

Nutritional composition, total phenolic compounds and antioxidant activity of quinoa (*Chenopodium quinoa* Willd.) of different colours

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) has been nutritionally highlighted when compared to other grains. In recent years the research on this pseudocereal has increased. In this work, six quinoa samples were studied: three from Peru, one from Brazil and two commercial samples. The samples were physically and physicochemically characterized, including macro- and micronutrient analysis, phenolic compounds content and antioxidant activity. Black, red and white samples showed as main difference the size, weight, ashes and dietary fibre content. Black samples were the smallest and lightest and had the lowest starch content but presented the highest levels of ashes and dietary fibre. The protein content (16.9 %) in the white Brazilian variety was higher than the others. Red and black samples had the highest levels of most minerals analysed. The antioxidant capacity measured by the DPPH method was higher for black and red samples in comparison with the white ones. However, the white Brazilian variety showed a significantly higher antioxidant capacity measured by the ABTS assay. With regard to the phenolic content, a difference was found between the samples which ranged from 55.5 to 95.5 g GAE 100 g⁻¹. The colour of the grain was found as not related to a higher content of phenolic compounds. Because their compositions are generally similar to light-coloured grains, and in some parameters such as dietary fibre and content of some micronutrients are superior, the grains of dark-coloured quinoa varieties (RPP, BCP) would have to be explored to develop foods that take advantage of this colour diversity.

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Introduction

Quinoa (*Chenopodium quinoa* Willd.) has been cultivated by the inhabitants of the Andes for human consumption for centuries and in recent years it has increased its sowing and market value in part to its excellent nutritional characteristics

(Navruz-Varli and Sanlier 2016). The light-coloured varieties had been the most preferred by the consumers and also the most studied. Several studies had already reported the nutritional profile of these varieties (Koziol 1992; Ando *et al.* 2002; Aluwi *et al.* 2017) indicating also the presence of some anti-nutrients like phytates and saponins.

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The relationship between the content of saponins and the colour of the quinoa grains has been studied showing that the whites are sweeter (lower saponins content) than the yellow ones (Souza *et al.* 2004).

With regard to the phenolic acid content of quinoa of different colours, the published results were inconclusive. Some have reported that the content of phenolic acids and flavonoids did not differ much between samples of different colours, highlighting only some variety while others of similar colour presented similar values to the rest (Repo-Carrasco-Valencia *et al.* 2010). On the other hand, comparing one cream-coloured quinoa sample with other white ones, the first has been reported to have a higher content of total polyphenols and scavenging activity (Repo-Carrasco-Valencia *et al.* 2011). Other authors have pointed out that for red quinoa varieties, the ones that were redder would have greater antioxidant activity and higher content of phenolic compounds (Abderrahim *et al.* 2015).

It is reported that the content and characteristics of starch and protein from different quinoa lines vary due to the environment and genetic and agronomic differences (Lindeboom 2005). In the same way, the variation in the mineral content of quinoa of different varieties is due to the interactions of genotype and environmental parameters (Prado *et al.* 2014). The content of certain minerals was higher in varieties that were of a determined colour. Physically, some coloured varieties were reported to be heavier and larger than the white ones (Apaza *et al.* 2013). Thus, our aim was to determine some characteristics of different quinoa varieties in order to confirm possible relationships between their values and the colour.

Experimental

Quinoa samples

Three samples of Quinoa (*Chenopodium quinoa* Willd.) grains were obtained from the National Agricultural Research Institute (INIA) of Peru: INIA 415 “Pasankalla”, INIA 420 “Negra Collana” and INIA “Salcedo”, coded as RPP, BNP and WSAp for this study. RPP, BNP and WSAp

were released in 2006, 2008 and 1995 respectively (Apaza *et al.* 2013). Two commercial samples from Arequipa, Peru were purchased for the study (coded as RCP and BCP for this study) and a Brazilian quinoa sample (BRS “Syetetuba”, coded as WSyB, released in 2006 according to Spehar *et al.* 2011) was obtained from the farm of the Federal University of Brasilia. RPP and BNP samples were sown in 2011 and harvested in 2012 (Puno, Peru), WSAp was sown in 2013 and harvested in 2014 (Puno, Peru), WSyB was harvested in 2014 (Brasilia, Brazil), the commercial varieties (RCP and BCP) were both acquired in 2014. The samples of quinoa grains are shown in Fig. 1.



Fig. 1. Quinoa grains of different colors. RPP: INIA 415 “Pasankalla”, RCP: Red Peruvian commercial sample, BNP: INIA 420 “Negra Collana”, BCP: Black Peruvian commercial sample, WSyB: BRS “Syetetuba”, WSAp: INIA “Salcedo”.

