Original article

**Phenolic, apparent antioxidant and nutritional composition of quinoa (Chenopodium quinoa Willd.) seeds**

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**Abstract** Quinoa, a gluten-free pseudocereal, has a nutrient and phytochemical profile which may benefit health. Our aim was to investigate the variability in the phenolic and apparent antioxidant content of different quinoa varieties to identify a variety with a high phytochemical content to use in a quinoa-enriched bread. The results showed that free phenolics contributed most (50–83%) to total phenolic content compared with conjugated or bound forms. Apparent antioxidant activities measured by FRAP, ABTS⁺ and DPPH of free, conjugated and bound extracts were broadly similar, except for free antioxidants when determined by FRAP, which were higher. Phenolic content was positively correlated with FRAP and ABTS⁺ apparent antioxidant activity. Quinoa samples had a high protein content (13.5 g/100 g dry weight), with a well-balanced amino acid profile. Incorporation of quinoa into baked products such as bread is proposed as a way to deliver this healthy whole-grain cereal into the diet.

**Keywords** Quinoa, composition, nutrient content, quinoa-enriched bread.

**Introduction**

Quinoa (Chenopodium quinoa Willd.) is a grain-like crop grown originally in countries of the Andean region of South America which was domesticated for human consumption and as an animal feed for 3000–4000 years (Vega-Gálvez et al., 2010). Due to increasing popularity, the grain was recently introduced as a cereal crop in non-indigenous regions such as Europe, North America, Australia, China and Japan. Quinoa is a member of the family Amaranthaceae rather than Poaceae (Gramineae), but because the seeds are mostly milled into flour and used as a cereal crop, it is referred to as a pseudocereal. Although quinoa is not a member of the grass family, the grain is included in the ‘whole grain’ category due to its similar nutrient composition to grass seeds (Prego et al., 1998). Minerals, lipid and protein are found mostly in the embryo and endosperm. The quinoa seed outer coats are formed from a dry, very thin, two-layered pericarp. The seeds are round and flattened, about 1.5–4.0 mm in diameter and 0.5 mm in thickness; around 350 seeds weigh 1 gram, and their colour ranges from white to grey and black, with many other colours including yellow, red, purple and violet depending on the phytochemical content. Currently, there are approximately 250 quinoa varieties identified, which are classified by the colour of the plant and seeds, and plant morphology (Jančurová et al., 2009; Vega-Gálvez et al., 2010). Quinoa seeds are consumed in many different ways as dried seeds in salads and mueslis or cooked and used in a similar way to rice, puffed/extruded to make breakfast cereals, or milled into flour for used in baked products including breads.
pancakes and tortillas. In addition, quinoa seeds can be fermented to make beers and the South American traditional drink ‘chichi’. Homemade breads made with added quinoa flour are growing in popularity as part of the daily menu as a way to diversify the range of foods containing quinoa with potential benefits to human health (De Carvalho et al., 2014). The sensory value of quinoa-enriched bread is important, since appearance, smell, taste and flavour of bread greatly affect consumers’ preferences for cereal products. Although breadmaking methods have been described in a few studies, little is known about the sensory characteristics of bread with the addition of quinoa flour (Chlopicka et al., 2012; Bilgici & Ibanoglu, 2015). There is current interest in encouraging the consumption of quinoa since a number of recent studies have shown beneficial effects on biomarkers of cardiovascular disease risk (e.g. Li et al., 2018; Pourshahidi et al., 2020; Obaroakpo et al., 2020; Martínez-Villaluenga et al., 2020; Karimin et al., 2020).

Due to its high nutritional quality, the Food and Agricultural Organisation of the United Nations (FAO) announced that 2013 should be ‘The International Year of the Quinoa’ in order to raise the profile of the food and encourage its use. Quinoa also contains a large number of phytonutrients, including the family of saponins. These compounds, primarily located in the pericarp layer, give quinoa seeds an unpleasant bitter taste, making the removal of saponins via washing or mechanical abrasion before human consumption necessary (Prego et al., 1998; Vega-Gálvez et al., 2010). Quinoa is gluten-free making it a food of choice for individuals with coeliac disease seeking to follow a gluten-free diet.

Quinoa, like most seeds, has a high carbohydrate content, but it is also a good source of high-quality protein, due to its amino acid profile. Quinoa seeds have good levels of unsaturated fatty acids, and a high dietary fibre content; they are a good source of minerals and other important components such as vitamin C and phenolic compounds making quinoa an excellent alternative to common cereals (Ando et al., 2002; Tang et al., 2015). Phenolics, including flavonoids, phenolic acids and tannins, are bioactive secondary plant metabolites that possess a variety of physiological properties such as apparent antioxidant, anti-inflammatory, antimicrobial, cardiovascular protective and anti-carcinogenic activities (Scalbert et al., 2011). To date, only the free form of phenolics in quinoa seeds has been quantified in most of the published studies, but it is well known that phenolics in grains exist conjugated to small molecules such as peptides and oligosaccharides, and they are also in bound forms attached to cell wall materials (Dini et al., 2010). Compositional data for quinoa are still currently scarce compared with other common cereals, such as wheat, maize and rice (Sosulski et al., 1982; Abugoch James, 2009; Alvarez-Jubete et al., 2009). The potential possible compositional differences (i.e. phenolics, protein, fibre and fat) attributable to the genetic backgrounds are largely unknown.

Therefore, the aim of this study was to investigate the degree of variability in the nutritional composition and free, conjugated and bound phenolic contents and apparent antioxidant activity of these fractions from different quinoa varieties. To our knowledge, this is the first study to report the phenolic content and free, conjugated and bound profiles of apparent antioxidant activity of quinoa seeds determined using a range of different analytical methods, since the literature in the last decade has focused on measurement of free forms using various extraction solvents to obtain maximum extraction.

Materials and methods

Quinoa samples

In this study, a total of thirteen quinoa varieties which were available in the UK at the time of the research were purchased either directly from the supplier online or through commercial supermarket retail outlets. Samples came from various regions, including Peru, Ecuador, Bolivia, USA, UK, Netherlands and China. Samples were milled and stored frozen at −80°C until use.

