

Comparison of nutritional and nutraceutical properties of *Chenopodium quinoa* cultivated in Mexico and Ecuador

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd.) is considered a superfood due to its nutritional qualities and potential health benefits. In this work, the nutritional and nutraceutical properties were compared for white Ecuador quinoa seeds (WEQ) and red Ecuador quinoa seeds (REQ) with respect to the white Mexican quinoa seeds (WMxQ). Quinoa seeds from Peru have been introduced and adapted to the geographic and climatic conditions by the State of Morelos, Mexico, improved by mass selection in field to obtain plants with desirable phenotypic characteristics. The nutritional properties of seeds were evaluated through a proximate analysis, mineral content, amino acids profile, and fatty acid profile of quinoa oil. The nutraceutical properties were determined quantifying total phenols, total betalains and antioxidant capacity. The results evidenced that WEQ seeds had the highest protein content (16.59 g 100 g⁻¹ dw). The P, Ca, and Mg contents were high in WMxQ (424.6, 60.3, and 152.3 mg 100 g⁻¹ in dw respectively). Arginine, glutamic acid, and alanine were the most abundant amino acids in the three cultivars. In all quinoas, unsaturated fatty acids were most predominant (73.6% to 78.0%). The Mexican quinoa seeds showed the highest total phenolic content (2.9 mg gallic acid equivalent g⁻¹ in fw). The concentration of betalains in the REQ was 83.3 mg 100 g⁻¹ fw. The results demonstrate that nutritional and phenolic contents varies according to the color seeds and region. Quinoa of Mexico is high in phenolic compounds and may be used as a source of natural antioxidants.

Key words: Antioxidant capacity, betalains, free amino acids, nutraceutical potential, quinoa.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.), member of the Amaranthaceae family, is a native species of the Andean region in South America and routinely used in the preparation of soups, salads, desserts, and sweet drinks, among others (Ceyhun and Sanlier, 2019). Quinoa is mainly produced in Bolivia, Ecuador and Peru, but its cultivation has spread to others regions of Europe, Asia, and North America (Rojas et al., 2011). This species due to its wide genetic variability and broader adaptability grows from sea level until to an altitude of 4000 m and can tolerate adverse agro-ecological and edaphic conditions (Vega-Gálvez et al., 2010). In 1990, Peruvian quinoa was introduced in Mexico and has adapted to dry climate (Coahuila, 1300 m a.s.l.), sub-humid climate (State of Mexico, from 2300 to 2600 m a.s.l.), and humid subtropical climate (Morelos, 940 m a.s.l.) Quinoa that was adapted and produced in the State of Morelos shown higher yield of seeds (2 t ha⁻¹) and higher forage production.

Quinoa is considered a superfood due to its nutritional qualities (Maradini Filho et al., 2017), thus it is becoming increasingly popular choice of food among the health-conscious populations and millennials. Quinoa seeds are a rich source of protein (Lee and Sim, 2018), free sugars (Pereira et al., 2019), organic acids (Pellegrini et al., 2018), vitamins, minerals, and essential amino acids (Vilcacundo and Hernández-Ledesma, 2017). In addition to macronutrients, quinoa also contains bioactive compounds such as phenols and betalains responsible for the various seed colors and are related to the antioxidant capacity (Tang and Tsao, 2017). The nutritional composition and phenolic contents of quinoa seeds vary considerably according to the geographic location, environmental factors, soil type, fertilization, and genetic background (Miranda et al., 2011). A detailed landscape of the chemical composition of Morelos region adapted germplasm would better understand its nutraceutical properties, stability in response to Genotype × Environment interactions, and allow its exploitation through genetic improvement. Previous studies compared quinoas cultivated in Denmark, Poland, Chile, USA, Argentina, and Korea and reported high contents of proteins (from 12% to 16%), lipids (from 4.2% to 13.0%), and ash (from 2.7% to 4.0%) with respect to quinoas of Peruvian and Bolivian origin (Lee and Sim, 2018; Sobota et al., 2020). Although several studies have evaluated quinoa varieties grown in different parts of the world, little is known about the ones adapted to the Mexican environment.

Therefore, the aim of this study was to compare the nutritional and nutraceutical properties of three quinoa cultivars; one adapted in Mexico and the other two from the Andean region of Ecuador. The outcome of this study would contribute to the knowledge of the potential benefits of quinoa seeds to the health of consumers in these two regions of the world.

