

# Characterization of selected quinoa (*Chenopodium quinoa* Willd.) lines from Buenos Aires Province, Argentina, using intrinsic chemical parameters of the grains

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## ABSTRACT

The so-called sea-level ecotypes of quinoa grow satisfactorily under the agro-ecological conditions of the central region of Buenos Aires Province (Argentina). A previous selection derived from a quinoa genetic improvement project allowed the identification of 14 lines with good agronomic behavior, considering grain yield, the weight of one thousand grains, harvest index, plant height, and growth habits. This study aimed to characterize these 14 quinoa lines according to the specific chemical parameters of the grains to select those with particular compositional features, projecting them as crops of potential agro-industrial interest for the region. The parameters evaluated in the grains were: moisture, lipids, protein, ash, and carbohydrates using standardized techniques; Na, K, Ca, Mg, P, Fe, and Zn by absorption spectrophotometric techniques; composition of tocopherols and phenolic acids by HPLC and contents of saponins by spectrophotometry. In addition, total phenolic compounds contents and *in vitro* antioxidant capacity were estimated by Folin-Ciocalteu and DPPH methods, respectively. A multivariate statistical analysis was applied to establish the differences between the grains from the 14 selected quinoa lines according to the evaluated parameters. The first component of the biplot graph separated the quinoa grains with the highest protein, ash, mineral, and saponin content (RU5-9, RU5-1, and NLG-3) from the other variables evaluated. The second component clearly showed a distinction between the quinoa grain samples rich in lipids and tocopherols (RU5-14 and RU5-4) and those with a higher content of phenolic compounds, which were positively correlated with the *in vitro* antioxidant activity (NLG-4 and RU5-2). These results demonstrated that the evaluated parameters could help select quinoa lines with specific composition characteristics to be cultivated in the Buenos Aires region and eventually used for breeding programs for this species.

**Keywords:** sea-level genotype quinoa, quinoa grain composition, antioxidant, tocopherols, phenolic acids, saponins.

**Abbreviations:** db: dry basis, HPLC: High-Performance Liquid Chromatography, TPC: total phenolic compounds, DPPH: 2,2-diphenyl-1-picrylhydrazyl.

## RESUMEN

Los ecotipos de quinoa (*Chenopodium quinoa* Willd.) del nivel del mar han demostrado buena adaptación a las condiciones agroecológicas de la región central de la provincia de Buenos Aires. Una selección previa derivada de un proyecto de mejoramiento genético de quinoa permitió identificar 14 genotipos con buen comportamiento agro-

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nómico, considerando su rendimiento de granos, peso de mil granos, índice de cosecha, altura de planta y hábitos de crecimiento. Este estudio tuvo como objetivo caracterizar dichos genotipos según composición proximal, tocoferoles, principales ácidos fenólicos, saponinas y capacidad antioxidante *in vitro* de los granos, con el fin de seleccionar aquellos con las características adecuadas para ser proyectados como cultivos de potencial interés agroindustrial para la región. Los parámetros evaluados fueron: humedad, lípidos, proteínas, cenizas y carbohidratos, mediante técnicas estandarizadas; Na, K, Ca, Mg, P, Fe y Zn por espectrofotometría de absorción atómica; composición de tocoferoles y ácidos fenólicos por técnicas de HPLC y, finalmente, contenido de saponinas, compuestos fenólicos totales y capacidad antioxidante por métodos espectrofotométricos. Se aplicó un análisis estadístico multivariado de los datos obtenidos para establecer diferencias entre genotipos. El primer componente del gráfico biplot separó aquellas muestras con mayor contenido de proteínas, cenizas, minerales y saponinas (RU5-9, RU5-1 y NLG-3) de las demás variables evaluadas. El segundo componente mostró claramente una distinción entre aquellas muestras ricas en lípidos y tocoferoles (RU5-14 y RU5-4) y aquellas con mayor contenido de compuestos fenólicos y mayor actividad antioxidante *in vitro* (NLG-4 y RU5-2). Los resultados obtenidos mostraron variabilidad genética con respecto a los parámetros químicos, nutricionales y funcionales evaluados, lo cual muestra que podrían ser de utilidad como herramienta para la selección de genotipos con características nutricionales y funcionales específicas.

**Palabras clave:** ecotipos de quinoa de nivel del mar, antioxidantes, tocoferoles, ácidos fenólicos, saponinas.

## INTRODUCTION

Quinoa (*Chenopodium quinoa Willd.*) is a dicotyledonous species belonging to the *Poaceae* family. This native crop of the Andes was an essential food for the Incas and continues today to be for the Quechua and Aymara peoples of the rural regions. It was called "the mother grain" by the Incas, which sustained the community and was considered sacred (Abugoch James, 2009). The nutritional value of quinoa has been revalued in recent years thanks to the contributions to the scientific knowledge on this grain. In addition, the FAO has declared this species as a crop that makes a significant contribution to world nutrition and agriculture (Martínez, 2014). Besides its intrinsic nutritional value, it is source of health-promoting bioactive compounds that can reduce the risk of contracting several diseases and stimulate psychophysical well-being (Vega-Gálvez *et al.*, 2010). Another aspect about quinoa worth mentioning is that it does not contain gluten-forming proteins, which makes its inclusion in gluten-free diets possible (Alvarez-Jubete *et al.*, 2009).

Quinoa is an annual plant of 1 to 3,5 m high. It presents a 15 to 70-cm length panicle (inflorescence) that may render up to 200 g of grains (Repo de Carrasco and Encina Zelada, 2008). According to the genotypes and the phenological stage, the coloring of the plant varies from green to red. Colors like dark purple, yellow, orange, and other shades have been differentiated (Repo-Carrasco-Valencia *et al.*, 2010). Being a member of the *Amaranthaceae* family, the coloration that both plants and seeds can acquire is due to betalains, pigments with a structure, and a biosynthesis that is different from that of anthocyanins (Gandía-Herrero and García-Carmona, 2013). The quinoa ecotypes that best adapt to the agro-climatic characteristics of the central region of the province of Buenos Aires are the so-called "sea level ecotypes", which are characterized by their moderate biomass production, medium-sized grains (<3 mg), relatively short crop cycles (< 180 days) and potential yields close to 2,000 kg/ha, grown as an extensive crop in rainfed conditions (Cogliatti y Heter, 2016; Hasta, 2017).

Quinoa has multiple applications, all parts of the plant being usable. The primary purpose of this crop is to produce grain for human feeding. Another use is for cattle feeding, where the entire plant is used fresh, silaged, or pelletized. Moreover, the post-harvest remnants of the plant may be finely chopped or ground to elaborate concentrates and food supplements for animals. Finally, quinoa grains are also used for poultry breeding (Bhargava *et al.*, 2006).