Phenolic, antioxidant and nutrient analysis

Phenolic compound extraction
Free, conjugated and bound phenolic compounds were isolated from flour samples based on the method described by Li et al. (2008).

Free phenolic compounds. 25 mg of quinoa flours were blended with 1 mL of 80% chilled ethanol for 5 min in 2-mL Eppendorf tubes using a multi-tube rotator mixer (Stuart SB3), followed by sonication for 10 min. The mixtures were then centrifuged at 7200×g (Eppendorf 5430) for 15 min, and the supernatant was removed into a clean Eppendorf tube. The extraction was repeated four times under the same conditions. All supernatants were combined, then evaporated to dryness at 45°C under a gentle stream of nitrogen gas and finally dissolved with deionised water to a volume of 350μL. After centrifugation at 19,100×g for 5 min, the supernatant was transferred to a clean vial. The extracts were stored at −80°C until future use.

Conjugated phenolic compounds. 10 mg of whole-grain flours were mixed with 1 mL of 80% chilled ethanol for 5 min in 2-mL Eppendorf tubes using a multi-tube...
rotator mixer, followed by being sonicated for 10 min. After centrifugation at 7200×g for 15 min, the supernatant was removed into a clean Eppendorf tube. The extraction was repeated four times under the same conditions. All supernatants were combined and then evaporated to dryness at 45 °C under a gentle stream of nitrogen gas. The dried extracts were then hydrolysed with 400 µL of 2 M NaOH at room temperature for 4 h and then acidified to pH 2 with 12 M HCl (80 µL). The solution was extracted four times with 500 µL of ethyl acetate. After centrifugation at 19 100×g for 5 min, the upper layer was transferred to a clean Eppendorf tube, and combined ethyl acetate extracts were evaporated to dryness at 45 °C under a gentle stream of nitrogen gas. Phenolic compounds were reconstituted with deionised water to a final volume of 350 µL of water followed by centrifugation at 19,100×g for 5 min; the supernatant was transferred to a clean vial and then stored at −80 °C until use.

**Bound phenolic compounds.** The residues from free or conjugated phenolics extraction above were digested with 800 µL of 2 M NaOH for 4 h before acidification to pH 2 with 12 M HCl (120 µL). The mixture was extracted four times with 800 µL ethyl acetate, and the supernatants combined as described above and then evaporated to dryness at 45 °C under a gentle stream of nitrogen gas. Phenolic compounds were reconstituted with deionised water to a final volume of 350 µL followed by centrifugation at 19,100×g for 5 min; the supernatant was transferred to a clean vial and then stored at −80 °C until use.

**Folin–Ciocalteu phenolic content (FC assay)**

The phenolic content of quinoa free, conjugated and bound extracts was determined using the Folin–Ciocalteu method as described by Zhang et al. (2006). Before the measurement, commercial Folin–Ciocalteu phenol reagent (Sigma-Aldrich, Poole Dorset) was diluted 1:10 (v/v) with deionised water. Gallic acid (GA) was used as the reference standard against which to assess the phenolic contents, which were then expressed as GA equivalents (GAE)/g of dried sample. Serial dilutions of a stock GA standard in deionised water were carried out to provide a calibration curve at 500, 250, 125, 62.5 and 31.25 µg mL⁻¹. 10 µL of GA standard solutions, blank or each extract was added into the 96-well microplate. 130 µL of the diluted Folin–Ciocalteu phenol reagent was added. Five minutes later, 100 µL of 7.5% sodium carbonate solution was added, and the resulting solution thoroughly mixed. The absorbance values were measured at 765 nm using a spectrophotometer (Multiskan Ascent 96/384 Plate Reader) after incubation at 40 °C for 30 min. Final results were given as mg of GAE/g sample dry weight.

Analysis of each extract of free, conjugated or bound phenolics was repeated four times in this study.

**Apparent antioxidant assays**

**Ferric reducing ability of plasma (FRAP assay).** The FRAP assay was performed using the method of Benzie & Strain (1996). Briefly, 10 mM of ferric 2,4,6-Tripyridyl-s-Triazine (Fe³⁺TPTZ) solution was prepared by dissolving 0.0781 g of Fe³⁺TPTZ in 40 mM hydrochloric acid. A FRAP working solution was prepared by mixing 50 mL of 300 mM acetate buffer (pH 3.6), 5 mM of 20 mM ferric chloride (FeCl₃·6H₂O) and 5 mL of 10 mM Fe³⁺TPTZ solution. Standard solutions of ferrous sulphate in the range 200–1000 µmol L⁻¹ were prepared by dilution. μL of standards, blank or sample extracts were mixed with 300 µL of FRAP working reagent in a 96-well microplate and incubated at 37 °C for 4 min. The absorbance of samples was measured at 593 nm after incubation. The final results were expressed as µmol Fe²⁺ Equivalent (E)/g sample dry weight. All reagents were prepared fresh immediately before use.

**2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS⁺⁺ assay).** A modification of the 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay from Re et al. (1999) was used. An ABTS cation radical (ABTS⁺⁺) stock solution was obtained by mixing 7 mM ABTS with 2.45 mM potassium persulphate 9:1 (v/v) which was then stored at room temperature in the dark overnight before use. The ABTS⁺⁺ stock solution was then diluted with 5 mM phosphate buffer solution (PBS), pH 7.4, until the absorbance of the mixture at 734 nm was 0.7 (±0.02). Trolox standard solutions in the range 0.1–0.5 mM were made by diluting 2.5 mM Trolox in ethanol. 10 µL of Trolox standard, blank or sample extracts were mixed with 290 µL of ABTS⁺⁺ working solution in a 96-well microplate. The decrease in absorbance was measured at 734 nm. The final results were expressed as µmol Trolox Equivalents (TE)/g sample dry weight.