MATERIAL AND METHODS

Plant materials

White Ecuador quinoa (*Chenopodium quinoa* Willd.) seeds (WEQ) ‘INIAP-Tunkahuan’ released by Instituto Nacional de Investigaciones Agropecuarias de Ecuador (INIAP) were purchased directly from the farmers in Ibarra (0°19'12" N, 78°12'36" W), Imbabura Province, and red Ecuador quinoa seeds (REQ) ‘red-INCA’ were purchased in local market of Quito, Ecuador. White Mexico quinoa seeds (WMxQ), were provided by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Experimental Station of Tlaltizapan (18°41'19.8" N, 99°07'35.1" W), Morelos, Mexico. The different quinoa samples were acquired in the same season year. In the case of Ecuador, the quinoa is grown in Highland soil with an annual rainfall from 200 to 500 mm and temperature from 13 to 18 °C, while in Mexico, the quinoa was produced with low pluvial precipitation (< 100 mm annual) and average temperature of 22 °C. Seeds were washed, dried, and ground using a blender (NB-101S, Housewares, Los Angeles, California, USA) until obtaining a fine powder (20 mesh). Quinoa flours were stored in polyethylene bags at -20 °C until use. The color was measured in the whole seeds.

Chemicals

Folin-Ciocalteu phenolic reagent, anhydrous sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium acetate trihydrate, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), ferric chloride hexahydrate, quercetin, potassium persulfate, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich products (St. Louis, Missouri, USA). Aluminum chloride and potassium acetate were acquired from Fermont S.A. de C.V. and Meyer S.A. de C.V., Mexico, respectively. All reagent were analytical grade.

Determination of color

The color was determined with a Hunter lab colorimeter (MiniScan XE Plus 45/0-L, HunterLab, Reston, Virginia, USA) in CieLab scale (L*, a*, b*). Values of a* and b* were calculated, the tone angle (Hue; h*) parameter and color saturation (Chroma; C*) applying the Equations 1 and 2, while the L* value it was taken as lightness (McGuire, 1992):

$$h^* = \tan^{-1} \frac{b^*}{a^*} \quad (1)$$

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

Proximate and chemical analysis

The proximate composition was determined according to the methods described by AOAC (1998), moisture (934.01), protein (2001.11, using N × 6.25), fiber (962.09), ash (942.05), and fat (920.39). Mineral content was determined as described by Gavlak et al. (2013) using a microwave (Microwave Reaction System, Anton Paar, Mexico). A quinoa flour sample (0.15 g) was placed in a microwave digestion tube and added 0.5 mL concentrated nitric acid (16.8 N) and 2.0 mL 30% hydrogen peroxide to each tube. The tubes with sample and reagents were taken to predigestion for 30 min and then placed in the microwave. An atomic absorption spectrophotometer (GBC, SavantAA, Mexico) was used to Ca, Mg, Fe and Zn, a flame photometer (Lachat Instrument, Burlington, Canada) to K, and an ultra-violet spectrophotometer (Jenway, 6715 UV/Vis, Staffordshire, UK) to P.

Free amino acids profile identification

Free amino acids were analyzed according to the method proposed by Joshi et al. (2006). Quinoa seeds (10 mg) were frozen in liquid nitrogen and ground to a fine powder after mixing with 20 mM HCl (10 µL mg⁻¹ sample), the mixtures were centrifuged at 14 000 × g at 4 °C for 20 min. The supernatants were derivatized using an AccQ-Fluor reagent kit (Waters, Milford, Massachusetts, USA). Free amino acids were carried out using UPLC system (Acquity H-class, Waters) coupled with Xevo TQ mass spectrometer using an electrospray ionization (ESI) probe. Samples (10 µL) were injected onto column (AccQTag, 2.1 mm i.d. × 100 mm, 1.7 particles, Waters) and the data were collected using Empower software (Waters). The mobile phase of water phase (A) (0.1% formic acid v/v) and acetonitrile (B) (0.1% formic acid v/v), with a stable flow rate at 0.5 mL min⁻¹ and column temperature setting at 60 °C. L-Norleucine was used as an internal control, and the amino acids were quantified in nmol mg⁻¹ quinoa seeds.

Fatty acid composition

Quinoa flour samples (20 g) were subjected to extraction (14 h) with *n*-hexane in Soxhlet equipment. After, hexane was removed under reduced pressure in a rotary evaporator (R-300, Büchi, Flawil, Switzerland) to obtain the oil. Fatty acid methyl esters (FAME's) were obtained by esterification and transesterification of the oil (López-Yerena et al., 2018) with some modifications. The FAME's were analyzed in a gas chromatograph (6890, Agilent, Santa Clara, California, USA) with an ATSiIar column (0.25 mm i.d. × 30 m × 0.25 µm film thickness, Croydon, England), and a flame ionization detector (FID). The initial oven temperature was 170 °C with ramps of 10 °C per min at a final temperature of 240 °C. The injector and detector temperatures were set at 250 °C and hydrogen gas was used at a flow rate of 1.8 mL min⁻¹. The results were expressed as a percentage of the area (% area).