Regarding the production area, quinoa has been cultivated throughout the Andean region, mainly in Peru, Bolivia, and Ecuador, for over 7,000 years by pre-Inca and Inca cultures. Historically, it has been cultivated from the north of Colombia to the south of Chile, at up to 4,000 m above sea level. However, the best yields were achieved at sea-level heights from 2,500 to 3,800 m, with an annual rainfall varying from 250 and 500 mm and an average temperature of 5 to 14°C (Mujica and Jacobsen, 2007). In the last decades, the search for alternative crops that could adapt to climate change and soil degradation, in addition to the interest in the nutritional properties of quinoa and its derivatives, has led to a considerable increase in the surface area destined for its cultivation globally. This increase has occurred in traditional producing countries (Peru and Bolivia) and other South American countries, such as Chile, Argentina, Brazil, Colombia, and Ecuador. The same has occurred in Canada, the US, European countries, India, China, and Australia, although, in the latter, to a lesser extent.

The broad genetic variability of quinoa allows the different cultivars to adapt to agroecological conditions, including drought, high altitudes, extreme temperatures, high salinity, sandy soils, and unsuitable conditions for other crops (Brady *et al.*, 2007). Therefore, quinoa tolerates various soil acidity conditions, from pH 6,0 to 8,5. Furthermore, the development of the plant may not be affected by temperatures of about -1°C to 35°C. It can develop even in regions where the annual rainfall varies between 200 and 400 mm (Mujica *et al.*, 2001), which is why FAO promoted its crop in arid regions. The sowing season in the Andean highlands takes place in August, extending until

December, and, in some areas, from January to March (Valencia-Chamorro, 2003).

The present work aimed to characterize selected quinoa lines using grain chemical parameters related to their contents of macro and micro components and other chemical properties to differentiate those with specific features to be cultivated in the region as potential staples.

## MATERIAL AND METHODS

### Reagents

All the reagents used in the present work were of analytical grade, unless another type is specified. The standards used in the spectrophotometric techniques and HPLC solvents were of appropriate purity and characteristics to the analytical methodology applied.

### Vegetal material and sample preparation

In this study, 14 quinoa lines that had been previously selected from a genetic improvement project (Hasta, 2017) were evaluated. The grains were collected from plants cultivated during 2019 at the experimental farm of the Faculty of Agronomy (UNCPBA, Azul, Buenos Aires Province, 36° 49' 53" South latitude and 59° 53' 23" West longitude). The plants were grown in rows spaced 0.4 m, in dryland conditions, on a typic argiudol soil and fertilized with diammonium phosphate at sowing and urea at 30 days to achieve a final nutrient availability of 150 kg of N/ha and 20 ppm of P. The evaluated lines were identified as REGALONA, FARO, KVL32, NL6, LV2, and RU5 after their classification according to the selected agronomic parameters (table 1). All the tests were carried out using the grains of the selected quinoa lines which had been previously grounded by a horizontal blade at a cereal grain mill (model TDMC, Tecno

Dalvo, Argentina) for 30 seconds. From the resulting powder, the determinations described below were made in triplicate.

### Moisture

The water content was determined gravimetrically using a thermobalance (model DBS 60-3, Kern & Sohn GmbH, Germany). Approximately 2 g of powered grains were placed in an aluminum dish and then exposed under an infrared lamp at a temperature of 105°C until a constant weight was obtained. Moisture was calculated as the weight loss during drying and expressed as a percentage.

### Proteins

The crude protein content was assessed by the Kjeldahl method (AOAC, 1990), using automatic equipment for digestion and distillation (B-316, Büchi, Switzerland). Dry powdered samples were used, and the protein content was obtained by multiplying the nitrogen mass by 6.25.

### Lipids

The lipid content was determined using Soxhlet equipment and *n*-hexane, following a standard method (AOCS, 2013). Approximately 15 g of dry quinoa flour were placed in Whatman N° 3 paper cartridges. The process was carried out for 4 h at continuous reflux cycles at 80°C. The solvent was then removed by a rotary evaporator (R-114, Büchi, Flawil, Switzerland) at 55°C.

### Ashes

This determination was based on an AOAC technique (2005). Approximately 4 g of quinoa flour were placed in a porcelain

Line	GY (kg/ha)	GW (g/1000)	HI	PH (cm)	CC (Days)
FARO-1	3399	2.2	31.0	105	126
FARO-2	2464	2.8	24.5	89	135
FARO-3	1845	2.2	22.1	99	126
KVL 32-4	1610	2.0	28.2	77	126
LV2-3	2112	2.7	23.5	108	135
NLG-3	1998	2.8	23.4	104	124
NLG-4	1886	2.6	23.4	99	153
RU5-1	2518	2.5	27.1	115	131
RU5-2	2668	2.3	25.9	115	126
RU5-4	1940	2.2	24.6	111	126
RU5-9	2197	2.8	17.9	113	139
RU5-11	1319	2.3	17.0	109	131
RU5-14	2296	2.1	26.8	111	126
REGALONA	2047	2.2	23.8	95	154

**Table 1.** Grain yield (GY), thousand-grain weight (GW), harvest index (HI), plant height (PH) and crop cycle (CC) of the selected quinoa lines (Hasta, 2017).

crucible and heated on a wire mesh with a burner until a carbonaceous residue was obtained. Then, it was introduced into a muffle furnace at 550°C until a whitish residue was produced.

### Mineral determinations

The Ca, Mg, Zn, Fe, Na, and K levels were assessed by Atomic Absorption Spectrometry applying a technique based on bibliography (Viñas *et al.*, 1993). Briefly, 50 mg of processed quinoa samples were weighed into digestion tubes, and 500 µL of 1:1 (v/v) concentrated nitric and perchloric acids mixture was added. The sealed tubes were allowed to stand for 6 h at ambient temperature and then for 12 h at 95°C in a thermoblock (TD 200 P3, Falc Instruments, Italy). After organic matter digestion, the tube contents were diluted with bidistilled water, then homogenized, and centrifuged at 500 x g. For Ca and Mg assays, the supernatants were diluted with 0.2% LaCl<sub>3</sub> solution, while for Fe, Zn, Na, and K analysis, dilutions were made with bidistilled water. The measurements were achieved using an atomic absorption spectrophotometer (M 906, GBC, Australia) and the external standard calibration method. In addition, the P concentration was determined by absorption spectrophotometry (Ultrospect III, Pharmacia LKB, Sweden), according to Jaszczyńska *et al.* (2003).