Saponin removal

The saponin content in the samples was evaluated by a semiquantitative method described by Koziol

(1990). This method is based on the tensioactive properties of the saponins, which form a stable foam whose height is related to the saponin content in the grains after being dissolved in water and shaken. The percentage of saponins was obtained with Eq. 1.

$$\text{saponin (\%)} = \frac{0.646 \times (\text{height of foam [cm]}) - 0.104}{(\text{weight of the sample [g]}) \times 10} \quad (1)$$

Preparation of quinoa samples

The quinoa grains were washed with distilled water (at 37 °C) in the ratio 1:2 (quinoa: distilled water). Then the grains were shaken by hand for 20 min with distilled water and rinsed. This procedure was performed twice and then the grains were dried at 37 °C for 12 hours in a hot air oven. The dried grains were stored in polypropylene bags in a cold storage chamber (10 °C) for two months before use (August 2014). For the colour evaluation, four sub-samples were taken from these stored grains. For the determination of size distribution and weight of thousand grains eight sub-samples were taken. Three, two and six sub-samples were taken to the physical-chemical, elemental and antioxidant activity analysis respectively. For all cases, each of these sub-samples were processed according to the corresponding methodology described in this work.

Colour evaluation

The colour of the quinoa grains was evaluated using the colourimeter Chroma Meter 200b (Minolta Co., Osaka, Japan) by the CIE 1976 L*a*b* system obtaining the values of L* (lightness), a* and b* (opponent colour axes). The hue angle (H°) and chroma (C*) were calculated according to the Eq. 2 and 3.

$$H^\circ = \text{tg}^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\text{Chroma (C}^*) = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

Size distribution and weight of thousand grains

The dried quinoa grains after saponin removal were analyzed by size. For this purpose, Tyler/mesh sieves number 12, 14 and 20, corresponding to openings of 1.41 mm, 1.19 mm, and 0.841 mm

respectively, were used. The amounts retained in each sieve were weighed and expressed in percentages. In addition, 100 whole grains without impurities were counted manually, in 8 replicates, for the determination of the weight of one thousand grains. After weighing using a Sartorius BL210S analytical balance (Sartorius, Germany), the mean, standard deviation and coefficient of variation of the measurements were calculated. The result of a thousand grains was calculated by multiplying by 10 the average weight of the repetitions if the coefficient of variation was less than 4 %. As the weight of a thousand grains of a sample varies according to the moisture content the results obtained were standardized to 10 % moisture, which was the average moisture obtained from the grains at the time of evaluation.

Physical-chemical analysis

The grains previously washed were ground in a Marconi MA-090 hammer mill (Marconi Ltd., Piracicaba, Brazil) with a 20 mesh screen (0.841 mm). The milled grains were evaluated for moisture using an A&D MX-50 Moisture Analyzer (A&D Co. Ltd., Tokyo, Japan). The ash content (calcination in muffle at 550 °C for two hours), lipids (extraction with ethyl ether using Soxhlet extractor) and crude protein (Kjeldhal digestion with the factor 6.25 for conversion of the total nitrogen content) were determined according to the methods 923.03, 920.39C and 979.09A described by AOAC (2005). The starch content of the milled grains of each sample was determined by the Megazyme Total Starch Kit (AA / AMG) K-TSTA 09 (Megazyme, Ireland).

Macro- and microminerals content

Macrominerals phosphorus, potassium, calcium, magnesium, sulfur and microminerals iron, zinc, copper, manganese, aluminum, sodium, and lithium were determined using a methodology based on the method 953.01 by AOAC (2005). For all elements, nitroperchloric digestion was used, except for boron, which was extracted by dry digestion in muffle according to Malavolta *et al.* (1997). The quantification of the elements was performed in an atomic absorption spectrophotometer,

the lithium was quantified in a coupled plasma mass spectrometer (iCAP 7400 ICP-OES Analyzer, Thermo Fisher Scientific, USA). The results were expressed as mg 100 g⁻¹ of dry basis product.

Determination of phenolic compounds

The content of total phenolic compounds (TPC) was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton *et al.* 1999) using gallic acid as standard. The hydroalcoholic extracts were obtained according to the methodology of Repo-Carrasco-Valencia *et al.* (2010), with modifications. A sample containing 5 g of milled grains was dissolved in 20 mL of 95 % ethanol. After homogenization for 1 min the samples were stored at -4 °C for 18 hours. Then the extracts were centrifuged at 29,000 g for 20 min in an Eppendorf 5810 R refrigerated centrifuge at 4 °C (Eppendorf AG, Hamburg, Germany) and filtered using Whatman filter paper No. 2 and stored in amber bottles under refrigeration at 7 °C until the time of analysis. Aliquots of 0.5 mL of the hydroalcoholic extract were transferred to test tubes, where 4 mL of distilled water and 0.5 mL of the Folin-Ciocalteu solution were added. Then the tubes were shaken in a vortex mixer and put aside for 3 min. After the addition of 0.5 mL of a 4 % (w/v) sodium carbonate solution, the tubes were vortexed again and held for two hours at room temperature and protected from light. Immediately after, the absorbance reading was performed in a FEMTO 700S spectrophotometer at 740 nm (Sao Paulo, Brazil). The results were expressed in milligrams of gallic acid equivalent (GAE) per gram (dry basis).