Extraction and fractionation of samples

A quinoa flour sample (1.0 g) was successively extracted with 25 mL methanol:water (50:50 v/v, pH 2) and acetone:water (70:30, v/v) solution. The extract was shaken at room temperature (22 °C) for 1 h and then centrifuged (5810 R, Eppendorf, Hamburg, Germany) at 2100 × g, at 4 °C for 10 min. The supernatants were mixed, filtered (ashless filter paper, Whatman grade 41, GE Healthcare, Buckinghamshire, UK), adjusted to 50 mL, and stored at -18 °C until use. From this extract, the free phenolics, flavonoids, and antioxidant capacity were determined. The pellet obtained from the previous extractions was re-extracted with 10 mL methanol:concentrated sulfuric acid (90:10, v/v) and refluxed for 10 h, then centrifuged (2100 × g, 4 °C, 10 min). The supernatant was recovered and adjusted to 10 mL with the extraction solvent, filtered, and stored at -18 °C until use. From this extract, the phenolics, flavonoids, betalains, and antioxidant capacity were determined (Abderrahim et al., 2015).

Total phenolics, flavonoids, and betalains content

The total phenolic content (TPC) quantification was carried out according to Hernández-Rodríguez et al. (2016) using Folin-Ciocalteu reagent. A gallic acid standard curve with a linear range (concentration 0.00 to 0.01 mg gallic acid mL⁻¹) was prepared. Results were expressed as mg gallic acid equivalent (GAE) g⁻¹ quinoa flour.

The total flavonoids content (TFC) was determined according to the method described by Chang et al. (2002). A quercetin standard curve with a linear range (concentration 0 to 0.01 mg quercetin mL⁻¹) was prepared. The results were expressed as mg quercetin equivalent (QE) g⁻¹ quinoa flour.

Total betalains were quantified by the method of Castellanos-Santiago and Yahia (2008) adapted to the microplates. Total betalains content (BC) was calculated from the equation:

$$BC \text{ (mg g}^{-1}\text{)} = [(A \times DF \times MW \times Vd)/\epsilon \times L \times Wd]$$

where A is the maximum absorbance at 535 and 435 nm for betacyanins and betaxanthin respectively, DF is the dilution factor, MW (g mol^{-1}) is molecular weight of betaxanthin (308 g mol^{-1}) and betacyanin (550 g mol^{-1}), Vd (mL) is volume of sample capacity, ϵ is molar extinction coefficient of betacyanins ($\epsilon = 60\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$) and betaxanthin ($\epsilon = 48\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$), L (cm) is length of the path-light in the well (0.50 cm) and Wd (g) is sample weight. The absorbances were measured in a Microplate reader (software Gen5, Bioteck Instruments Inc., Winooski, Vermont, USA). The results were expressed as mg betalain 100 g^{-1} quinoa flour.

Antioxidant capacity

The antioxidant capacity (AC) of the extract was determined by 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Re et al., 1999), ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Cheng et al., 2006) assays, adapted to the microplates. The results were expressed as mg trolox equivalent (TE) g^{-1} quinoa flour. The inhibitory concentration 50% (CI_{50}) was calculated by plotting the percentages of degraded DPPH against the sample concentration.

Statistical analysis

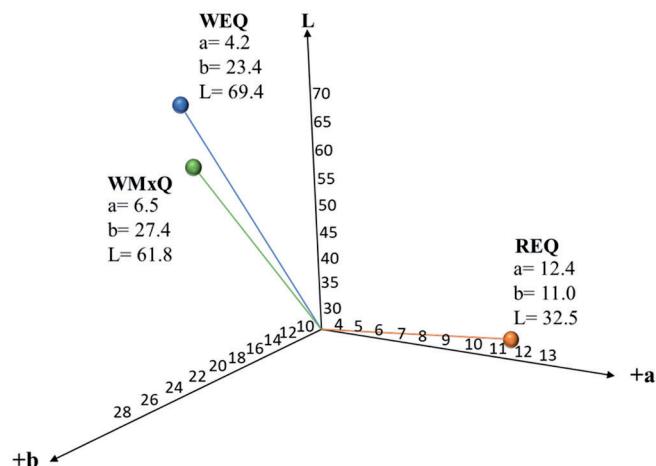
Results are presented as mean value \pm standard deviation of three replicates per sample and analyzed by one-way ANOVA. To compare means Tukey's test was used and differences were considered significant at $p \leq 0.05$. Statistical analysis was performed using SAS program 2005 (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Color study

Figure 1 shows the color parameters L^* , a^* and b^* of three quinoa cultivars. The results evidenced positive values for a^* and b^* coordinates. The lightness value (L^*) that indicates color brilliance revealed a significant difference ($P \leq 0.05$) between quinoa cultivars. The positive a^* coordinate was higher in the red seeds based on the value of hue (40.81°) and chroma (16.22), also showed a significant difference ($P \leq 0.05$), the tone of this seeds was purple-red, less pure, and less bright. The b^* positive coordinate was high in the WMxQ cultivar, according to the hue (76.26°) and chroma (27.33) values which were significantly different ($P \leq 0.05$) between the studied cultivars. The combination of these coordinates

Figure 1. Graphical representation of color parameters L^* , a^* , and b^* of three quinoa cultivars ($n = 3$).