### Phenolic compounds extraction

The phenolic extracts from quinoa grains were obtained following the methodology described by Carciochi *et al.* (2015). Briefly, about 2 g of quinoa flour were weighed into a 50 mL centrifuge tube, and then 20 mL of ethanol 80% (v/v) were added. The mixture was homogenized and agitated in an orbital shaker at 160 rpm for 30 min at 60°C (MaxQ HP, Fisher Scientific, USA). After this, tubes were centrifuged for 10 min at 8900 x g (Sorvall Legend X1, Thermo Scientific, USA), and the supernatant was withdrawn. A second extraction of the residue was performed under the same conditions. Finally, the supernatants were pooled and stored at -18°C for further analysis.

### Total phenolic compounds assay

The total phenolic compounds (TFC) content of the samples was estimated using the Folin-Ciocalteu method, according to Singleton *et al.* (1999). To 100 µL of the extract resulting from the previous procedure, 300 µL of saturated sodium carbonate solution was added. The mix was homogenized and left to stand two hours under darkness before absorbance measurement at 765 nm in a spectrophotometer (UV-7804 C, Viswagene Biotech Pvt. Ltd., India). The results were expressed as gallic acid equivalents (GAE) for 100 g of dry grains, using a calibration curve constructed from standard gallic acid solutions.

### In vitro antioxidant activity measurement

A test for estimating the *in vitro* antioxidant activity of the quinoa grains extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was performed following the protocol of Brand-Williams *et al.* (1995). 50 µL of the extracts were mixed with 1950 µL of 100 µM methanolic solution of DPPH. After 30 min of incubation under darkness, the absorbance value at 517 nm was registered. The antioxidant activity was estimated

from the decrease in absorbance value as:

$$\% \text{ DPPH radical inhibition} = \frac{[(\text{Abs}(t=0) - \text{Abs}(t=30)) / \text{Abs}(t=0)] \times 100}$$

Where:

Abs(t=0): initial absorbance

Abs(t=30): absorbance at 30 min

Afterward, the results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in µmol Trolox/100 g sample (Vollmannova *et al.*, 2013).

### Determination of the main phenolic acids

Identification and quantification of the main phenolic acids reported in the quinoa grain extracts (gallic acid, p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, and ferulic acid) were carried out by reverse phase HPLC method using a modified procedure of Ross *et al.* (2009). 10 mL of the extract were evaporated to dryness in a rotary evaporator (R-114, Büchi, Flawil, Switzerland) at 40°C. The solid residue was then resuspended in 0,5 mL of a 1:1 methanol: water mix and the solution was filtered by a 0.45 µm nylon membrane (Gamafil, Argentina) before being injected into an HPLC system (Serie 1050, Hewlett Packard, California, USA) equipped with a column LiChrospher® 100, RP-18 end-capped, 150 mm x 4.6 mm, 5 µm particle (Merck, Germany) and a UV-Vis detector settled at 270 nm. Two solvents were used for the mobile phase: A: 0.1% (v/v) aqueous formic acid solution and B: 0.1% (v/v) methanolic solution of formic acid. Elution of analytes was performed using a gradient with the following proportion of B in the eluent (v/v): 10% during 10 min, 10% to 35% (lineal) from 10 min to 40 min, 35% during 5 min, 35% to 100% (lineal) from 45 min to 60 min, 100% B during 5 min, and finally 100% to 10% (lineal) from 65 min to 67 min to prepare the column for the next injection. The flow rate was 1 mL/min, and all the phenolic compounds were quantified as aglycones using the standard external method. The range of acids standard concentration for each phenolic acid used was 4-80 µg/mL. The final results for quinoa grain samples were expressed as mg/100 g of dry seeds.

### Tocopherols determination

The identification and quantification of tocopherols were performed by HPLC methodology with fluorescence detection, using the lipidic fraction obtained after the extraction with n-hexane of the quinoa grains according to IUPAC (1992) and AOCS (2017) standard methods. About 0.5 g of lipidic fraction were weighed into a 5.0 mL volumetric flask, and the volume was completed with n-hexane, completing the dissolution in an ultrasonic bath (8891-26, Cole Parmer, USA). A membrane filtered volume of 20 µL was injected into an HPLC system (Serie 1050, Hewlett Packard, California, USA) equipped with a column Lichrosorb Si 60, 250 mm x 4.6 mm, 5 µm particle (Merck, Germany). The detection was carried out with a fluorescence detector (Agilent 1100, California, USA) settled at excitation and emission wavelengths of 290 nm and 330 nm, respectively. The flow rate was 1 mL/min, and the identification of α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol was made using the retention times of the corresponding standards and the respective concentrations calculated using the standard external method as mg/100 dry sample.

## Saponins analysis

For the extraction and quantification of total saponin content of the quinoa grains, the method proposed by Monje and Raffailac (2006) was followed. Briefly, 1 g of quinoa flour was put into a plastic tube, and 10 mL of 50% (v/v) ethanol was added. The mixture was then agitated in an orbital shaker at 160 rpm for 30 min (MaxQ HP, Fisher Scientific, USA). Then, the suspension was paper-filtered, the solution collected in a 10 mL volumetric flask, and the volume was completed with the same ethanolic solvent. The extracts were kept at -18°C until use. For saponin quantification, 1750 µL of Lieberman-Burchard reagent (mixture of 16.7% acetic anhydride in concentrated sulphuric acid) were added to 500 µL of quinoa extract, and the solution was incubated at 20°C for 30 min. Then, the absorbance at 528 nm was measured (M 906, GBC, Australia). The values were used to estimate saponin levels using a calibration curve made with standard saponin solutions (Sigma, St. Louis, USA).

## Statistical analysis

The experimental results were performed in triplicate and were presented as mean ± SD. The statistical analysis was carried out using the InfoStat program (2016, InfoStat group, Argentina), through which the analysis of variance was performed with the comparison of means of the treatments using the Tukey test ( $p < 0.05$ ). The regression and principal component analyses were also carried out with the same software.

## RESULTS AND DISCUSSION

### Macro and micronutrient composition

Table 2 shows the results from the compositional analysis of the different lines of quinoa studied in this work. Some values

extracted from the bibliography corresponding to this same species, amaranth (*Amaranthus* spp.), and the main cereals of intensive cultivation worldwide are shown in table 3. Carbohydrates represented the main fraction of the quinoa grains, and the NL6-4 presented the highest value among the grain samples studied (mean content 79.76%, dry basis). Carbohydrates in quinoa resulted in higher levels than amaranth (62.2%) and wheat (72.5%) but lower than rice (89.8%).