Evaluation of antioxidant capacity

The evaluation of the antioxidant capacity of the hydroalcoholic extracts obtained for the determination of phenolic compounds was performed using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assays. For the DPPH assay the methodology proposed by Brand-Williams *et al.* (1995) was used. An aliquot (500 µL) of solution was

transferred to a test tube containing 3 mL of 95 % ethanol and 300 µL of the DPPH solution. The solution was held out of the light for 45 min previously to the absorbance reading at 515 nm using a spectrophotometer and compared with a blank. A standard curve was obtained using trolox solutions from 5 to 50 µM. The trolox equivalent antioxidant capacity (TEAC) was obtained with Eq. 4 and expressed as µmol TEAC g⁻¹ db (dry basis).

$$DPPH \text{ reduced } (\%) = \frac{Absorbance_{blank} - Absorbance_{sample}}{Absorbance_{blank}} \times 100 \quad (4)$$

The methodology proposed by Re *et al.* (1999) was used to evaluate the reduction of the ABTS radical. An aliquot (30 µL) was transferred to a test tube containing 3.0 mL ABTS (7 mM). The solutions were kept in the dark for 6 min and the absorbance was read at 734 nm. The standard curve was obtained from 500 to 2000 µM using trolox solutions. The results were expressed in µmol TEAC g⁻¹ db using a blank as a reference.

Statistical analysis

The results were subjected to analysis of variance (ANOVA) and the Tukey test for comparison of means ($p < 0.05$) using the SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Saponin content

The quinoa grains of the different samples were evaluated for saponin content before and after saponin removal. The grains presented low initial saponin content, from nearly zero (≤ 0.01 % db for RPP and BCP) to ≤ 0.04 % db (RCP, BNP and WSAp), except for WSAp which presented a high content (0.34% db). BNP and RPP were considered as free from saponins by Apaza *et al.* (2013) like WSAp according to Spehar *et al.* (2011), which differed from our results. Koziol (1992) suggested, as possible explanation, the different environmental conditions of grain production or the different methodologies used to determine the saponin

Table 1. Lightness (L^*), opponent colour axes (a^* and b^*), chroma (C^*) and hue angle (H°) of quinoa grains. Four replicates. Values followed by the same letter in the column do not differ (Tukey test, $p < 0.05$).

Sample	L^*	a^*	b^*	Chroma (C^*)	H°
RPP	33.4±1.4 ^c	16.2±1.2	25.4±2.8	30.2±2.9 ^a	57.4±1.5 ^b
RCP	30.8±1.5 ^d	18.9±0.9	22.1±2.1	29.1±2.1 ^a	49.4±1.7 ^c
BNP	14.1±1.8 ^e	7.3±1.5	4.9±0.9	8.8±1.7 ^c	34.2±4.4 ^e
BCP	13.2±2.6 ^e	6.9±1.6	5.7±1.5	9.1±1.6 ^c	39.4±9.3 ^d
WSyB	66.0±0.9 ^b	2.9±2.7	23.1±0.7	23.2±0.7 ^b	82.8±0.5 ^a
WSaP	76.1±1.2 ^a	2.1±0.3	24.8±1.0	24.9±1.0 ^b	85.3±0.7 ^a

content. Our results showed that after saponin removal procedure, the saponin content was 0.01 % (dry basis) for BSyB and 0 % for the other cultivars. According to this author the saponins are the main antinutritional factors of the quinoa grain but they can be removed by wet or abrasive methods, reaching levels not exceeding 0.01 %, as observed in our samples because such substances are concentrated in the outer layers of the grains. It has been reported that some plant-based saponins can form complexes with iron and zinc, which would reduce absorption in rats (Southon *et al.* 1988). However, saponins have very low toxicity to other mammals (Malinow *et al.* 1982) and do not affect protein availability in the case of quinoa (Ruales and Nair 1992).