2, 2-diphenyl-1-picrylhydrazyl (DPPH⁺) assay. A modification of the 2,2-diphenyl-1-picrylhydrazyl (DPPH⁺) method of Van Hung et al. (2009) was used. Briefly, 10 mg DPPH⁺ powder was dissolved in 100 mL of methanol to make a DPPH⁺ stock solution which was stored in the fridge overnight. A DPPH⁺ working solution was prepared by mixing 30 mL of this stock solution with 70 mL of methanol to form the final concentration of 0.076 mM. Trolox standard solutions in the range 0.25–1.25 mM were made by diluting 2.5 mM Trolox in methanol. 10 µL of Trolox standard, blank or samples was pipetted into the 96-well plate and 390 µL of the DPPH⁺ working solution...
added and mixed well. The absorbance was measured at 517 nm after incubation at 30 °C for 30 min. The final results were expressed as µmol Trolox Equivalents (TE)/g sample dry weight.

Nutrient composition analysis of quinoa seeds
The quinoa samples were analysed for energy and nutrient content to British Standards by accredited companies as described in Li et al. (2018).

Calculations and statistical analyses
In this study, extraction of free, conjugated and bound phenolics was replicated four times, and measurement of each extract by FC assay or the three antioxidant activity assays was also repeated four times. Data analysis was based on the four replicate mean values using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) expressing the results as means ± standard deviations (SD). Using the Shapiro–Wilk test confirmed normality in the data. A 2-sample independent t-test was used to determine the differences between phenolic fractions (e.g. free phenolics vs conjugated phenolics), and P values less than 0.05 were considered significantly different.

Results and discussion
Phenolic content and apparent antioxidant activity content of isolated fractions of different quinoa varieties

Phenolic content
The phenolic content and the apparent antioxidant contents of the different isolated fractions are shown in Table 1. Individual values for each variety are shown in Table S1. The free phenolic content of the 13 quinoa varieties ranged by more than twofold between 0.89 and 2.13 mg GAE/g, with an average of 1.44 mg GAE/g. These values are comparable to those found for coloured quinoa seeds of 1.23–3.41 mg GAE/g reported by Abderrahim et al. (2015) and between 0.88 and 3.03 mg GAE/g by Inglett et al. (2015) However, the values were lower than those reported by Gomez-Caravaca et al. (2014) and by Tang et al. (2015) (2.53 mg GAE/g and 4.2 mg GAE/g, respectively). The conjugated phenolic content, although much lower, covered a similar range and variability between varieties (CV 27% for Free and 28% for conjugated phenolics). On average, the concentration of phenolics in the bound fraction was the lowest but the bound phenolic contents of the three red quinoa varieties (0.62, 0.63 and 0.80 mg GAE/g sample) were much higher than that seen in the white quinoa varieties and the values were much more variable between varieties (CV 78%). The bound phenolic values determined in this study were much lower than those of 2.97 and 1.99 mg GAE/g reported by Gomez-Caravaca et al. (2014) and Inglett et al. (2015), respectively. The total phenolic content (TPC, sum of free, conjugated and bound phenolics) of quinoa was, on average, 2.18 mg GAE/g, which was lower than the values reported by Inglett et al. (2015) and Gomez-Caravaca et al. (2014) (3.84 mg GAE/g and 5.24 mg GAE/g, respectively). The phenolics in quinoa were mainly found in the free form, which contributed about 50–83% of the TPC across all varieties. In this regard, the results in this study are consistent with those shown by Hung & Morita (2008) for buckwheat. However, they contrast with data for corn, rice, wheat, oat and rye which suggest that phenolics are primarily present in bound forms linked to cell wall materials. Although the phenolic compounds were mainly present in the free form, this study also suggests that the TPC of quinoa could be underestimated in previously published studies without including the contribution of conjugated and bound phenolic compounds. Distribution of the phenolics between fractions may have an impact on their digestion and metabolism in the gastrointestinal tract. For example, some studies have shown that free phenolic compounds may be digested.

Table 1 Mean ± SD of phenolic content (PC) and antioxidant activity of extracts of 13 quinoa varieties.

|          | PC (mg GAE/g dw) | FRAP (µmol Fe²⁺E/g dw) | ABTS⁺⁺ (µmol TE/g dw) | DPPH (µmol TE/g dw) |
|----------|-----------------|-------------------------|-----------------------|---------------------|
| Free     | 1.44 ± 0.38     | 3.49 ± 1.52             | 8.61 ± 1.67           | 5.22 ± 2.28         |
| Conjugated | 0.48 ± 0.12***   | 1.54 ± 0.65***          | 5.02 ± 1.30***       | 6.77 ± 3.08         |
| Bound    | 0.26 ± 0.25***#  | 1.25 ± 1.58**           | 4.08 ± 3.98***       | 5.05 ± 2.65         |
| Total PC* | 2.18 ± 0.45     | 6.27 ± 2.67             | 17.71 ± 3.75         | 17.04 ± 4.99        |

*Total PC; Total phenolic content = Free + Conjugated + Bound.  
**mg GAE/g dw; mg gallic acid equivalents/g dry weight.  
#µmol Fe²⁺E/g dw; µmol ferrous ion equivalents/g dry weight.  
#µmol TE/g dw; µmol Trolox equivalents/g dry weight.  
***<0.01, ****<0.001, conjugated and bound phenolic content compared with free phenolic content.  
*<0.05, bound phenolic content compared with conjugated phenolic content.
in the upper gastrointestinal tract, while the bound fractions could survive stomach and intestinal digestion, allowing them to be released in the colon and, therefore, potentially play a protective role in the distal gut.

On average, the TPC of quinoa was higher than those found in common cereals including wheat (0.56 mg g⁻¹), barley (0.88 mg g⁻¹), rye (1.03 mg g⁻¹) and millet (1.39 mg g⁻¹) (Ragaee et al., 2006), suggesting that quinoa may serve as an excellent source of phenolic compounds in the diet. The wide variations in the level of different phenolic forms among quinoa varieties included in this study which would impact on the levels present in the diet depending on the variety chosen for consumption. The variability might be explained by differences in the genetic background, and environmental conditions under which the varieties were grown. This information (other than the country of origin) was not available for the samples analysed here but should be an important consideration for future studies and in the selection of varieties for production and consumption.