WMxQ: White Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa; L^* : lightness (%); $+a$: x, red-purple tone component; $+b$: y, yellow tone component.

with the L value revealed the yellow-cream shade slightly lighter but with less lightness than the WEQ. Pellegrini et al. (2018) reported a hue of 89.71° and chroma of 16.69 in white quinoas, in this study, the hue value was low while the chroma value was higher than found by these authors. The L value* in red quinoa was lower than values reported by Laqui-Vilca et al. (2018), who obtained values for lightness between 48.1% and 53.5% in red quinoa seeds pericarp. The food color is an indicator of natural pigments, these pigments can be used as food coloring and be considered as functional ingredients due to their implication in human health (Shetty and Geethalekshmi, 2017). The color of quinoa seeds varies from white to black with yellow, red, pink, and violet hues. Red quinoa have significant betalains content; in particular of betanins and isobetanins that are pigments with biological activities for the benefit of health (Tang et al., 2015).

Proximate and chemical analysis

Quinoa, with high nutritional potential, is a species of C₃ photosynthetic pathway, unlike other cereals, it adapts and tolerates agroclimatic changes (García-Parra et al., 2020). The content of protein, essential amino acids, fiber, carbohydrates, and unsaturated fatty acids determines the nutritional quality of quinoa seeds. The chemical and proximate composition of quinoa seeds is shown in Table 1. The protein (16.5 g 100 g⁻¹) content in WEQ seeds was significantly ($P \leq 0.05$) higher than WMxQ. Fat content in WEQ and REQ (7.2 and 7.5 g 100 g⁻¹, respectively) were higher than for WMxQ. In contrast, ash and crude fiber levels were high in WMxQ (2.75 and 2.73 g 100 g⁻¹, respectively) and REQ (2.53 and 2.80 g 100 g⁻¹, respectively). The REQ had the highest content of carbohydrate (73.4 g 100 g⁻¹). Unlike our results, much lower protein (11.2 to 13.0 g 100 g⁻¹) and fat (4.8 to 5.4 g 100 g⁻¹) contents have been reported in the quinoa seeds grown in Peru, Bolivia, and Spain (Nowak et al., 2016; Pellegrini et al., 2018; Pereira et al., 2019). Protein content in the range of 13% to 17% has been reported by most studies (Maradini Filho et al., 2017). This content in quinoa seeds is higher than in traditional cereals such as wheat (7.4%), barley (10.5%), and rice (13.3%) (Tang and Tsao, 2017) and is considered a high-quality protein because provide all essential amino acids, particularly by the presence of lysine, methionine, and threonine, which are lacking in other cereals such as wheat and corn (Hernández-Ledesma, 2019). The consumption of 100 g quinoa seeds accounts for 46.5% of daily protein requirement according to Food and Agriculture Organization of the United Nations (FAO) guidelines. Furthermore, the quinoa contains important bioactive compounds as peptides and phenolic compounds that are related to the prevention of cardiovascular and immunological diseases, cancer, diabetes, among many others (Tang and Tsao, 2017). The Highland Northern of Ecuador soils are of volcanic origin and contain significant amounts of organic matter, a source of available N for the plants (Huygens et al., 2008) by this reason the Ecuadorian seeds contain the highest concentration of protein. Additionally, oil and crude protein contents is affected by Genotype × Environment interaction (Arslanoglu et al., 2011).

Table 1. Proximate analysis and mineral content of three quinoa cultivars.

| | WMxQ | WEQ | REQ |
|--------------|----------------|----------------|----------------|
| Moisture | 7.39 ± 0.18a | 6.99 ± 0.02b | 7.10 ± 0.02ab |
| Protein | 15.74 ± 0.03b | 16.59 ± 0.12a | 13.70 ± 0.04c |
| Fat | 6.53 ± 0.10b | 7.26 ± 0.12a | 7.52 ± 0.18a |
| Ash | 2.75 ± 0.04a | 2.25 ± 0.22b | 2.53 ± 0.03ab |
| Crude fiber | 2.73 ± 0.05a | 1.64 ± 0.24b | 2.80 ± 0.08a |
| Carbohydrate | 72.23 ± 0.13b | 72.25 ± 0.08b | 73.43 ± 0.07a |
| P | 424.66 ± 18.9a | 296.33 ± 9.29b | 235.66 ± 5.13c |
| K | 254.66 ± 2.52b | 241.66 ± 4.72c | 334.66 ± 6.81a |
| Ca | 60.33 ± 3.21a | 21.00 ± 1.00b | 14.66 ± 1.52c |
| Mg | 152.33 ± 4.04a | 124.33 ± 3.05b | 97.66 ± 2.52c |
| Fe | 3.97 ± 0.11b | 4.78 ± 0.11a | 3.85 ± 0.05b |
| Zn | 2.08 ± 0.06b | 2.52 ± 0.19a | 2.07 ± 0.00b |

Moisture content is expressed as g 100 g⁻¹ fresh weight; protein, fat, ash, crude fiber, and carbohydrate contents are expressed as g 100 g⁻¹ dry weight; minerals content are expressed as mg 100 g⁻¹ dry weight. For each row, means ± standard deviation followed by different letters are significantly different ($n = 3$, $P \leq 0.05$).