Color evaluation

The studied quinoa samples presented different colour parameters as shown in Table 1. In the literature consulted the saponin content was related to the colour; reporting that the yellow cultivars had more saponin than the white ones (Souza *et al.* 2004). The values observed for H° (above 80) suggest that the samples considered white could be

classified with a tendency to yellow. However, even though the white Brazilian sample (WSyB) had the highest content of saponins, the Peruvian white sample (WSaP) had a similar content to the samples of different colour, so it is not possible to confirm with our data the relationship between colour and content of saponins in quinoa grains of different colours. The differences observed for H° , among the grains of the same colour, RPP and RCP and BNP and BCP samples, may be due to the commercial samples (RCP and BCP) are not standardized and a mixture of different colours may have occurred.

Medina *et al.* (2010) tried to use image analysis to determine the geographic origin of different quinoa grains but proved that it was impossible to use the colour of the grains for this purpose. However, it has been stated that the colour of grains of dark quinoa varieties is an indicator of the presence of betacyanins (low L^* values), while the colour of lighter varieties is related to a higher betaxanthin content (Escribano *et al.* 2017). Thus, the main use of measuring the colour of quinoa grains has been as a characteristic associated with their composition. As regards manufactured products (based on quinoa flour),

Table 2. Distribution of quinoa grains by size and weight of one thousand grains. For size distribution $n = 4$ whereas for the weight of one thousand grains $n = 8$. Values followed by the same letter in the row do not differ (Tukey test, $p < 0.05$).

Tyler mesh No. (aperture in mm)	Percentage with respect to the weight of one thousand grains [%]					
	RPP	RCP	BNP	BCP	WSyB	WSaP
12 (1.41)	86.2	83.9	12.6	19.6	78.2	85.7
14 (1.19)	13.4	13.9	81.0	74.7	21.2	13.7
20 (0,841)	0.4	2.2	6.4	5.4	0.4	0.4
Bottom (< 0.841)	0.0	0.0	0.0	0.3	0.2	0.2
One thousand grains weight [g]	3.71±0.06 ^c	4.11±0.09 ^a	2.30±0.07 ^e	2.06±0.05 ^f	3.88±0.06 ^b	3.12±0.09 ^d
Coefficient of variation [%]	1.6	2.1	3.2	2.3	1.6	2.9

Table 3. Physical-chemical composition of quinoa grains. Three replicates. Values followed by the same letter in the column do not differ (Tukey test, $p < 0.05$).

Quinoa samples	Ash [%]	Protein [%]	Lipid [%]	Total Dietary Fibre [%]	Starch [%]
RPP	2.51±0.02 ^b	14.0±0.3 ^b	7.33±0.11 ^a	14.30±0.01 ^b	56.02±0.03 ^b
RCP	2.58±0.02 ^b	12.5±0.6 ^b	6.70±0.14 ^b	13.90±0.06 ^b	54.56±0.01 ^d
BNP	2.96±0.10 ^a	14.1±0.1 ^b	6.67±0.10 ^b	18.20±0.00 ^a	49.25±0.07 ^c
BCP	3.01±0.09 ^a	14.0±0.2 ^b	7.34±0.11 ^a	19.70±0.22 ^a	47.22±0.09 ^f
WSyB	2.32±0.01 ^c	16.9±0.2 ^a	6.84±0.08 ^b	8.70±1.67 ^c	55.39±0.05 ^c
WSaP	2.36±0.02 ^c	13.7±0.1 ^b	7.29±0.03 ^a	10.50±0.45 ^c	59.72±0.05 ^a

it has been observed that there is an effect on the colour of the final product in relation to the percentage of flour used, but it was not indicated whether the pigments of quinoa grains played a role in this characteristic (Demir and Kiliç 2017).

Size distribution and weight of thousand grains

The red and white samples showed larger grains than the black ones (Table 2). This is consistent with the data from Apaza *et al.* (2013) who reported the average diameter of BNP, RPP and WSaP as 1.60, 2.10 and 2.00 mm, respectively. The same authors also reported the weight of thousand grains for BNP, RPP and WSaP as 2.03, 3.51 to 3.72 and 3.10 to 3.70 g, respectively. This trend is followed by our data, which showed that black samples are lighter than the others. Values above 3.0 g per thousand grains are considered as large grains (Spehar *et al.* 2011) and considered more desirable in the growing quinoa market.