The difference between the results presented in this study and the literature may be due to the different extraction methods, including time of extraction, solid-liquid ratio, temperature, particle size and other factors, with the extraction solvent being an important factor. The polarities of phenolic compounds range considerably and a wide range of solvents, including water, methanol, ethanol, acetone and chloroform, ethyl acetate and their mixtures have been applied individually or in combination to achieve maximum extraction amounts from grains. Particularly, it appears that aqueous/organic mixed solvents are more efficient for extraction of phenolic compounds than pure organic solvents alone (Zielinski & Kozlowska, 2000). The extraction solvents used in this current study were based on the previously published paper of Zielinski & Kozlowska, (2000). The extraction solvents used in this current study which would impact on the levels present in the diet depending on the variety chosen for consumption. The variability might be explained by differences in the genetic background, and environmental conditions under which the varieties were grown. This information (other than the country of origin) was not available for the samples analysed here but should be an important consideration for future studies and in the selection of varieties for production and consumption.

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**Apparent antioxidant activity**

Many methods and strategies have been proposed and developed to evaluate total apparent antioxidant activity in foodstuffs and plant tissues. The most common spectrophotometric methods based on reaction with electron-donating or hydrogen radical (H•)-producing antioxidant compounds are FRAP, ABTS•⁺ and DPPH which were applied in this study.

As shown in Table 1, the FRAP and ABTS•⁺ apparent antioxidant activities of the free fraction were significantly higher than those of conjugated and bound extracts (P < 0.05), but no differences were found between conjugated and bound flour extracts (P > 0.05). The FRAP apparent antioxidant activities of free extracts were similar to those reported in the study by Nsimba et al. (2008) but were higher than those reported by Tejeda et al. (2008) The ABTS•⁺ values of free fraction reported here were similar to the range of values of between 9.40 and 14.74 μmol TE/g dw reported by Repo-Carrasco-Valencia & Serna (2011), but were markedly higher than the levels reported by Tejeda et al. (2008). There were no significant differences (P > 0.05) in the DPPH apparent antioxidant activities of free, conjugated and bound extracts in this study between the 13 quinoa varieties, although conjugated extracts had numerically higher DPPH antioxidant values compared with free and bound extracts (Table 1). The DPPH apparent antioxidant activity of free extracts was in agreement with the results of Dini et al. (2010), who reported that bitter and sweet raw quinoa seeds had values of 6.71 and 2.87 μmol TE/g dw, respectively, and they were also similar to the range of 0.49–5.08 μmol TE/g dw reported by Inglett et al. (2015), although the DPPH apparent antioxidant activity of bound extracts was much lower than values shown by this group.

The high apparent antioxidant activity reported for many natural foods including fruits, vegetable and whole-grain cereal products is often attributed to the polyphenolic content of the food. The literature is full of such statements which have then often been translated into supposed associations between ‘antioxidant activity’ in a food/diet and ‘antioxidant status’ of an individual consuming that food. It is often suggested that an individual’s antioxidant status is, or can be, affected by the amount of high antioxidant activity foods consumed. Antioxidants, especially phenolic compounds, have been hypothesised to protect cell constituents by combating oxidative stress, reducing
the risk of various degenerative diseases relating to oxidative stress, such as cardiovascular disease, cancer and osteoporosis (Scalbert et al., 2011). However, this relationship has been questioned, with more recent studies suggesting that polyphenolics may have beneficial effects through acting as signalling molecules and not as ‘antioxidants’. For example, growing evidence suggests that dietary-derived flavonoids may exert beneficial effects on long-term potentiation, and consequently memory and cognitive performance, via their interactions with signalling pathways, including the phosphatidylinositol-3 kinase/protein kinase B/Akt, protein kinase A and protein kinase C (Spencer, 2008).

**Correlation between phenolic content and apparent antioxidant activity**

In this study, possible correlations between the phenolic content (PC) and apparent antioxidant activity of the quinoa varieties were determined for the different assays used, and these are shown in Table 2. The correlation between PC and apparent antioxidant activity for FRAP and ABTS+• measurements was statistically positive ($r > 0.5$, $P < 0.05$), but this was not the case for the relationship between DPPH and PC. Additionally, relatively high and significantly positive correlations were observed between the FRAP and ABTS+• methods for measuring antioxidant activity of the different quinoa extracts ($r \geq 0.679$, $P < 0.05$), but not when comparing FRAP and DPPH, or ABTS+• and DPPH antioxidant activities. This is probably because the principles behind the FRAP and ABTS+• methods are similar; thus, higher correlations among values by these methods are expected. The lack of significant correlations between DPPH assay and other methods may be because of the relatively higher stability and lower reactivity of the DPPH radical, which only reacts with more reactive reducing substances. In one study, the correlation coefficient between total phenolic content and FRAP values ($R^2 = 0.483$), although higher than DPPH scavenging activity ($R^2 = 0.205$), was relatively weak (Nsimba et al., 2008). These were contrary to the results reported by Dini et al. (2010) that antioxidant activity of sweet and bitter quinoa seeds was highly positively correlated with the TPC ($R^2 \geq 0.95$).

The relationship between PC and antioxidant activity has been widely determined in various foods including fruits and vegetables with some, but not all studies showing linear correlations between PC and apparent antioxidant activity (Babbar et al., 2011). The unclear relationships between PC and apparent antioxidant activity may be because (1) phenolic compounds are not the only components responsible for apparent antioxidant activity in the foodstuff; other compounds contributing to measures include ascorbic acid, vitamin E, tocopherol, carotenoids and sterols, (2) the antioxidant activity of most pure phenolic compounds or vitamins was lower than those of the complex fruit extracts on a dry weight basis and the apparent antioxidant activity may not only be dependent on the level of the individual antioxidants, but interactions between them, and (3) different methods to determine antioxidant activity with various analytical mechanisms may lead to different observations as described above. This latter point is particularly important since the different methods used to determine apparent antioxidant activity here, and in other studies, are based on methodologies which also interact with biomolecules which do not necessarily possess true antioxidant properties (Cao & Prior, 1998; Prior & Cao, 1999; Re et al., 1999; Prior, 2004; Nagah & Seal, 2005).

