamnetin	Rhamnetin	Total
Quinoa samples						
Ccoito		38.1 ± 2.3	16.3 ± 1.6			54.5 ± 4.0
INIA-415 Pasankalla		35.7 ± 0.2	0.45 ± 0.11			36.2 ± 0.3
Roja de Coporaque	0.22 ± 0.04	55.5 ± 4.2	16.9 ± 1.1			72.6 ± 5.3
Witulla	0.86 ± 0.11	23.5 ± 0.8	44.7 ± 1.2			69.0 ± 2.1
03-21-0093	0.90 ± 0.13	32.6 ± 0.1	14.2 ± 0.7			47.7 ± 1.0
Salcedo INIA		11.6 ± 0.1	54.2 ± 0.5			65.8 ± 0.6
Commercial 1	1.24 ± 0.07	36.8 ± 0.6	10.2 ± 0.3	2.08 ± 0.06		50.3 ± 1.0
Commercial 2	0.51 ± 0.08	47.1 ± 2.4	21.5 ± 1.1			69.2 ± 3.6
Huaripongo	0.88 ± 0.20	53.2 ± 4.1	14.2 ± 0.8	0.89 ± 0.11		69.2 ± 5.2
03-21-1181	0.67 ± 0.12	28.5 ± 2.7	11.5 ± 0.3	1.02 ± 0.10		41.7 ± 3.2
Mean ± SD	*0.5 ± 0.5*^{a}	*36 ± 13*^{a}	*20 ± 20*^{a}	*0.4 ± 0.7*^{a}	*0 ± 0*^{a}	*58 ± 13*^{a}
Kañiwa samples						
Kello		84.3 ± 1.2		60.0 ± 1.3		144.3 ± 2.5
Wila		68.7 ± 5.8		14.2 ± 0.8		83.0 ± 6.6
Guinda		25.1 ± 2.0		29.5 ± 1.3		54.6 ± 3.3
Ayara		21.4 ± 1.4	5.97 ± 0.02		18.7 ± 2.0	46.1 ± 3.5
Commercial sample	0.18 ± 0.01	78.6 ± 6.6	2.24 ± 0.33	24.8 ± 2.4		105.8 ± 9.3
Mean ± SD	*0.04 ± 0.08*^{b}	*60 ± 30*^{a}	*2 ± 3*^{b}	*30 ± 20*^{b}	*4 ± 8*^{a}	*90 ± 40*^{a}

Means within a column with the same superscript letter are not significantly different ($\alpha = 0.05$).

Table 5
Contents of betacyanins in kiwicha grains (mg/100 g).

Sample	Amaranthine	Iso-amaranthine	Betanin	Total
1	nd[a]	nd	nd	nd
2	nd	nd	nd	nd
3	1.0 ± 0.2	0.8 ± 0.2	0.1 ± 0.2	1.9 ± 0.4
4	nd	nd	nd	nd

[a] nd ⩽ 0.1 mg/100 g.

The betacyanin content is presented in Table 5. Of the analysed kiwicha samples only the pink variety contained betacyanins above LOQ (0.1 mg/100 g). The total amount of betacyanins was low (1.9 ± 0.4 mg/100 g dw) compared to the values (mean 91.4 ± 4.0 mg/100 g fresh weight) reported in different vegetative parts, i.e. seedlings, leaves and inflorescences, of *Amaranthus* (Cai et al., 2001). To our knowledge no data on betacyanins in kiwicha seeds have previously been reported in the literature.

Our results indicate that the Andean indigenous crops, quinoa and kañiwa, are very good sources of flavonoids. Kiwicha does not contain quantifiable amounts of these compounds. The levels of flavonoids in quinoa and kañiwa were superior to those in flavonoid-rich berries such as lingonberry and cranberry. The phenolic acid content of Andean indigenous crops is comparable to the content of these substances in oat, barley, corn and rice. Overall, our study demonstrates that Andean indigenous crops have excellent potential as sources of health-promoting bioactive compounds such as flavonoids. More studies are required with the aim of identifying the most promising varieties in this respect.

Further studies should be conducted to determine phenolic compound composition and anti-oxidant content and activity in processed Andean grains.

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References

AOAC (1995). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.

Barba de la Rosa, A. P., Fomsgaard, I. S., Laursen, B., Mortensen, A. G., Olvera-Martínez, L., Silva-Sánchez, C., et al. (2009). Amaranth (*Amaranthus hypochondriacus*) as an alternative crop for sustainable food production: Phenolic acids and flavonoids with potential impact on its nutraceutical quality. *Journal of Cereal Science, 49*, 117–121.

Berganza, B., Alvaro, L., Moran, W., Rodrıguez, G., Coto, N., Santamarıa, M., et al. (2003). Effect of variety and location on the total fat, fatty acids and squalene content of amaranth. *Plant Foods for Human Nutrition, 58*, 1–6.