On the other hand, the protein content of the quinoa lines ranged between 13.8% and 16.5%, corresponding to NL6-4 and RU5-4, respectively. In particular, NL6-4 presented the lowest protein level with a significant difference from the rest of the lines. Table 3 shows that these values are similar to those published for the same species and amaranth and higher than the protein contents of cereals. It is worth mentioning that, compared with other food grains, the amino acid composition of quinoa protein presents more similarities with human and cow milk proteins (Risi, 1991). Concerning lipid content, higher values were found for LV2-3 (3.35%) and RU5-4 (4.17%). These values are in the range reported for this parameter by other authors in quinoa and were higher than the average values published for cereals like rice (0.4%) and wheat (2.6%) but lower than corn (4.7%) and amaranth (8.81%) (table 3).

On the other hand, the ashes content of grains from the 14 quinoa lines showed significant differences ranging from 3.21% to 6.84% for RU5-14 and RU5-9, respectively. These levels were higher than those in rice, corn, and wheat but similar to those in amaranth. In particular, RU5-9 exhibited the highest ash content compared to the other species and even to different values published for the same species (table 3).

The analysis of the main mineral profile present in quinoa ashes resulted in the values shown in table 4. In agreement with the study of Abugoch James (2009), the most abundant minerals

Line	Ashes	Proteins	Lipids	Carbohydrates*
RU5-1	3.51 ± 0.09 <sup>a,b,c</sup>	15.64 ± 0.16 <sup>b</sup>	1.56 ± 0.29 <sup>a</sup>	79.28 ± 0.30 <sup>e,f</sup>
RU5-2	3.41 ± 0.13 <sup>a,b,c</sup>	15.35 ± 0.31 <sup>b</sup>	2.17 ± 0.05 <sup>ab</sup>	79.07 ± 0.05 <sup>e,f</sup>
RU5-4	3.27 ± 0.05 <sup>ab</sup>	16.53 ± 0.72 <sup>b</sup>	4.17 ± 0.11 <sup>b</sup>	76.02 ± 0.45 <sup>ab</sup>
RU5-9	6.84 ± 0.05 <sup>e</sup>	16.35 ± 0.81 <sup>b</sup>	2.33 ± 0.01 <sup>ab</sup>	74.48 ± 0.21 <sup>a</sup>
RU5-11	3.34 ± 0.01 <sup>ab</sup>	15.65 ± 0.13 <sup>b</sup>	2.67 ± 0.06 <sup>ab</sup>	78.34 ± 0.07 <sup>c,d,e,f</sup>
RU5-14	3.21 ± 0.02 <sup>a</sup>	15.62 ± 0.43 <sup>b</sup>	3.67 ± 0.25 <sup>ab</sup>	77.50 ± 0.66 <sup>c,d</sup>
FARO-1	4.52 ± 0.05 <sup>c,d</sup>	15.58 ± 0.18 <sup>b</sup>	1.65 ± 0.30 <sup>a</sup>	78.25 ± 0.48 <sup>c,d,e</sup>
FARO-2	4.34 ± 0.09 <sup>a,b,c,d</sup>	16.25 ± 0.22 <sup>b</sup>	2.20 ± 0.25 <sup>ab</sup>	77.20 ± 0.48 <sup>b,c</sup>
FARO-3	4.79 ± 0.04 <sup>d</sup>	15.49 ± 0.18 <sup>b</sup>	2.74 ± 0.25 <sup>ab</sup>	76.98 ± 0.22 <sup>b,c</sup>
NL6-3	3.61 ± 0.10 <sup>a,b,c,d</sup>	15.47 ± 0.52 <sup>b</sup>	1.50 ± 0.00 <sup>ab</sup>	79.42 ± 0.61 <sup>e,f</sup>
NL6-4	3.36 ± 0.13 <sup>a,b,c,d</sup>	13.84 ± 0.27 <sup>a</sup>	3.03 ± 0.03 <sup>ab</sup>	79.76 ± 0.47 <sup>f</sup>
LV2-3	4.51 ± 0.10 <sup>b,c,d</sup>	15.14 ± 0.02 <sup>b</sup>	3.35 ± 0.22 <sup>b</sup>	76.99 ± 0.29 <sup>b,c</sup>
KVL32-4	3.34 ± 0.10 <sup>ab</sup>	15.37 ± 0.30 <sup>b</sup>	2.43 ± 0.24 <sup>ab</sup>	78.86 ± 0.15 <sup>d,e,f</sup>
REGALONA	3.77 ± 0.07 <sup>a,b,c,d</sup>	15.41 ± 0.36 <sup>b</sup>	3.08 ± 0.25 <sup>ab</sup>	77.74 ± 0.17 <sup>c,d</sup>

Data expressed in % as mean ± SD (n = 3), db. Different letters in the same row indicate significantly different values ( $p < 0.05$ ). \*Values estimated by difference.

**Table 2.** Centesimal composition of grains from the selected quinoa lines.

Component	Quinoa	Amaranth <sup>f</sup>	Corn <sup>g</sup>	Wheat <sup>h</sup>	Rice <sup>h</sup>
Proteins	11.52 <sup>a</sup> - 16.5 <sup>b</sup>	14.6	10.2	10.5	7.8
Lipids	1.42 <sup>b</sup> -9.7 <sup>c</sup>	8.81	4.7	2.6	0.4
Carbohydrates	61.2 <sup>d</sup> - 72.6 <sup>e</sup>	62.2	81.1	72.5	89.8
Ashes	1.92 <sup>b</sup> -3.49 <sup>a</sup>	3.25	1.7	1.8	0.7

<sup>a</sup> De Oliveira Lopes *et al.*, 2009, <sup>b,g</sup> Koziol, 1992, <sup>c</sup> Ando *et al.*, 30, 2002, <sup>d</sup> Chauhan *et al.*, 1992, <sup>e</sup> Ruales, Nair, 1993, <sup>f</sup> Valcárcel-Yamani and Da Silva Lannes, 2012, and <sup>h</sup> Repo-Carrasco *et al.*, 2003.

**Table 3.** Some centesimal compositional values reported for quinoa, amaranth, and main cereals (as %, db).

in quinoa grains were potassium, phosphorus, and magnesium. It is worth mentioning that the ashes corresponding to RU5-9, FARO-2, and KVL32-4 presented the highest calcium content of all the quinoa lines analyzed. Konishi *et al.* (2004) studied the mineral distribution in quinoa grains and demonstrated that phosphorus and magnesium are located in the embryonic tissue, while calcium and potassium are present in the pericarp. So, the polishing usually applied for saponin elimination of grains can decrease their calcium content. Comparing the values of the mineral contents of the quinoa lines analyzed in this work with some reported in the literature for the same species, amaranth, and the cereals wheat and rice (table 5), some considerations may be mentioned. The levels of calcium and magnesium showed significant differences among all the quinoa lines, with the highest levels found for KVL32-4 and FARO-3, respectively. These values were higher than those published for the same species, rice, and wheat, but lower than amaranth. Furthermore, RU5-1 and LV2 showed the highest phosphorus contents. In addition, NL6-3 threw the minimum zinc content

while maximum values corresponded to RU5-9, KVL32-4, and NL6-4, similar to those in rice and wheat. Concerning iron contents, KVL32-4 presented the maximum value among all the lines, superior to rice, wheat, and amaranth. The maximum sodium content corresponded to FARO-2, higher than the value published for amaranth, lower than rice and wheat, and below the range reported for other quinoa varieties. Finally, the maximum potassium content was found in RU5-9, superior to rice, wheat, and amaranth.