**Nutrient composition analysis of quinoa seeds**

As shown in Table 3, there was considerable variation in the proximate composition and amino acid content between the thirteen quinoa seed samples. Individual values for each variety are shown in Tables S2 and S3. All measurements were made on raw flour samples so the impact of cooking and food processing which may affect the phytochemical and nutrient composition (Mhada et al., 2020) has not been considered.

The total protein content of the varieties analysed ranged from 11.80 to 14.66 g/100 g, with an average of 13.51 g/100 g, which is in line with that found for six Peruvian quinoa genotypes at 11.32 to 14.72 g/100 g by Repo-Carrasco-Valencia et al. (2010) and from 11.31 to 16.18 g/100 g for quinoa from different regions of Chile by Miranda et al. (2012). In comparison with the common cereals, these results show that the protein content in quinoa is higher than that in rice, corn, sorghum, barley and rye, and is similar to that of wheat (Koziol, 1992; Jancurova et al., 2009). The nutritional quality of protein of foods is mainly

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**Table 2 Correlations between phenolic content (PC) and antioxidant activity, and between different measures of antioxidant activity.**

| Cereal species | PC vs FRAP | PC vs ABTS+• | PC vs DPPH | FRAP vs ABTS+• | FRAP vs DPPH | ABTS+• vs DPPH |
|---------------|------------|--------------|------------|----------------|---------------|----------------|
| Free          | 0.848**    | 0.603*       | 0.457      | 0.679*         | 0.625         | 0.401          |
| Conjugated    | 0.517*     | 0.696*       | -0.453     | 0.689**        | -0.170        | -0.292         |
| Bound         | 0.979*     | 0.976**      | 0.379      | 0.982**        | 0.446         | 0.468          |
| Total PC*     | 0.797**    | 0.545*       | 0.110      | 0.629**        | 0.466         | 0.636          |

*P value of linear regression correlation coefficient.

*P < 0.05.

**P < 0.01.
Table 3 Mean ± SD of proximate composition and amino acid content of 13 quinoa accessions, expressed on a dry matter basis

| Energy (Kcal/100 g) | Mean | SD |
|---------------------|------|----|
| (kJ/100 g)          | 359 ± 5 | 1514 ± 25 |
| g/100 g             |      |     |
| Protein             | 13.51 ± 0.85 |     |
| Ash                 | 2.44 ± 0.46 |     |
| Moisture            | 10.86 ± 1.17 |     |
| Available carbohydrate | 57.19 ± 2.63 |     |
| Total sugars        | 4.31 ± 0.98 |     |
| Sodium              | <0.1 |     |
| Salt                | <0.1 |     |
| Insoluble dietary fibre | 8.27 ± 1.43 |     |
| Soluble dietary fibre | 1.26 ± 0.44 |     |
| Total dietary fibre | 9.53 ± 1.65 |     |
| Fat                 | 6.46 ± 0.79 |     |
| Saturated fat       | 0.74 ± 0.10 |     |
| Monounsaturated fat | 1.83 ± 0.27 |     |
| Polyunsaturated fat | 3.63 ± 0.48 |     |
| Essential amino acids |      |     |
| Histidine           | 0.36 ± 0.02 |     |
| Isoleucine          | 0.49 ± 0.03 |     |
| Leucine             | 0.79 ± 0.04 |     |
| Lysine              | 0.78 ± 0.04 |     |
| Methionine          | 0.26 ± 0.01 |     |
| Phenylalanine       | 0.48 ± 0.03 |     |
| Threonine           | 0.49 ± 0.03 |     |
| Tryptophan          | ND |     |
| Valine              | 0.61 ± 0.03 |     |
| Non-essential amino acids |      |     |
| Alanine             | 0.55 ± 0.03 |     |
| Arginine            | 1.13 ± 0.08 |     |
| Aspartic acid       | 1.07 ± 0.05 |     |
| Cystine             | 0.20 ± 0.01 |     |
| Glutamic acid       | 1.77 ± 0.12 |     |
| Glycine             | 0.68 ± 0.05 |     |
| Proline             | 0.49 ± 0.03 |     |
| Serine              | 0.61 ± 0.03 |     |
| Tyrosine            | 0.38 ± 0.03 |     |

ND, not determined.

determined by the composition of essential amino acids (EAA) present in the foodstuff. As shown in Table 3, quinoa contains high levels of the EAA (tryptophan was lost during hydrolysis in the method used here and cannot be reported), making the protein high quality and similar to casein in milk (Vega-Gálvez et al., 2010). Thus, quinoa is an unusual plant food in providing a ‘complete protein’ containing all the EAA for the human diet at approximately 5.75 g/100 g (Stukic et al., 2012). The values for quinoa protein are close to those suggested by FAO/WHO/UNU, with a well-balanced EAA profile with good levels of lysine and sulphur-containing amino acids, in contrast to the protein content of common cereals such as wheat and maize, which are much lower in lysine. Processing of quinoa impacts on protein quality by affecting digestibility and it is increased considerably with cooking (Koziol, 1992; Ruales & Nair, 1992). Additionally, protein quality may be partly influenced by antinutritional factors present in some cereals, which reduce their nutritional value (Filho et al., 2017). Some antinutritional factors, including saponins, phylic acid, tannins, nitrates, oxalates and trypsin inhibitors, have been identified in quinoa seeds (see, e.g., Gómez-Caravaca et al., 2011; Gómez-Caravaca et al., 2014; Vilca-Cundo & Hernández-Ledesma, 2017) but their impact on overall protein quality has not been determined.

Available carbohydrate levels in the quinoa seeds tested here ranged between 48.8 g/100 g and 61.7 g/100 g, with an average of 57.2 g/100 g, which was lower than the value of 65.6 g/100 g reported Koziol (1992) and the range of between 68.8 and 75.8 g/100 g reported by Repo-Carrasco-Valencia & Serna (2011), but compares favourably with the values of between 56.1 to 62.5 g/100 g reported by Miranda et al. (2012).