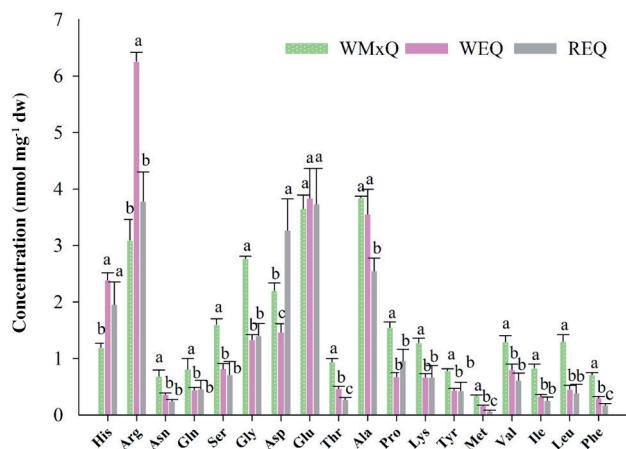
WMxQ: White Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa.

According to our results, P was the main micronutrient in all the analyzed samples (235.6 - 424.6 mg 100 g^{-1}), followed by K (241.6 - 334.6 mg 100 g^{-1}), Mg (97.6 - 152.3 mg 100 g^{-1}), and Ca (21.0 - 60.3 mg 100 g^{-1}). WMxQ had the highest contents of P, Ca, and Mg; WEQ had the highest contents of Fe (4.7 mg 100 g^{-1}) and Zn (2.4 mg 100 g^{-1}), showing significant differences ($P \leq 0.05$) among studied cultivars; and REQ showed the highest K content (334.6 mg 100 g^{-1}). Although Fe and Zn contents in the three cultivars were lower than other published studies (Lee and Sim, 2018), they were much higher than the regionally popular cereals such as wheat, corn and rice (Nowak et al., 2016). It is known that the chemical and mineral composition of quinoa seeds is significantly affected by geographical areas, N bioavailability, and genetic background (Miranda et al., 2011).

Analysis of free amino acids

According to some studies, the content of amino acids in quinoa depends on the region of cultivation and genetic variability of seeds (Miranda et al., 2012; Lee and Sim, 2018). This variation can be explained by the genetic characteristics of the Ecuadorian seeds, that are cultivated in Highland volcanic soils (2500 m a.s.l.), while that the Mexican quinoa grows at low altitude (950 m a.s.l.) In the studied samples, 18 amino acids were detected, including eight essential (Figure 2). WMxQ accumulated the highest concentration of almost all free amino acids. The most abundant free amino acids in the three quinoa cultivars were arginine, glutamic acid, and alanine. The arginine content in WEQ (6.25 nmol mg^{-1}) was significantly higher than WMxQ (3.09 nmol mg^{-1}); glutamic acid content showed nonsignificant differences ($P > 0.05$) among WMxQ, WEQ, and REQ cultivars. Alanine content was similar between WMxQ (3.84 nmol mg^{-1}) and WEQ (3.55 nmol mg^{-1}), while red seeds were low in arginine and alanine contents. Arginine is a critical amino acid for children and adolescents due to its role in stimulating the production and release of growth hormones (Abugoch-James, 2009) and also in production of creatine, a compound involved in the muscle regeneration process (Kreider et al., 2017). The essential amino acids (histidine, lysine, methionine, valine, isoleucine, leucine, phenylalanine, and threonine) represented about 27% of the total amino acids analyzed. Consistent with other studies (Lee and Sim, 2018), our results confirmed the abundant accumulation of arginine, glutamic acid, alanine, histidine, aspartic acid, glycine, and lysine in all three cultivars. Food consumption with a balanced composition of amino acids may replace amino acids lost in natural biochemical processes, contributing to the prevention of age-related diseases and improving health (Rose, 2019).

Figure 2. Free amino acids profile of three quinoa cultivars.



Means \pm standard deviation followed by different letters are significantly different for each aminoacid ($n = 3$, $P \leq 0.05$).

dw: Dry weight; WMxQ: white Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa.