Physical-chemical analysis

All the samples had moisture levels below 12 % (from 8.3 to 11.2 %), being thus suitable for storing according to Spehar (2006). The black samples presented the highest values for the ash content (Table 3), around 3 % db, followed by the red and white samples. These values were similar to those found by Repo-Carrasco-Valencia *et al.* (2010) for the cultivars RPP (2.5 %) and WSaP (2.4 %). All the samples analysed had a similar protein content, with only one of the white samples (WSyB) standing out. With respect to lipid content,

a sample of each colour (RPP, BCP, WSaP) was particularly noteworthy. The protein content of the samples was also similar to those observed by Miranda *et al.* (2012), who reported values from 11.32 to 16.10 % db. Repo-Carrasco-Valencia *et al.* (2010) reported values for lipids ranging from 5.2 to 6.8% for white, black and red cultivars. Meanwhile, Ando *et al.* (2002) found 6.5 % of lipids in the white cultivar Real, which was slightly lower than our results for samples of the same colour. The content of lipids of all samples was higher than other cereals such as rice, corn, wheat and barley, which present 2.2 %, 4.7 %, 2.3 % and 1.9 %, respectively (Kozioł 1992). The two white samples had the lowest values of dietary fibre, followed by the two red samples. The dietary fibre content of black samples is comparable to that of hulled barley and superior to that of raw white rice (17.3 % and 1.3 % respectively, according to USDA (2017)). The variation in the ash, protein, lipid, and fibre contents among quinoa samples can be attributed to the production site (climate, soil, temperature, management) and to the genetic characteristics of the cultivars (Lindeboom 2005; Repo-Carrasco-Valencia *et al.* 2011).

The main component found in the samples was starch (Table 3). The observed results for starch are close to the ranges already reported in previous studies. The composition of quinoa grains of different colours (red, yellow and white) was evaluated by Bruin (1964), reporting the starch content ranged from 58.1 to 64.2 % and the white samples showed the highest levels. On the other hand, Lindeboom (2005) evaluated 8 lines of quinoa grains founding that the starch content varied from 48.3 to 62.5 % db, but the colour of the grains was not specified.

Table 4. Contents of macrominerals and microminerals in mg 100 g⁻¹ db, except for Li. Two replicates. Values followed by the same letter in the row do not differ (Tukey test, $p < 0.05$).

Element	Quinoa samples					
	RPP	RCP	BNP	BCP	WSyB	WSaP
P	578±13 ^a	588±13 ^a	512±14 ^b	490±7 ^b	510±12 ^b	492±4 ^b
K	699±1 ^b	593±5 ^c	815±33 ^a	693±1 ^b	559±10 ^c	663±1 ^b
Ca	73±3 ^b	109±0 ^a	71±7 ^b	118±1 ^a	69±5 ^b	77±2 ^b
Mg	243±7 ^{bc}	264±5 ^a	226±6 ^{cd}	246±0 ^{ab}	239±5 ^{bcd}	220±1 ^d
S	185±5 ^a	174±5 ^{ab}	158±14 ^{ab}	144±1 ^b	177±16 ^{ab}	164±2 ^{ab}
Fe	5.63±0.15 ^{cd}	5.25±0.03 ^d	7.28±0.18 ^{bc}	17.2±1.10 ^a	8.59±0.09 ^b	4.74±0.03 ^d
Zn	3.70±0.05 ^b	3.79±0.04 ^b	2.98±0.06 ^d	3.36±0.04 ^c	5.12±0.08 ^a	3.17±0.03 ^{cd}
B	1.34±0.05 ^a	1.16±0.03 ^b	1.01±0.01 ^{cd}	0.92±0.01 ^d	1.11±0.02 ^{bc}	0.80±0.02 ^e
Cu	0.47±0.01 ^b	0.50±0.01 ^b	0.47±0.04 ^b	0.57±0.00 ^a	0.48±0.01 ^b	0.51±0.01 ^{ab}
Mn	6.90±0.15 ^c	3.41±0.02 ^e	9.48±0.11 ^a	7.99±0.06 ^b	2.53±0.07 ^f	5.53±0.10 ^d
Al	2.21±0.05 ^{dc}	2.82±0.25 ^c	1.84±0.01 ^d	5.87±0.20 ^a	4.54±0.19 ^b	1.79±0.07 ^d
Na	17.5±0.29 ^c	36.9±0.56 ^b	11.8±0.32 ^d	49.3±0.17 ^a	17.1±0.06 ^c	16.6±0.38 ^c
Li (µg kg ⁻¹)	0.14±0.01 ^b	0.58±0.02 ^a	0.14±0.02 ^b	0.65±0.08 ^a	0.17±0.02 ^b	0.19±0.00 ^b