#### Total phenolic contents and *in vitro* antioxidant activity

Table 6 shows the TPC and the *in vitro* antioxidant activity values found in the grains of the 14 selected quinoa lines. Minimum and maximum levels of TPC (expressed as mg/100 g in gallic acid equivalent, GAE, dry basis) corresponded to RU5-9 (194.15) and NL6-4 (278.12), respectively. These values were comparable to those reported by Tang *et al.* (2015) but higher than the levels found by Miranda *et al.* (2011), who evaluated

Lines	Mg	Ca	P	Fe	Zn	Na	K
RU5-1	215.22 ± 0.05 <sup>a</sup>	81.36 ± 0.04 <sup>c</sup>	370.62 ± 4.10 <sup>3k</sup>	7.45 ± 2.11 <sup>b</sup>	3.41 ± 1.51 <sup>a,b,c</sup>	4.81 ± 0.88 <sup>d</sup>	452.97 ± 0.37 <sup>f</sup>
RU5-2	256.24 ± 0.21 <sup>e</sup>	143.18 ± 0.12 <sup>h</sup>	343.10 ± 8.10 <sup>3g</sup>	40.29 ± 1.92 <sup>g</sup>	3.61 ± 0.41 <sup>a,b,c</sup>	1.92 ± 0.63 <sup>a</sup>	174.24 ± 0.12 <sup>a</sup>
RU5-4	224.17 ± 0.02 <sup>b</sup>	67.82 ± 0.16 <sup>a</sup>	285.82 ± 0.01 <sup>b</sup>	11.42 ± 2.80 <sup>c,d</sup>	2.76 ± 2.33 <sup>ab</sup>	1.96 ± 0.74 <sup>a</sup>	247.07 ± 0.29 <sup>b</sup>
RU5-9	252.44 ± 0.71 <sup>d</sup>	206.41 ± 0.12 <sup>k</sup>	351.79 ± 7.10 <sup>4h</sup>	34.04 ± 1.46 <sup>f</sup>	4.28 ± 0.44 <sup>c</sup>	3.88 ± 0.21 <sup>b,c,d</sup>	1015.55 ± 0.42 <sup>m</sup>
RU5-11	255.29 ± 0.10 <sup>e</sup>	130.42 ± 0.03 <sup>f</sup>	267.24 ± 6.10 <sup>4a</sup>	12.08 ± 1.14 <sup>d</sup>	2.78 ± 4.63 <sup>ab</sup>	3.89 ± 0.62 <sup>b,c,d</sup>	373.48 ± 0.35 <sup>e</sup>
RU5-14	295.62 ± 0.61 <sup>k</sup>	125.24 ± 0.05 <sup>e</sup>	329.30 ± 4.10 <sup>4f</sup>	5.93 ± 2.38 <sup>a</sup>	2.87 ± 0.66 <sup>ab</sup>	3.47 ± 0.51 <sup>b,c,d</sup>	330.48 ± 0.11 <sup>d</sup>
FARO-1	290.54 ± 0.37 <sup>j</sup>	156.57 ± 0.04 <sup>i</sup>	316.65 ± 8.10 <sup>3d</sup>	12.03 ± 2.12 <sup>d</sup>	3.13 ± 2.34 <sup>ab</sup>	2.68 ± 0.43 <sup>ab</sup>	533.74 ± 0.20 <sup>j</sup>
FARO-2	272.38 ± 1.81 <sup>g</sup>	207.91 ± 0.18 <sup>k</sup>	304.93 ± 0.01 <sup>c</sup>	15.65 ± 2.56 <sup>e</sup>	2.83 ± 1.39 <sup>ab</sup>	8.23 ± 0.43 <sup>e</sup>	521.22 ± 0.15 <sup>g</sup>
FARO-3	328.40 ± 0.19 <sup>l</sup>	168.49 ± 0.03 <sup>j</sup>	287.84 ± 7.10 <sup>3b</sup>	11.92 ± 1.07 <sup>c,d</sup>	2.89 ± 0.89 <sup>ab</sup>	3.76 ± 0.80 <sup>b,c,d</sup>	636.81 ± 0.40 <sup>j</sup>
NL6-3	260.07 ± 0.12 <sup>f</sup>	73.07 ± 0.09 <sup>b</sup>	352.50 ± 6.10 <sup>3h</sup>	14.04 ± 1.03 <sup>e</sup>	2.40 ± 2.10 <sup>a</sup>	3.81 ± 0.45 <sup>b,c,d</sup>	852.11 ± 0.46 <sup>k</sup>
NL6-4	229.93 ± 0.07 <sup>c</sup>	67.27 ± 0.05 <sup>a</sup>	323.10 ± 4.10 <sup>3e</sup>	44.57 ± 1.82 <sup>h</sup>	4.41 ± 0.13 <sup>c</sup>	3.29 ± 0.72 <sup>b,c,d</sup>	529.10 ± 0.28 <sup>h</sup>
LV2-3	273.05 ± 0.25 <sup>g</sup>	121.14 ± 0.07 <sup>d</sup>	372.25 ± 9.10 <sup>3k</sup>	10.45 ± 1.04 <sup>c</sup>	3.42 ± 1.12 <sup>b,c</sup>	3.23 ± 0.50 <sup>b,c</sup>	864.56 ± 0.47 <sup>l</sup>
KVL32-4	281.24 ± 0.46 <sup>i</sup>	227.22 ± 0.04 <sup>l</sup>	366.10 ± 5.10 <sup>3j</sup>	48.88 ± 1.67 <sup>i</sup>	4.32 ± 0.01 <sup>c</sup>	1.89 ± 0.76 <sup>a</sup>	334.77 ± 0.12 <sup>d</sup>
REGALONA	275.93 ± 0.02 <sup>h</sup>	136.52 ± 0.13 <sup>g</sup>	362.00 ± 0.01 <sup>i</sup>	7.74 ± 2.01 <sup>b</sup>	3.14 ± 0.62 <sup>a,b,c</sup>	4.63 ± 0.28 <sup>c,d</sup>	284.15 ± 0.23 <sup>c</sup>

Values expressed in mg/100g, db as means ± SD (n = 3). Different letters in the same row indicate significantly different values (p < 0.05).