Fatty acid composition

The fatty acids profile in the studied quinoa cultivars is displayed in Table 2. In all three cultivars, unsaturated fatty acids were most predominant (73.6% to 78.0%). Linoleic acid was the primary fatty acid ranging from 45.4% to 53.9%, followed by oleic acid (23.8% to 28.0%). The results evidenced that WMxQ and WEQ contained higher linoleic acid concentration than Peruvian quinoa seeds (30%) (Pereira et al., 2019). In addition, the content of linoleic acid in quinoas cultivated in WMxQ and WEQ was higher than the reported in peanut, almond, olive, coconut, wheat germ and rice germ oil (Orsavova et al., 2015). Linoleic acid is important in the human diet because it reduces the risk of cardiovascular diseases and the incidence of diabetes type II (Abugoch-James, 2009). The fatty acids composition of seeds in the present study was comparable to those reported for different quinoa varieties cultivated in USA (Wood et al., 1993).

Free and bound phenols and flavonoids content

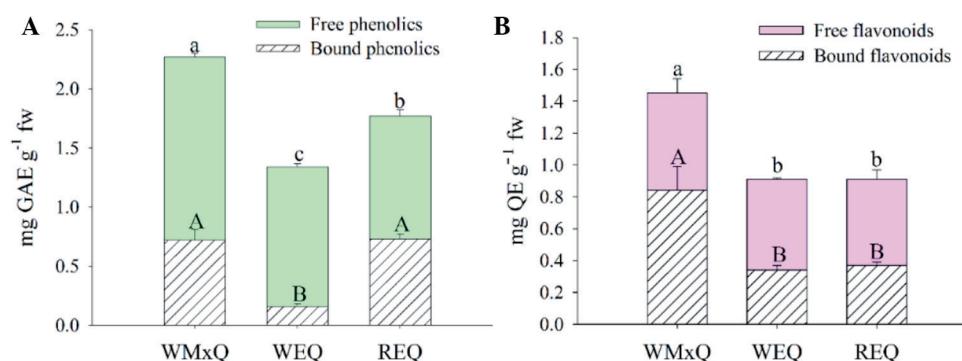
The results of total phenolic content (TPC) in free and bound forms are shown in Figure 3A. The TPC is the sum of the free and bound fractions; the free phenolic content in WMxQ was 40.9% higher than WEQ and showed significant differences ($P \leq 0.05$) between cultivars. While, in the bound fraction, WMxQ showed 77.7% more TPC than WEQ. The highest TPC in WMxQ (2.9 mg GAE g⁻¹ fw), with respect to Ecuadorian seeds, can be explained given their genotypic difference in addition to limited irrigation conditions and high temperature exposure, factors that could increase the production of free phenolic compounds (Piovesana et al., 2018). According to Fischer et al. (2013), water restriction during the vegetative cycle and genetic variability of the genotype have a significant effect on the increase of TPC and antioxidant capacity. The free fraction of WMxQ was slightly higher than those reported by Tang et al. (2015) for white seeds (2.2 mg GAE g⁻¹). Abderrahim et al. (2015) reported TPC values from 2.5 to 2.7 mg GAE g⁻¹ for red quinoa seeds, these results were higher than our results. The TPC in WMxQ and WEQ was also slightly lower than obtained from quinoa seeds cultivated in Peru, USA, and Korea (3.4, 4.3 and 4.5 mg GAE g⁻¹, respectively) (Lee and Sim, 2018).

Table 2. Fatty acids profile by gas chromatography on quinoa seeds cultivated in different regions.

| Cultivars | Saturated | | | | Unsaturated | | | | NI |
|-----------|---------------------|----------------------|----------------------|-------|------------------------|------------------|---------------------|-------|--------|
| | Palmitic (C16:0) | Esterinic (C18:0) | Araquidic (C20:0) | Total | Palmitoleic (C16:1) | Oleic (C18:1) | Linoleic (C18:2) | Total | |
| | % Area | | | | % Area | | | | % Area |
| WMxQ | 10.02 | 0.56 | 0.40 | 10.99 | 0.22 | 23.81 | 53.99 | 78.02 | 10.99 |
| WEQ | 9.65 | 0.62 | 0.43 | 10.70 | 0.19 | 25.76 | 52.13 | 78.08 | 11.22 |
| REQ | 10.32 | 0.72 | 0.53 | 11.57 | 0.20 | 28.04 | 45.42 | 73.66 | 14.77 |

NI: Not identified; WMQ: White Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa.

Figure 3. Free and bound phenols (A) and flavonoids (B) content of three quinoa cultivars.



Means \pm standard deviation followed by different letters are significantly different in free or bound compounds ($n = 3$, $P \leq 0.05$).

GAE: Gallic acid equivalent; fw: fresh weight; QE: quercetin equivalent; WMxQ: white Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa.