Macro- and micromineral content

A high content of P and K was observed, confirming that these two elements represent the greatest amount of minerals in quinoa grains (Bruin 1964; Mota *et al.* 2016). Red samples showed the highest phosphorus content with respect to the others samples. RPP and RCP had more P content than varieties from Chile (285.6 to 526.36 mg 100 g⁻¹ db according to Miranda *et al.* 2012) or Bolivia (123.72 to 330.96 mg 100 g⁻¹ db according to Prado *et al.* 2014). The content of P in quinoa is higher than that found in maize (210 mg 100 g⁻¹), wheat (323 mg 100 g⁻¹), rice (95 mg 100 g⁻¹) and barley (221 mg 100 g⁻¹) (USDA 2017). It was reported that the potassium content of quinoa milled grains was 639.3 mg 100 g⁻¹ (Ando *et al.* 2002), whereas (Prado *et al.* 2014) showed a wider range (from 498.47 to 995.24 mg 100 g⁻¹). Those results were consistent with ours although no colour effect has been observed and just BNP stood out from the rest. Quinoa has higher potassium contents than other cereals and pseudocereals of interest as corn, wheat, rice and barley (USDA 2017) and amaranth (Nascimento *et al.* 2014). It has been reported that even after quinoa grains were cooked, potassium and phosphorus remained the main elements (Mota *et al.* 2016). The commercial samples RCP and BCP had the higher calcium content, with no grain

colour effect observed. Other studies had reported broader ranges in the concentration of this mineral. Varieties analyzed by Miranda *et al.* (2012) ranged from 25.15 to 116.60 mg 100 g⁻¹, whereas Prado *et al.* (2014) reported a range from 77.10 to 211.29 mg 100 g⁻¹. The saponin removal process could decrease the percentage of calcium because this mineral is more concentrated in the pericarp followed by the embryo and perisperm (Konishi *et al.* 2004).

Magnesium was present in more concentration than reported for other quinoa varieties (Miranda *et al.* 2012; Prado *et al.* 2014) but close to 250 mg 100 g⁻¹, informed by Koziol (1992). However, it has been recorded values as high as 502.0 mg 100 g⁻¹ (Konishi *et al.* 2004). In comparison, the magnesium content of hard red winter wheat and hulled barley was reported as 126 and 35 mg 100 g⁻¹ respectively (USDA 2017).

Although there is no recommended intake for sulphur, and that deficiencies of this mineral are not known (Sizer and Whitney 2003), our results were consistent with those of Bruin (1964), who reported a range from 150 to 220 mg 100 g⁻¹. The genetic characteristics influence the mineral composition of the quinoa grains, as well as the type of soil where they are cultivated due to the chemical or mineral composition of the soils and fertilizers used (Vega-Gálvez *et al.* 2010; Nascimento *et al.* 2014). In relation to the

microminerals evaluated (Table 4), it was observed that the iron content was in the range previously reported by Miranda *et al.* (2012), who reported a range from 4.82 to 7.19 mg 100 g⁻¹, except for BCP which stood out from the rest with 17.2 mg 100 g⁻¹. However, Prado *et al.* (2014) reported a wider range from 4.76 to 24.04 mg 100 g⁻¹, evidencing the great variation that can exist between the different samples of quinoa grains, as well as in this study. It was reported that the hard red winter wheat and hulled barley had just 3.19 and 3.60 mg 100 g⁻¹ of iron respectively, whereas the raw medium-grain white rice had 0.80 mg 100 g⁻¹ (USDA 2017).

The zinc content in the samples was similar to the range reported by Miranda *et al.* (2012) (from 2.73 to 5.01 mg 100 g⁻¹) and Prado *et al.* (2014) (from 1.65 to 4.22 mg 100 g⁻¹). One white sample (WSyB) had more zinc content than the red and black samples although the other white sample (WSaP) show a similar value to the black ones, so it was not possible to establish a colour-based relationship with our data.

Red samples (RPP and RCP) had the highest boron content. These results were similar to those of Bruin (1964), who reported a range from 0.92 to 1.29 mg 100 g⁻¹, but lower than those of Karyotis *et al.* (2003) who reported a completely different range (from 3.4 to 4.7 mg 100 g⁻¹). Boron is considered to be essential for animals, but the requirement for humans is still under study (Sizer and Whitney 2003).

As far as the authors know, the presence of aluminium in quinoa was analysed previously only by Bruin (1964), who reported a range from 6 to 10 mg 100 g⁻¹. Our results are lower than those but since aluminium is not known to have a biological function in animal or human organisms (Stahl *et al.* 2017), it is not considered an essential nutrient but an anti-nutrient.

A black sample (BCP) had the higher copper content although the other black showed a similar value than the ones of different colour. Overall, our results were in the range reported by Prado *et al.* (2014) (from 0.35 to 1.12 mg 100 g⁻¹) for varieties harvested in Bolivia. However, copper content of quinoa harvested in Chile showed a different and higher range: from 0.75 to 1.52 mg 100 g⁻¹ (Miranda *et al.* 2012).

Both black samples (BNP and BCP) presented higher content of manganese than the red ones and lastly by the white ones. Our results for black and red samples were higher than those reported by Prado *et al.* (2014) (from 1.55 to 3.85 mg 100 g⁻¹) and the black samples even surpassed the levels reported by Miranda *et al.* (2012) (from 2.36 to 6.47 mg 100 g⁻¹).