Quinoa has been regarded as a potential alternative to oilseed crops because of the quantity and quality of its lipid content. Quinoa analysed in this study had a lipid content ranging from 4.8 g/100 g to 7.5 g/100 g, with an average of 6.5 g/100 g, which is similar to the value reported by Filho et al. (2017) who reported a lipid content of around 7%. This is between 2 and 3 times higher than in buckwheat (4.2 g/100 g) and other common cereals, such as wheat (1.8 g/100 g) and maize (2.5 g/100 g), but is much lower than soy (18.9 g/100 g) (Hager et al., 2012). The majority (84%) of fatty acids found in quinoa were unsaturated fats, as expected for a plant food, similar to values around 87% reported by Ando et al. (2002) As reported by Filho et al. (2017), the fatty acids found in quinoa provide a good source of the essential fatty acids linoleic acid and α-linolenic acid; quinoa also contains good level of the fat-soluble antioxidant vitamins, including α- and γ-tocopherol, although these observations could not be confirmed in the current study.

Quinoa seeds are a good source of dietary fibre (Zhu, 2020). In the present study, values ranged from 7.6 g/100 g to 12.6 g/100 g, with an average value of 9.5 g/100 g for AOAC total dietary fibre determinations. These values were lower than those found for red quinoa seeds (8.97 g/100 g) reported by Repo-Carrasco-Valencia et al. (2010), the values between 11.6 and 15.1 g/100 g reported by Miranda et al. (2012) and the values between 13.6 and 16.0 g/100 g reported by Repo-Carrasco-Valencia & Serna (2011), although the proportions of soluble and insoluble dietary fibre were similar. The level of dietary fibre in quinoa seeds was lower than that in barley (15.6%) and rye (15.1%) and similar to wheat (10.7%), but was much higher
than that for rice (2.8%), corn (7.3%) and sorghum (3.7%).

Sensory properties of quinoa-enriched bread – pilot study

Homemade breads made with added quinoa flour are growing in popularity as part of the daily menu as a way to diversify the range of foods containing quinoa with potential benefits to human health (De Carvalho et al., 2014). The sensory value of quinoa-enriched bread is important, since appearance, smell, taste and flavour of bread greatly affect consumers’ preferences for cereal products. Although breadmaking methods have been described in a few studies, little is known about the sensory characteristics of bread with the addition of quinoa flour (Chlopicka et al., 2012; Bilgiçli & Ibanoglu, 2015). We therefore completed a preliminary study to explore the acceptability of quinoa-enriched bread as a possible route for introducing quinoa into the diet. Full details of the bread manufacture and sensory analysis are shown in Supplementary Material. Briefly, refined wheat bread (control) was compared with breads in which 20% and 30% of the wheat flour was replaced with milled BIOFAIR (place of origin, UK) quinoa. This variety was selected from the 13 varieties analysed because it had the highest levels of antioxidant activity and polyphenolics which may affect the sensory characteristics of the bread. The levels of quinoa were chosen so that a portion of bread could deliver 20 g of quinoa into the diet. 20% and 30% quinoa-enriched breads were progressively smaller, denser and darker than the control refined wheat bread (Figure S1). Since quinoa is gluten-free, the reduced gluten content of the test breads resulted, as expected, in a reduced bread volume with a denser structure. A panel of 41 volunteers found that quinoa-enriched breads were significantly darker, had more aroma and were less soft with increasing quinoa content (Figure S2). Addition of quinoa increased the flavour of the bread and at the highest inclusion rate the bread was considered less enjoyable than the control bread or the 25% quinoa-enriched breads which were rated similarly (Figure S2).

Breads are basic food commodities in many countries and are mainly prepared from wheat flour containing gluten which is responsible for its textural properties. Because of the lack of gluten in quinoa, the use of 100% quinoa flour in bread formulation results in lower baking quality (Alencar et al., 2017); thus, quinoa can only partially substitute wheat flour in breadmaking and in other baked products or high carbohydrate foods such as pasta (Caratini & Rosentrater, 2019). Previous substitution levels of quinoa flour for refined wheat flour in bread formulations were 10%–20% (Bilgiçli & Ibanoglu, 2015) and 50% (Turkut et al., 2016). Bread made from 100% quinoa flour is available for coeliac patients. However, sensory evaluation of quinoa-containing bread has received less attention. Stikic et al. (2012) reported that 15% quinoa-wheat bread had a higher sensory score than 10% quinoa-wheat, with the lowest score for 20% quinoa-wheat bread. Chlopicka et al. (2012) also showed that 15% quinoa-wheat bread was scored as being more tasty than 20% quinoa-wheat bread. Wheat bread made with 10% quinoa flour has found to be acceptable based on dough volume, dough stability, weight, structure, taste and colour (Lorenz & Coulter, 1991; Enriquez et al., 2003). Overall, this preliminary sensory analysis suggests that 20% quinoa-enriched bread would be an acceptable way of introducing quinoa into the diet. Based on these observations, we subsequently used this level of inclusion to make a quinoa-enriched bread for use in a dietary intervention study (Li et al., 2018).

Conclusion

The present study demonstrates for the first time that phenolic compounds in quinoa not only exist in a free form, but also in conjugated and bound forms that can be liberated by alkaline and/or acid hydrolysis. Quinoa is a good source of these phenolic compounds but there is considerable variability between sources of the seed. The phenolic content of quinoa could be underestimated if conjugated and bound forms are not determined. A correlation between phenolic content and apparent antioxidant activity was observed as expected, but the nutritional relevance of this relationship remains to be evaluated. Proximate analysis of the quinoa varieties evaluated showed considerable variation between sources which could be due to differences in agronomic and environmental conditions during quinoa production which must be taken into consideration when developing products for human consumption. Quinoa has a high nutritive value compared with other common cereals, especially in protein quantity and quality making it an ingredient of choice for product formulations. However, the variability between sources of the grain may limit its use in a commercial environment. Quinoa flour can be used in combination with other cereal flours to improve the nutritional quality of products and preliminary data suggest that inclusion values up to 20% are likely to produce bread with a good consumer acceptability, although this, and its use in other baked products requires further investigation.

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**Author contribution**

Liangkui Li: Conceptualization (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (supporting); Writing-original draft (equal). Georg Lietz: Conceptualization (supporting); Project administration (supporting); Supervision (supporting); Writing-review & editing (equal). Chris Seal: Conceptualization (supporting); Project administration (lead); Supervision (equal); Writing-review & editing (equal).