The most abundant flavonoids in quinoa seeds are flavonols like quercetin, myricetin, and kaempferol derivatives. Quercetin has the highest antioxidant capacity, therefore provides multiple benefits to human health (Tang et al., 2015). Free flavonoids content in WMxQ ($1.45 \text{ mg QE g}^{-1}$ fw) was significantly higher ($P \leq 0.05$) than WEQ ($0.91 \text{ mg QE g}^{-1}$ fw), which is equivalent to 37.2% more flavonoids regarding the cultivars from Ecuador (Figure 3B). Regarding bound flavonoids, WMxQ exceeded by 59.5% and 55.9% to WEQ and REQ, respectively. The TFC was the highest in the soluble phenolic fraction that in the extract by acid-hydrolysis. The free flavonoid values in WMxQ and WEQ were slightly lower than those reported by Lee and Sim (2018) for white quinoa seeds (between 1.4 and 1.9 mg QE g^{-1}). The free flavonoid content in REQ was comparable with the values from 0.81 to $1.34 \text{ mg QE g}^{-1}$ presented by Abderrahim et al. (2015). It is important to consider that the variation of polyphenols in quinoa seeds could be related to crop conditions, varieties, and environmental factors (Miranda et al., 2011).

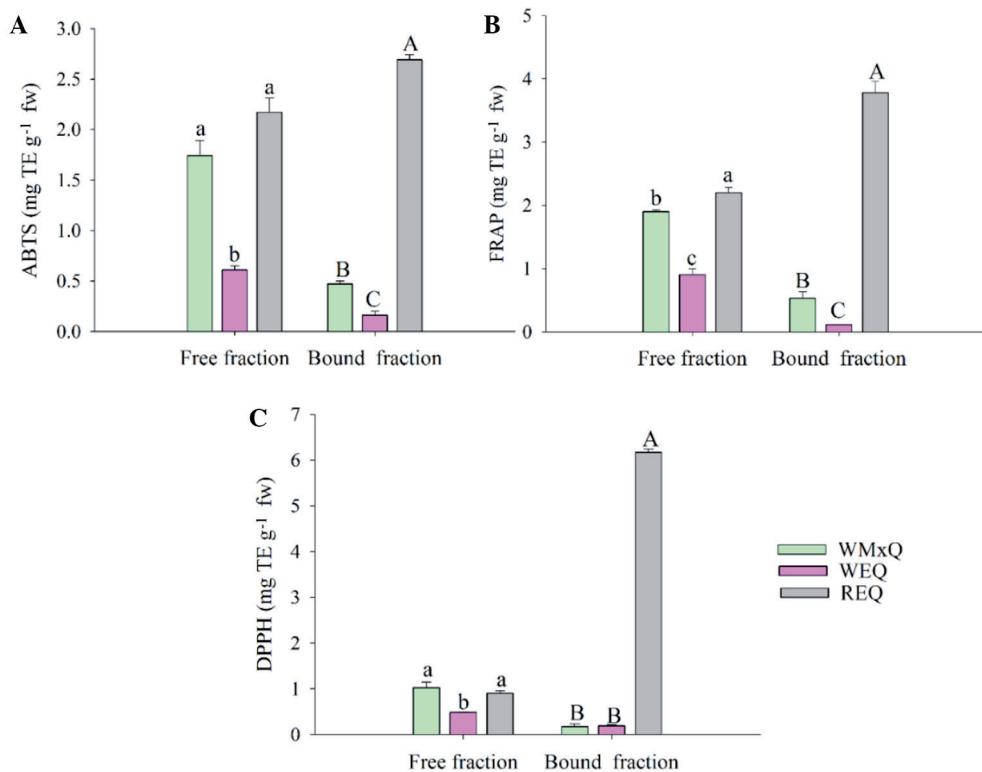
Content of free and bound betalains in red quinoa

Betalains are the pigments involved in the different colors of quinoa seeds, these pigments are divided into two groups, betacyanins (Bc) and betaxanthins (Bx). Total betalain content is the sum of Bc and Bx present in the sample and was determined in free and bound fraction of red seeds extract. Betalains were not detected in the free fraction extract; however, in the bound extract betalains, betaxanthins ($44.9 \text{ mg } 100 \text{ g}^{-1}$ fw) and betacyanins ($38.4 \text{ mg } 100 \text{ g}^{-1}$ fw) were identified at 435 and 535 nm, respectively. The total betalains content ($83.3 \text{ mg } 100 \text{ g}^{-1}$ fw) in red quinoa seeds from Ecuador was higher compared with the values reported by Li et al. (2015) for amaranth seeds from Mexico, Guatemala and Andean region (0.07 to $0.96 \text{ mg } 100 \text{ g}^{-1}$). The Bc and Bx contents in the present study were higher than those determined by Abderrahim et al. (2015) for quinoa seeds, these authors used the conventional extraction method with methanol:water (20:80 v/v, room temperature). On the other hand, Laqui-Vilca et al. (2018) reported values between 70.7 and $89.6 \text{ mg } 100 \text{ g}^{-1}$ for Bc and 100.3 to $218.2 \text{ mg } 100 \text{ g}^{-1}$ for Bx in pericarp of red quinoa from Peru, these values were higher than values found in REQ. The latter article results were obtained mixing $50 \mu\text{g}$ quinoa with water to a final volume of $500 \mu\text{L}$, using the microwave-assisted extraction method. In this study, the total betalains content was extracted by acid-hydrolysis with heating, however, it is important to consider the time and temperature to avoid degradation of pigment. In addition, the results of this study demonstrate that the pigments responsible for the red quinoa seeds are betalains and not anthocyanins as reported by Pásco et al. (2009). The betalains are N compounds present in color seeds, its variation can be related with the high N concentration in volcanic origin soils.