With regard to sodium and lithium content, black and red samples showed the highest levels. In the case of sodium, our results were higher than those by Prado *et al.* (2014) (from 1.74 to 7.33 mg 100 g⁻¹) and Karyotis *et al.* (2003) (0.34 to 2.13 mg 100 g⁻¹) but lower than those reported by Bruin (1964) (from 11 to 22 mg 100 g⁻¹). Regarding the lithium, both commercial samples (BCP and RCP) had the highest content. Nascimento *et al.* (2014) observed lithium contents of 7.95 g 100 g⁻¹, being higher than those found in the present study. However, grains of Bolivia quinoa showed levels as high as 7.5 mg 100 g⁻¹ (Figuroa *et al.* 2013).

These authors have attributed the high lithium content to the Salar de Uyuni (Bolivia), which is considered to be among the areas with the highest lithium content in the world, so the dissimilar data found in the literature on the micromineral contents in quinoa grains could probably be explained by the type of soil and climate from which the cultivars come from.

Table 5. Content of total phenolic compounds present in quinoa grains. Six replicates. Values followed by the same letter in the column do not differ (Tukey test, $p < 0.05$).

Samples	TPC [mg GAE 100 g ⁻¹]
RPP	61.1±3.9 ^{bc}
RCP	65.4±4.0 ^b
BNP	95.9±3.6 ^a
BCP	55.5±3.7 ^c
WSyB	66.4±5.6 ^b
WSaP	59.6±4.4 ^{bc}

Content of total phenolic compounds

One of the black samples (BNP) showed the higher content of phenolic compounds (Table 5). The RPP cultivar was analysed reporting a content of 53.8 mg GAE 100 g⁻¹ (Repo de Carrasco and Zelada 2008), lower than that found in the present study. Fifteen quinoa cultivars evaluated by the same authors presented values ranging from 35.3

to 139.9 mg GAE 100 g⁻¹, a range that encompasses our results. Other studies presented values of phenolic compounds above 3.75 mg GAE g⁻¹, using a different methodology for the extraction process (using methanol and then acetone as extraction solvents, as reported by Paško *et al.* (2009). Repo-Carrasco-Valencia *et al.* (2011) evaluated four different quinoa (Blanca de Juli, Kcancolla, La Molina and Sajama) cultivars from Peru and observed phenolic compounds values higher than ours ranging from 142 to 197 mg GAE 100 g⁻¹.

It has been noted that the content of secondary plant metabolites, such as phenolic compounds, varies from generation to generation, depending on environmental factors (Verma and Shukla 2015). These factors include soil nutrients and rainfall (Borges *et al.* 2013). Because our samples were obtained from different locations in a different year, the effect of these factors on phenolic compounds should be considered, as some plant species have been reported to produce more phenolic compounds and antioxidant activity in highland and semi-arid climates (Kumar *et al.* 2017).

It was expected that the black and red samples had a higher TPC value than the white ones since a previous study reported that an improvement in TPC was accompanied by an increase in the pigments content in quinoa grains (Abderrahim *et al.* 2015). The same authors reported a negative correlation between TPC and lightness (L) value ($r = -0.619$, $p = 0.024$). However, our results did not allow us to confirm this relationship because the other black sample (BCP) had the lowest value of TPC, so other factors besides pigmentation must have influence on the content of phenolic compounds in quinoa grains. In this regard, Escribano *et al.* (2017) quantified some pigments (amaranthin, iso-amaranthin, betanin, iso-betanin, dopaxanthin, dopamine-BX, proline-BX and one unknown-BX) of RPP, BNP, WSaP and 26 other quinoa varieties of different colors, detecting a concentration of amaranthin and iso-amaranthin of 0.8 mg kg⁻¹ (for each pigment) for RPP only, while the pigment content of the WSaP and BNP varieties was undetectable, although the colour varieties showed higher antioxidant activity. However, Tang *et al.* (2015b) presented data showing that the TPC content in Canadian

commercial samples of dark-coloured quinoa is greater than that of white samples, with ferulic acid being the main contributor to the TPC value of black samples. Therefore, it would be necessary to further quantify the content of ferulic acid and other phenolic compounds to elucidate whether the storage conditions or genotype of the present BCP sample are responsible for its low TCP value.