**Conflicts of interest**

The authors declare no conflicts of interest.

**Peer review**

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**References**

Abderrahim, F., Huanatico, E., Segura, R., Arribas, S., Gonzalez, M.C. & Condezo-Hoyos, L. (2015). Physical features, phenolic compounds, betalains and total antioxidant capacity of coloured quinoa seeds (Chenopodium quinoa Willd.) from Peruvian Altiplano. Food Chemistry, 183, 83–90.

Albuogoq James, L.E. (2009). Quinoa (Chenopodium quinoa Willd.): Composition, Chemistry, Nutritional, and Functional Properties. In: Advances in Food and Nutrition Research (edited by S.L. Taylor). Pp. 1–31, vol. 58. New York, NY: Elsevier, Academic Press.

Alencar, N.M.M., de Morais, E.C., Steel, C.J. & Bolini, H.M.A. (2021). The data that support the findings of this study are on behalf of Institute of Food, Science and Technology (IFSTTF). The Authors. © The Authors.

Bilgili, N. & Ibanoglu, Ş. (2015). Effect of pseudo cereal flours on some physical, chemical and sensory properties of bread. Journal of Food Science and Technology, 52, 7525–7529.

Cao, G. & Prior, R. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clinical Chemistry, 44, 1309–1315.

Caratini, C. & Rosentrater, K.A. (2019). Developing and testing gluten free spaghetti using quinoa. Journal of Food Research, 8, 20–32.

de Carvalho, F.G., Ovidio, P.P., Padovan, G.J., Jordão Junior, A.A., Marchini, J.S. & Navarro, A.M. (2014). Metabolic parameters of postmenopausal women after quinoa or corn flakes intake – a prospective and double-blind study. International Journal of Food Science and Nutrition, 65, 380–385.

Chlopicka, J., Pasko, P., Gorinstein, S., Jedryas, A. & Zagrodzki, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereals breads. LWT - Food Science and Technology, 46, 548–555.

Dini, I., Tenore, G.C. & Dini, A. (2010). Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter Chenopodium quinoa seeds. LWT - Food Science and Technology, 43, 447–451.

Enríquez, N., Peltzer, M., Raimundi, A., Tosi, V. & Pollio, M.L. (2003). Characterization of wheat and quinoa flour blends in relation to their breadmaking quality. Anales de la Asociacion Quimica Argentina, 91, 47–54.

Gómez-Caravaca, A.M., Iafelice, G., Verardo, V., Marconi, E. & Caboni, M.F. (2014). Influence of pearling process on phenolic and saponin content in quinoa (Chenopodium quinoa Willd). Food Chemistry, 157, 174–178.

Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A. & Caboni, M.F. (2011). Simultaneous determination of phenolic compounds and saponins in quinoa (Chenopodium quinoa Willd) by a liquid chromatography–diode array detection–electrospray ionization–time-of-flight mass spectrometry methodology. Journal of Agricultural and Food Chemistry, 59, 10815–10825.

Huger, A.-S., Wolter, A., Jacob, F., Zannini, E. & Arendt, E.K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. Journal of Cereal Science, 56, 239–247.

van Hung, P., Maeda, T., Miyatake, K. & Morita, N. (2009). Total phenolic compounds and antioxidant capacity of wheat graded flours by polishing method. Food Research International, 42, 185–190.

Hung, P.V. & Morita, N. (2008). Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities. Food Chemistry, 109, 325–331.

Jancurova, M., Minarovicova, L. & Dandar, A. (2009). Quinoa - a novel cereals with high protein content and its potential uses. Czech Journal of Food Sciences, 27, 71–79.

van der Kamp, J.W., Poutanen, K., Seal, C.J. & Richardson, D.P. (2014). The HEALTHGRAIN definition of ‘whole grain’. Food Science and Technology, 58, 22100.

Karimian, J., Abedi, S., Shirinbakshmasoleh, M., Moodi, F., Moodi, V. & Ghavami, A. (2020). The effects of quinoa seed supplementation on cardiovascular risk factors: A systematic review and meta-analysis of controlled clinical trials. Phytotherapy Research, n/a. https://doi.org/10.1002/ptr.6901

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

Li, L., Lietz, G., Bal, W., Watson, A., Morrey, B. & Seal, C. (2018). Effects of quinoa (Chenopodium quinoa Willd.) consumption on markers of CVD risk. Nutrients, 10, 777.

Lorenz, K. & Coulter, L. (1991). Quinoa flour in baked products. Plant Foods for Human Nutrition, 41, 213–223.

Martínez-Villaluenga, C., Peñas, E. & Hernández-Ledesma, B. (2020). Pseudocereal grains: Nutritional value, health benefits and
current applications for the development of gluten-free foods. *Food and Chemical Toxicology*, **137**, 11178.

Mhada, M., Metougui, M.L., el Hazzam, K., el Kacimi, K. & Yasri, A. (2020). Variations of saponins, minerals and total phenolic compounds due to processing and cooking of quinoa (*Chenopodium quinoa* Willd.) seeds. *Foods*, **9**, 660.

Miranda, M., Vega-Galvez, A., Martinez, E. et al. (2012). Genetic diversity and comparison of physicochemical and nutritional characteristics of six quinoa (*Chenopodium quinoa* Willd.) genotypes cultivated in Chile. *Ciencia E Tecnología De Alimentos*, **32**, 835–843.

Nagah, A.M.S. & Seal, C.J. (2005). *In vitro* procedure to predict apparent antioxidant release from wholegrain foods measured using three different methods. *Journal of the Science of Food and Agriculture*, **85**, 1177–1185.

Nsimba, R.Y., Kikuzaki, H. & Konishi, Y. (2008). Antioxidant activity of various extracts and fractions of Chenopodium quinoa and Amaranthus spp. seeds. *Food Chemistry*, **106**, 760–766.

Obaroakpo, I.J., Nan, W., Hao, L. et al. (2020). The hyperglycemic regulatory effect of sprouted quinoa yoghurt in high-fat-diet and streptozotocin-induced type 2 diabetic mice via glucose and lipid homeostasis. *Food & Function*, **11**, 8354–8368.