Bressani, R., De Martell, E., & Godinez, C. (1993). Protein quality evaluation of amaranth in adult humans. *Plant Foods for Human Nutrition, 43*, 123–143.

Bressani, R. (1994). Composition and nutritional properties of amaranth. In O. Paredes-Lopez (Ed.), *Amaranth biology, chemistry and technology* (pp. 185–205). Boca Raton, FL: CRC Press.

Cai, Y., Sun, M., Wu, H., Huang, R., & Corke, H. (1998). Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species. *Journal of Agricultural and Food Chemistry, 46*, 2063–2070.

Cai, Y., Sun, M., & Corke, H. (2001). Identification and distribution of simple and acetylated betacyanins in the Amaranthaceae. *Journal of Agricultural and Food Chemistry, 49*, 1971–1978.

Cai, Y., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry, 51*, 2288–2294.

De Simone, F., Dini, A., Pizza, C., Saturnino, P., & Scettino, O. (1990). Two flavonol glycosides from *Chenopodium quinoa*. *Phytochemistry, 29*, 3690–3692.

Guzman-Maldonado, S., & Paredes-Lopez, O. (1998). Functional products of plants indigenous to Latin America. Amaranth and quinoa, common beans and botanicals. In G. Mazza (Ed.), *Functional foods. Biochemical and processing aspects* (pp. 293–328). Pennsylvania: Technomic Publishing Company.

Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. *International Journal of Molecular Sciences, 8*, 950–988.

Kanner, K., Harel, S., & Granit, R. (2001). Betalains – A new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry, 49*, 5178–5185.

Klimczak, M., Malecka, M., & Pacholek, B. (2002). Antioxidant activity of ethanolic extracts of amaranth seeds. *Nahrung/Food, 46*, 184–186.

Koziol, M. (1992). Chemical composition and nutritional evaluation of quinoa (*Chenopodium quinoa* Willd.). *Journal of Food Composition and Analysis, 5*, 35–68.

Mattila, P., Astola, J., & Kumpulainen, J. (2000). Determination of flavonoids in plant material by HPLC with diode array and electro array detections. *Journal of Agricultural and Food Chemistry, 48*, 5834–5841.

Mattila, P., & Hellström, J. (2007). Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis, 20*, 152–160.

Mattila, P., Pihlava, J.-M., & Hellström, J. (2005). Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry, 53*, 8290–8295.

Pasko, P., Barton, H., Zagrodzki, P., Gorinstein, S., Fołta, M., & Zachwieja, S. (2009). Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry, 115*, 994–998.

Pedreno, M. A., & Escribano, J. (2000). Studying the oxidation and the antiradical activity of betalain from beetroot. *Journal of Biological Education, 35*, 49–51.

Peñarrieta, M., Alvarado, A., Åkesson, B., & Bergenståhl, B. (2008). Total antioxidant capacity and content of flavonoids and other phenolic compounds in canihua (*Chenopodium pallidicaule*): An Andean pseudocereal. *Molecular Nutrition and Food Research, 52*, 708–717.

Ranhotra, G., Gelroth, J., Glaser, B., Lorenz, K., & Johnson, D. (1993). Composition and protein nutritional quality of quinoa. *Cereal Chemistry, 70*, 303–305.

Rastrelli, L., Saturnino, P., Schettino, O., & Dini, A. (1995). Studies on the constituents of *Chenopodium pallicaule* (Cañihua) seeds. Isolation and characterization of two new flavonol glycosides. *Journal of Agricultural and Food Chemistry, 43*, 2020–2024.

Repo-Carrasco, R., Espinoza, C., & Jacobsen, S.-E. (2003). Nutritional value and use of the Andean crops quinoa (*Chenopodium quinoa*) and kañiwa (*Chenopodium pallidicaule*). *Food Reviews International, 19*, 179–189.

Repo-Carrasco-Valencia, R., Peña, J., Kallio, H., & Salminen, S. (2009). Dietary fiber and other functional components in two varieties of crude and extruded kiwicha (*Amaranthus caudatus*). *Journal of Cereal Science, 49*, 219–224.

Scalbert, A., Manach, C., Morand, C., & Rémésy, C. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*, 287–306.

Shahidi, F., & Naczk, M. (1995). *Food phenolics*. Lancaster, USA: Technomic Publishing Co., Inc..

Stintzing, F., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science and Technology, 15*, 19–38.

Tapia, M. (1997). *Cultivos Andinos Subexplotados y Su Aporte a la Alimentación*. Santiago, Chile: Oficina Regional de la FAO para America Latina y el Caribe.

Zhu, N., Sheng, S., Li, D., Lavoie, E. J., Karwe, M. V., Rosen, R. T., et al. (2001). Antioxidative flavonoid glycosides from quinoa seeds (*Chenopodium quinoa* Willd). *Journal of Food Lipids, 8*, 37–44.