Evaluation of antioxidant capacity

The antioxidant capacity of the studied quinoa samples, evaluated by the DPPH method are shown in Table 6. It was previously reported that the antioxidant activity of the black quinoa grain samples was approximately 5.6 µM Trolox equivalent g⁻¹, while for the white and red samples it was between 4.4 and 4.8 µM Trolox equivalent g⁻¹ (Tang *et al.* 2015a), respectively. Our results shown a similar trend, being the coloured grains those with the highest antioxidant activities. However, an unidentified Bolivian quinoa sample presented 38.84 µM Trolox equivalent g⁻¹ (Paško *et al.* 2009), a value much higher than ours. A wide range of activity results was also reported by Repo de Carrasco and Zelada (2008), who evaluated the antioxidant capacity of 15 quinoa cultivars by the DPPH method, obtained values ranging from 117.5 to 2,400.5 µg Trolox g⁻¹, although the grain colour was not specified. The same authors presented values for amaranth in the range of 556.49 to 660.90 µg Trolox g⁻¹. For six varieties of barley, Ondrejovič *et al.* (2014) determined antioxidant activity of 200 to 1,400 µg Trolox g⁻¹. Regarding the percentage of inhibition, the black and red samples had higher values than the white ones which also presented the lowest contents of phenolic compounds and antioxidant capacity.

Analyzing the results of antioxidant capacity determined by the ABTS assay (Table 6), it can be observed that one white sample (WSyB) presented the highest antioxidant capacity, followed by one of the black samples (BCP). Repo-Carrasco-Valencia *et al.* (2011) studied four light-coloured quinoa cultivars (Blanca Juli, Kcancolla, La Molina 89 and Sajama) which presented higher values from 2,351.9 to 3,689.5 µg Trolox g⁻¹, being the yellow cultivar (La Molina 89) the one with the highest value.

Overall the results of antioxidant capacity are lower than those presented in the literature for quinoa. These large differences with respect to our results may be partly explained by the different extraction

Table 6. Antioxidant activity of quinoa grains using DPPH and ABTS and percentage of inhibition. Six replicates. Values followed by the same letter in the row do not differ (Tukey test, $p < 0.05$).

	Quinoa samples					
	RPP	RCP	BNP	BCP	WSyB	WSaP
DPPH [$\mu\text{M TEAC g}^{-1}$]	5.67 \pm 0.29 ^b	4.35 \pm 0.20 ^c	5.99 \pm 0.33 ^{ab}	6.18 \pm 0.31 ^a	3.33 \pm 0.39 ^d	1.95 \pm 0.16 ^e
DPPH [$\mu\text{g TEAC g}^{-1}$]	1,419.1	1,088.8	1,499.2	1,546.8	833.5	488.1
Inhibition [%]	45.0 \pm 2.4 ^a	34.4 \pm 1.6 ^b	47.6 \pm 2.7 ^a	47.4 \pm 2.6 ^a	23.5 \pm 3.3 ^c	12.0 \pm 1.3 ^d
ABTS [$\mu\text{M TEAC g}^{-1}$]	2.02 \pm 0.19 ^c	1.79 \pm 0.07 ^d	1.98 \pm 0.10 ^c	2.25 \pm 0.08 ^b	2.78 \pm 0.14 ^a	1.95 \pm 0.12 ^{cd}
ABTS [$\mu\text{g TEAC g}^{-1}$]	505.6	448.0	495.6	563.2	695.8	488.1
Inhibition [%]	12.92 \pm 1.85 ^{bc}	10.59 \pm 0.71 ^d	12.45 \pm 0.98 ^c	14.59 \pm 0.39 ^b	18.67 \pm 1.42 ^a	10.32 \pm 1.25 ^d

methodologies, so we could not accurately estimate the effect of colour.

Conclusions

This study has determined several nutritional characteristics of quinoa varieties of different colours (black, red and white) obtained from different sites (Arequipa and Puno regions, in Peru) in different harvest years (2012 and 2014). Physically, the red samples were larger and heavier than the white ones, and these in turn were larger and heavier than the black ones, confirming previous reports. Regarding the main anti-nutrient, one of the white samples (WSyP) had the highest content of saponins while BCP and RPP had a content of less than 0.01 % db. The lipid and protein content were similar for all the samples but black samples presented the lowest starch content. Black and red samples had more content of ashes and dietary fibre than the white ones indicating a possible correlation just for this two components. Samples of red and black colour stood out for their macrominerals content, especially RCP for Mg and BNP for K. The microminerals analysis revealed the same trend, with BNP standing out in most results although it was matched in aluminium content by WSyB. This white sample had the highest zinc content. The black samples showed the highest and lowest levels of phenolic compounds, while the red and white samples had similar results. Regarding the antioxidant activity, black and red samples showed the highest activities, highlighting BCP using the DPPH method. Using the ABTS method

there was no clear differentiation, although WSyB showed increased antioxidant activity and inhibition percentage. Thus, the relationship between the colour of the samples and the antioxidant activity could not be demonstrated with our results, although further studies are needed to assess the effect of environmental conditions on the concentration of secondary metabolites of interest, such as phenolic compounds. However, all the quinoa samples were found to be a good source of nutrients.

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