**Table 4.** Mineral composition of grain ashes from the selected quinoa lines.

Component	Quinoa	Amaranth <sup>c</sup>	Wheat <sup>b</sup>	Rice <sup>b</sup>
Mg	103 <sup>a</sup> -246.9 <sup>b</sup>	425.21	120	118
Ca	108.41 <sup>c</sup> -148.7 <sup>b</sup>	283.14	50	27.6
P	383.7 <sup>b</sup> - 469 <sup>d</sup>	55.59	380	284.5
Fe	1.4 <sup>d</sup> -13.2 <sup>b</sup>	29.35	5	3.7
Zn	2.7 <sup>e</sup> -7.44 <sup>f</sup>	n.r.	3.1	5.1
Na	12.2 <sup>b</sup> -31.3 <sup>e</sup>	4.14	10	12
K	7.5 <sup>e</sup> - 926.7 <sup>b</sup>	770.15	500	212

Values expressed in mg/100g, db. <sup>a</sup> Vilcacundo and Hernández-Ledesma, 2017, <sup>b</sup> Romo *et al.*, 2006, <sup>c</sup> Palombini *et al.*, 2013, <sup>d</sup> Sanders, 2009, <sup>e</sup> Dini *et al.*, 1992, <sup>f</sup> De Oliveira Lopes *et al.*, 2009. n.r: not reported.

**Table 5.** Some values of mineral elemental composition of quinoa, amaranth, wheat, and rice reported in the literature.

six quinoa genotypes from three different geographical regions of Chile. In another study, Repo de Carrasco and Encina-Zelada (2008) determined that the TPC of 15 quinoa grain varieties from the Puno region (Peru) ranged from 35.29 to 139.94 mg EAG/100g. These variations in the TPC of quinoa may be due to factors like botanical variety, pretreatment of the grains (like polishing and saponin removal), and extraction method, among others. Compared with cereals, the TPC obtained for the 14 quinoa lines was in the range published for wheat (Saura-Calixto

and Bravo, 2001). At the same time, these resulted in higher values than those corresponding to amaranth (Klimczak *et al.*, 2002). It is worth mentioning that many interferences may influence the Folin-Ciocalteu results. Therefore, reducing compounds and non-phenolic antioxidants, such as ascorbic acid, glucose, fructose, sulfites, and others, may be coextracted with the phenolic compounds from the grains, altering the results. Furthermore, tyrosine and tryptophan amino acids from proteins produce a blue color with the reagent (Peterson, 1979).

Concerning the *in vitro* antioxidant activity, table 6 presents the results assessed by the DPPH method corresponding to the quinoa grains of the 14 lines. Maximum and minimum levels corresponded to RU5-1 and NL6-4 (436.58 and 657.11  $\mu\text{mol ET}/100\text{ g}$ , respectively). Other authors have found values for this parameter varying in a broad range. So, Vollmannova *et al.* (2013) studied five quinoa genotypes in which the antioxidant activity values ranged between 260 and 1240  $\mu\text{mol TE}/100\text{g}$ . Moreover, Mamani Coaquira (2015) analyzed two varieties of quinoa from Peru with values of 121.1 and 279.4  $\mu\text{mol ET}/100\text{g}$ . The reported differences in the values obtained by this method can be affected by factors concerning the nature of the sample itself, like quinoa variety, cultivar, climate, agricultural conditions of cultivation, harvest, processing, and storage, as well as analytical variables inherent to the DPPH method, such as pH, metal ion presence and freshness of the reactive (Dawidowicz *et al.*, 2012; Kedare and Singh, 2011; Musa *et al.*, 2013).

#### Determination of the main phenolic acids of quinoa by HPLC

The ethanolic extracts from the grains of the different quinoa lines were submitted to reverse phase HPLC analysis to determine the main phenolic acids previously selected based on the results of Ross *et al.* (2009). Gallic acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, and ferulic acid were satisfactorily resolved in about 26 min of the HPLC analysis. The results confirmed the presence of all these compounds in the 14 extracts with the exception of caffeic acid (table 7). At this point, it is worth mentioning that the maximum absorption wavelengths of the phenolic acid depend strongly on the solvent used for dissolution and pH. In particular, caffeic acid, despite being a hydroxycinnamic acid like *p*-coumaric and

Line	TPC (mg GAE*/100g, d. b.)	AA ( $\mu\text{mol TE}^{**}/100\text{g}$ )
RU5-1	227.99 $\pm$ 8.79 <sup>b</sup>	436.58 $\pm$ 5.05 <sup>a</sup>
RU5-2	261.71 $\pm$ 6.68 <sup>l</sup>	534.96 $\pm$ 0.57 <sup>k</sup>
RU5-4	250.17 $\pm$ 16.08 <sup>h</sup>	489.85 $\pm$ 2.06 <sup>f</sup>
RU5-9	194.15 $\pm$ 8.58 <sup>a</sup>	484.30 $\pm$ 2.22 <sup>e</sup>
RU5-11	256.93 $\pm$ 13.96 <sup>k</sup>	511.89 $\pm$ 5.36 <sup>h</sup>
RU5-14	256.13 $\pm$ 4.21 <sup>jk</sup>	533.73 $\pm$ 4.67 <sup>k</sup>
FARO-1	235.85 $\pm$ 0.25 <sup>d</sup>	518.79 $\pm$ 2.52 <sup>i</sup>
FARO-2	242.65 $\pm$ 9.19 <sup>f</sup>	522.71 $\pm$ 0.74 <sup>j</sup>
FARO-3	241.29 $\pm$ 5.34 <sup>e</sup>	484.69 $\pm$ 2.84 <sup>e</sup>
NL6-3	254.99 $\pm$ 10.31 <sup>j</sup>	463.61 $\pm$ 3.97 <sup>c</sup>
NL6-4	278.12 $\pm$ 8.72 <sup>m</sup>	657.11 $\pm$ 4.30 <sup>l</sup>
LV2-3	252.47 $\pm$ 6.89 <sup>h</sup>	446.97 $\pm$ 0.90 <sup>b</sup>
KVL32-4	229.70 $\pm$ 1.58 <sup>c</sup>	469.20 $\pm$ 1.71 <sup>d</sup>
REGALONA	248.35 $\pm$ 8.92 <sup>g</sup>	499.10 $\pm$ 1.70 <sup>g</sup>

Values expressed in mg/100g, db as means  $\pm$  SD (n = 3). Different letters in the same row indicate significantly different values (p < 0.05).

\* Gallic acid equivalents. \*\* Trolox equivalents.