Antioxidant capacity

Figure 4 shows the results of antioxidant capacity (AC) as measured by ABTS, FRAP, and DPPH methods of three quinoa cultivars. The antioxidant capacity in REQ was significantly higher ($P \leq 0.05$) than white quinoas in all methods applied, this can be explained by the contribution of betalains to AC. The ABTS values (Figure 4A) in the free and bound phenolic fractions displayed significantly higher ($P \leq 0.05$) AC in WMxQ ($1.74 \text{ mg TE g}^{-1}$ fw) and with respect to WEQ. The FRAP analysis (Figure 4B) showed significant differences ($P \leq 0.05$) between studied quinoas. WMxQ (1.9 mg TE g^{-1} fw) presented 52.7% more AC than WEQ in the free phenolic fraction. In bound phenolic fraction, WMxQ (0.5 mg TE g^{-1} fw) showed 80% more AC than WEQ. The same behavior was observed by the DPPH method (Figure 4C), the AC in the free phenolic fraction of WMxQ (1.0 mg TE g^{-1} fw) was higher than WEQ. Mexican quinoa presented IC_{50} of 4.55 mg mL^{-1} , which is equivalent to 47.1% more AC regarding the white quinoa from Ecuador. However, in the bound phenolic fraction, WMxQ and WEQ showed nonsignificant differences. On the other hand, in the bound phenolic fraction, REQ showed IC_{50} value (0.784 mg mL^{-1} fw) lower than white quinoas, which is equivalent to 96.9% and 97.2% more AC for WMxQ and WEQ respectively. Tang et al. (2015) found that the AC was higher ($> 3000 \text{ mg kg}^{-1}$) in the hydrolysate fraction from red and black quinoas than free fraction, in the present study REQ had the same behavior. The AC obtained in the free phenolic fraction of the studied quinoas was lower than those presented by Pellegrini et al. (2018) for white seeds (1.9 to 4.5 mg TE g^{-1}) and red seeds (4.5 to 7.7 mg TE g^{-1}) by the ABTS, FRAP and DPPH methods. Regarding the DPPH results of quinoa seeds, the values in free phenolic fraction were comparable with IC_{50} (0.46 and 3.77 mg mL^{-1}) presented by Miranda et al. (2011). The difference in AC with other authors could be explained for the extraction method used, for the type of solvent, and time. The AC is associated with the phenolic content in free or bound forms, these compounds exert biological activities for the benefit of human health.

Figure 4. Antioxidant capacity by ABTS (A), FRAP (B) and DPPH (C) methods in quinoa cultivated in different regions.



Means \pm standard deviation followed by different letters are significantly different in free or bound compounds ($n = 3$, $P \leq 0.05$).
 TE: Trolox equivalent; fw: fresh weight; WMxQ: white Mexico quinoa; WEQ: white Ecuador quinoa; REQ: red Ecuador quinoa.

CONCLUSIONS

In the present study, the nutritional composition showed high contents of fiber, ash, P, Ca, Mg in the seeds of Mexican quinoa, while the seeds of white quinoa from Ecuador accumulated high contents of protein, fats, Fe, Zn, and amino acids such as arginine, histidine, alanine, and glutamic acid. Red quinoa showed higher content of carbohydrates, fiber, and K than white quinoas. All three studied quinoa cultivars showed presence of all essential amino acids (0.3 to 1.3 nmol mg⁻¹). The quinoa seeds oil was high in unsaturated fatty acids, mainly linoleic acid (45.4% to 53.9%). Regarding phenolics composition, Mexican quinoa had the highest phenolic content. Betalains were found only in the bound fraction of red quinoa. The acid hydrolysis method increased the extraction of betalains, compared to the assisted-microwave method previously reported. The seeds grown in Mexico showed almost 50% higher phenolic content than white quinoa seeds. Quinoa seeds adapted in Mexico have an improved nutritional and nutraceutical composition compared to white quinoa seeds from Ecuador.

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