Pourshahidi, L.K., Caballero, E., Osses, A., Hyland, B.W., Ternan, N.G. & Gill, C.I.R. (2020). Modest improvement in CVD risk markers in older adults following quinoa (*Chenopodium quinoa* Willd.) consumption: a randomized-controlled crossover study with a novel food product. *European Journal of Nutrition*, **59**, 3313–3323.

Prego, I., Maldonado, S. & Otegui, M. (1998). Seed structure and localization of reserves in *Chenopodium quinoa*. *Annals of Botany*, **82**, 481–488.

Prior, R.L. (2004). Plasma antioxidant measurements. *Journal of Nutrition*, **134**, 3184S–3185S.

Prior, R. & Cao, G. (1999). In vivo total antioxidant capacity: comparison of different analytical methods. *Free Radical Biology & Medicine*, **27**, 1173–1181.

Ragaei, S., Abdel-Aal, E.-S.M. & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry*, **98**, 32–38.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, **26**, 1231–1237.

Repo-Carrasco-Valencia, R., Hellström, J. K., Pihlava, J.-M. & Mattila, P.H. (2010). Flavonoids and other phenolic compounds in Andean indigenous grains: Quinoa (*Chenopodium quinoa*), kañawa (*Chenopodium pallidicaule*) and kiwacha (*Amaranthus caudatus*). *Food Chemistry*, **120**, 128–133.

Repo-Carrasco-Valencia, R.A.M. & Serna, L.A. (2011). Quinoa (*Chenopodium quinoa* Willd.) as a source of dietary fiber and other functional components. *Ciencia e Tecnología de Alimentos*, **31**, 225–230.

Ruales, J. & Nair, B.M. (1992). Nutritional quality of the protein in quinoa (*Chenopodium quinoa* Willd) Seeds. *Plant Foods for Human Nutrition*, **42**, 1–11.

Scalbert, A., Andres-Lacueva, C., Arita, M. et al. (2011). Databases on food phytochemicals and their health-promoting effects. *Journal of Agricultural and Food Chemistry*, **59**, 4331–4348.

Serpen, A., Capuano, E., Fogliano, V. & Gokmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural & Food Chemistry*, **55**, 7666–7681.

Sousliski, F., Krygier, K. & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *Journal of Agricultural and Food Chemistry*, **30**, 337–340.

Spencer, J.P.E. (2008). Food for thought. The impact of dietary flavonoids on memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society*, **67**, 238–252.

Stikic, R., Gliocchi, D., Demin, M. et al. (2012). Agronomical and nutritional evaluation of quinoa seeds (*Chenopodium quinoa* Willd.) as an ingredient in bread formulations. *Journal of Cereal Science*, **55**, 132–138.

Tang, Y., Li, X., Zhang, B., Chen, P.X., Liu, R. & Tsao, R. (2015). Characterisation of phenolics, betaines and antioxidant activities in seeds of three *Chenopodium quinoa* Willd. genotypes. *Food Chemistry*, **166**, 380–388.

Tejeda, L., Penarrieta, J., Alvarado, J., Ákesson, B. & Bergstenhåll, B. (2008). Determination of total antioxidant capacity and total phenolic compounds in Andean grade (*Quinoa, Canihua, Amaranth* and *Qentu*). *Revistas Boliviana de Quimica*, **25**, 70–74.

Turkut, G.M., Cakmak, H., Kumcuoglu, S. & Tavman, S. (2016). Effect of quinoa flour on gluten-free bread batter rheology and bread quality. *Journal of Cereal Science*, **69**, 174–181.

Vega-Galvez, A., Miranda, M., Vergara, J., Uribe, E., Puente, L. & Martinez, E.A. (2010). Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* Willd.), an ancient Andean grain: a review. *Journal of the Science of Food and Agriculture*, **90**, 2541–2547.

Vilcacundo, R. & Hernández-Ledesma, B. (2017). Nutritional and biological value of quinoa (*Chenopodium quinoa* Willd.). *Current Opinion in Food Science*, **14**, 1–6.

Zhang, Q., Zhang, J.Z., Shen, J.K., Silva, A., Dennis, D.A. & Barr, C.J. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, **18**, 445–450.

Zhu, F. (2020). Dietary fiber polysaccharides of amaranth, buckwheat and quinoa grains: A review of chemical structure, biological functions and food uses. *Carbohydrate Polymers*, **248**, 116819.

Zielinski, H. & Kozlowska, H. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry*, **48**, 2008–2016.

Li, L., Shewry, P.R. & Ward, J.L. (2008). Phenolic acids in wheat varieties in the HEALTHGRAIN diversity screen. *Journal of Agricultural & Food Chemistry*, **56**, 9732–9737.

Filho, A.M.M., Firozi, M.R., Borges, J.T.D.S, Pinheiro Sant’ana, H.M., Chaves, J.B.P. & Coimbra, J.S.D.R. (2017). Quinoa: Nutritional, functional, and antinutritional aspects. *Critical Reviews in Food Science and Nutrition*, **57**, 1618–1630.

Inglett, G., Chen, D. & Liu, S. (2015). Antioxidant activities of selective gluten free ancient grains. *Food and Nutrition Sciences*, **6**, 612–621.

Martinez-Villalunga, C., Peñas, E. & Hernández-Ledesma, B. (2020). Pseudocereal grains: Nutritional value, health benefits and current applications for the development of gluten-free foods. *Food and Chemical Toxicology*, **137**, 11178.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Test breads used in sensory analysis (left to right) Control white refined wheat bread, 20% quinoa bread and 30% quinoa bread

**Figure S2.** Results of the consumer (n = 41) test of refined wheat bread, 20% and 30% of quinoa breads evaluated on a ten-point scale (0 = extremely dislike to 10 = extremely like) by untrained participants.

**Table S1.** Summary of TPH, FRAP, TEAC and DPPH values for individual quinoa varieties

**Table S2.** Summary of energy and nutrient content for individual quinoa varieties

**Table S3.** Summary of amino acid content for individual quinoa varieties