**Table 6.** Total phenolic content (TPC) and *in vitro* antioxidant activity (AA) of the quinoa lines.

ferulic acids, which present a maximum absorption value at a wavelength of around 325 nm, can also absorb UV radiation of 270 nm. Indeed, this kind of molar absorptivity of the phenolic acid at this wavelength was sufficient to accurately detect significant amounts in the quinoa grain samples.

Repo-Carrasco *et al.* (2010), working with different Andean ecotypes and commercial varieties of quinoa grains, found the same phenolic acids as the present work, except for gallic and chlorogenic acids. Also, the authors determined that the maximum caffeic acid content among the quinoa grains samples represented 2.6% of the total acids. In agreement with the results of this study, we found that gallic acid was the most abundant compound in all the quinoa lines, with values ranging from 5.22 to 40.39 mg/100g, corresponding to RU5-9 and NL6-4, respectively. Moreover, Pasko *et al.* (2008) identified gallic acid as the predominant in quinoa grains, with a mean content of 32 mg/100g. The last column of table 7 shows the total content of the phenolic acids determined for each line. NL6-4 and RU5-9 presented the highest and lowest values of total phenolic acids, respectively, in correspondence with the results obtained for TPC by the Folin-Ciocalteu method. Tang *et al.* (2015) reported total phenolic acid contents of 46.7, 63.5, and 68.2 mg/ 100 g db, for red, white, and black quinoa grain types, respectively. Also, Pasko *et al.* (2008) informed a value of 49 mg/100 db for quinoa cultivated in Bolivia.

### Tocopherol determination

Considering that the tocopherol composition varies according to the genetic variability of plants (Repo-Carrasco-Valencia *et al.*, 2010), the concentration of main tocopherols in the lipidic

fraction obtained from grains of the selected quinoa lines was determined. Table 8 shows the contents of the different tocopherol isomers assessed by HPLC. The results revealed that the  $\alpha$  and  $\gamma$  forms were the most abundant in all quinoa lines. Besides, RU5-4 presented the greatest total content of tocopherol. In particular, regarding the contents of  $\alpha$ -tocopherol found by other authors in quinoa grains, they can differ widely depending on the sample evaluated. Thus, values from 1.6 mg/100 g db (Palombini *et al.*, 2013) and 2.6 mg/100 g db (Ruales and Nair, 1993), up to a maximum of 72.14 mg/100 g db (Repo-Carrasco *et al.*, 2003), have been reported. In addition, the levels of  $\alpha$ -tocopherol in quinoa resulted similar or higher in comparison with other no-cereal grains like buckwheat (*Fagopyrum esculentum*) (Ryan *et al.*, 2007), and amaranth (*Amaranthus* spp.) (Palombini *et al.*, 2013) and with the cereals barley (*Hordeum vulgare*) and maize (*Zea mays*) (Ryan *et al.*, 2007).

### Saponin analysis

The pericarp of the quinoa grain is rich in saponins, representing the principal antinutritional factor responsible for the characteristic bitterness and astringency that usually restrict its direct consumption. Moreover, sweet quinoa genotypes can be distinguished from bitter ones when their saponin content is less or more than 0.13% db (Vega-Gálvez *et al.*, 2010). Table 9 presents the saponin contents in the quinoa lines evaluated, with values ranging from 1.63 to 5.15 g/100 g db. Therefore, considering the previous classification criteria, all the quinoa accessions studied in the present work fall into the bitter genotype group. According to Tapia (1990), sensorial tests demonstrated that the maximum level of saponin in quinoa accept-

Line	Gallic acid	<i>p</i> -Hidroxi-benzoic acid	Vanillic acid	<i>p</i> -Coumaric acid	Ferulic acid	Total
RU5-1	18.38±0.02 <sup>b</sup>	0.36±0.01 <sup>c,d</sup>	0.73±0.04 <sup>a</sup>	1.02±0.02 <sup>b</sup>	4.55±0.00 <sup>e</sup>	25.05±0.49 <sup>c</sup>
RU5-2	34.75±0.11 <sup>d,e</sup>	0.29±0.12 <sup>b,c</sup>	1.15±0.09 <sup>a,b,c</sup>	1.55±0.13 <sup>f</sup>	5.88±0.02 <sup>h</sup>	43.61±0.21 <sup>i</sup>
RU5-4	25.95±0.01 <sup>b,c</sup>	0.53±0.02 <sup>e</sup>	1.58±0.00 <sup>b,c,d</sup>	0.90±0.02 <sup>a</sup>	1.96±0.03 <sup>a</sup>	30.92±0.57 <sup>e</sup>
RU5-9	5.22±0.14 <sup>a</sup>	0.13±0.18 <sup>a,b</sup>	1.15±0.02 <sup>a,b,c</sup>	1.16±0.16 <sup>c,d</sup>	5.38±0.00 <sup>g</sup>	13.03±0.35 <sup>a</sup>
RU5-11	38.44±0.02 <sup>e</sup>	0.36±0.01 <sup>c,d</sup>	2.00±0.00 <sup>d,e</sup>	1.25±0.13 <sup>d</sup>	4.86±0.15 <sup>f</sup>	46.91±0.42 <sup>j</sup>
RU5-14	26.36±0.00 <sup>c</sup>	0.75±0.01 <sup>f</sup>	2.33±0.04 <sup>e</sup>	2.02±0.00 <sup>i</sup>	6.70±0.12 <sup>i</sup>	38.16±0.49 <sup>h</sup>
FARO-1	27.19±0.2 <sup>c</sup>	0.54±0.11 <sup>e</sup>	1.70±0.17 <sup>c,d,e</sup>	1.57±0.01 <sup>f</sup>	7.39±0.02 <sup>k</sup>	38.39±0.14 <sup>h</sup>
FARO-2	27.23±0.14 <sup>c</sup>	0.31±0.13 <sup>c,d</sup>	1.44±0.06 <sup>a,b,c,d</sup>	1.46±0.43 <sup>e</sup>	3.41±0.00 <sup>b</sup>	33.84±0.35 <sup>f</sup>
FARO-3	9.72±0.02 <sup>a</sup>	0.79±0.05 <sup>f</sup>	1.69±0.01 <sup>b,c,d,e</sup>	2.22±0.07 <sup>j</sup>	4.99±0.03 <sup>f</sup>	19.42±0.35 <sup>b</sup>
NL6-3	26.41±0.00 <sup>c</sup>	0.52±0.06 <sup>e</sup>	1.59±0.00 <sup>b,c,d</sup>	1.74±0.91 <sup>g</sup>	5.31±0.16 <sup>g</sup>	35.57±0.21 <sup>g</sup>
NL6-4	40.39±0.02 <sup>e</sup>	0.09±0.00 <sup>a</sup>	4.02±0.03 <sup>f</sup>	4.18±0.00 <sup>k</sup>	9.52±0.02 <sup>i</sup>	58.21±0.35 <sup>k</sup>
LV2-3	27.72±0.23 <sup>c,d</sup>	0.86±0.17 <sup>f</sup>	1.44±0.33 <sup>a,b</sup>	1.42±0.12 <sup>e</sup>	4.12±0.13 <sup>c</sup>	35.57±0.14 <sup>g</sup>
KVL32-4	21.02±0.14 <sup>b,c</sup>	0.11±0.08 <sup>a</sup>	1.28±5.10 <sup>-3 a,b,c</sup>	1.11±0.46 <sup>b,c</sup>	4.25±0.03 <sup>d</sup>	27.77±0.21 <sup>d</sup>
REGALONA	35.11±0.002 <sup>a</sup>	0.42±0.01 <sup>d,e</sup>	1.67±0.22 <sup>b,c,d,e</sup>	1.89±0.64 <sup>h</sup>	7.24±1.10 <sup>d,j</sup>	46.34±0.42 <sup>j</sup>

Values expressed in mg /100g, db as mean ± SD (n = 3). Different letters in the same column indicate significantly different values (p < 0.05).

**Table 7.** Main phenolic acid contents in grains from the selected quinoa lines.



Line	Tocopherol isomer (mg/100g, db)			
	[α]	[β]	[γ]	[δ]
RU5-1	1.2 ± 4.10 <sup>-3</sup> b	0.01 ± 2.10 <sup>-3</sup> ab	0.85 ± 0.02 <sup>b</sup>	0.02 ± 4.10 <sup>-4</sup> ab
RU5-2	1.84 ± 0.02 <sup>d</sup>	0.02 ± 1.10 <sup>-5</sup> bc	1.51 ± 9.10 <sup>-3</sup> de	0.04 ± 2.10 <sup>-3</sup> ab
RU5-4	4.21 ± 0.10 <sup>h</sup>	0.04 ± 1.10 <sup>-4</sup> d	2.97 ± 1.16 <sup>g</sup>	0.1 ± 0.05 <sup>c</sup>
RU5-9	1.61 ± 0.03 <sup>c</sup>	0.02 ± 4.10 <sup>-3</sup> bc	1.06 ± 0.04 <sup>bc</sup>	0.02 ± 1.10 <sup>-3</sup> ab
RU5-11	2.5 ± 0.04 <sup>f</sup>	0.02 ± 6.10 <sup>-3</sup> bc	1.66 ± 0.03 <sup>e</sup>	0.04 ± 2.10 <sup>-5</sup> ab
RU5-14	3.17 ± 0.03 <sup>g</sup>	0.03 ± 7.10 <sup>-3</sup> cd	2.36 ± 0.04 <sup>f</sup>	0.07 ± 1.10 <sup>-3</sup> bc
FARO-1	2.17 ± 2.10 <sup>-3</sup> e	0.02 ± 4.10 <sup>-3</sup> bc	1.16 ± 0.04 <sup>c</sup>	0.02 ± 3.10 <sup>-3</sup> ab
FARO-2	1.89 ± 0.03 <sup>a</sup>	0.01 ± 0.05 <sup>a</sup>	1.12 ± 0.12 <sup>a</sup>	0.02 ± 0.02 <sup>a</sup>
FARO-3	3.22 ± 0.02 <sup>a</sup>	0.02 ± 0.03 <sup>a</sup>	1.68 ± 0.02 <sup>a</sup>	0.06 ± 0.01 <sup>a</sup>
NL6-3	0.95 ± 0.01 <sup>a</sup>	0.01 ± 0.04 <sup>a</sup>	0.86 ± 0.11 <sup>a</sup>	0.01 ± 1.10 <sup>-3</sup> a
NL6-4	2.03 ± 0.05 <sup>e</sup>	0.02 ± 6.10 <sup>-3</sup> ab	1.7 ± 0.05 <sup>e</sup>	0.03 ± 1.10 <sup>-3</sup> ab
LV2-3	2.1 ± 0.02 <sup>e</sup>	0.02 ± 8.10 <sup>-3</sup> bc	1.68 ± 0.03 <sup>e</sup>	0.04 ± 4.10 <sup>-3</sup> ab
KVL32-4	1.86 ± 0.02 <sup>d</sup>	0.03 ± 1.10 <sup>-3</sup> bc,d	1.29 ± 0.02 <sup>cd</sup>	0.03 ± 1.10 <sup>-3</sup> ab
REGALONA	2.15 ± 0.03 <sup>e</sup>	0.02 ± 1.10 <sup>-3</sup> bc	1.65 ± 1.10 <sup>-3</sup> e	0.03 ± 1.10 <sup>-3</sup> ab

Values expressed as mean ± SD (n = 3). Different letters in the same column indicate significantly different values (p < 0.05).

**Table 8.** Main tocopherol contents in the lipidic fraction of grains from the selected quinoa lines.

Line	Saponin (% db)
RU5-1	4.38 ± 0.19 <sup>d,e,f</sup>
RU5-2	3.44 ± 0.21 <sup>c,d</sup>
RU5-4	2.61 ± 0.26 <sup>a,b,c</sup>
RU5-9	2.11 ± 0.24 <sup>a,b</sup>
RU5-11	3.66 ± 0.07 <sup>c,d</sup>
RU5-14	1.63 ± 0.17 <sup>a</sup>
FARO-1	2.94 ± 0.50 <sup>b,c</sup>
FARO-2	3.02 ± 0.14 <sup>b,c</sup>
FARO-3	4.78 ± 0.28 <sup>e,f</sup>
NL6-3	5.15 ± 0.65 <sup>f</sup>
NL6-4	4.26 ± 0.14 <sup>d,e,f</sup>
LV2-3	2.72 ± 0.20 <sup>a,b,c</sup>
KVL32-4	2.11 ± 0.19 <sup>a,b</sup>
REGALONA	3.71 ± 0.05 <sup>c,d,e</sup>

Values expressed as mean ± SD (n = 3). Different letters in the same column indicate significantly different values (p < 0.05).

**Table 9.** Saponin contents of the grains from the selected quinoa lines.

able for human consumption was between 0.06% and 0.12%. According to these results, it is necessary to previously remove the saponins from the grains to make these quinoa lines edible.

The saponin contents of the quinoa lines studied agreed with other values reported in the bibliography. Stuardo and San

Martín (2008) determined saponin concentrations in the 0.1 to 5% range in quinoa grain samples from Bolivia. In another study, the total level of saponins in Chilean *Criolla* variety grains of quinoa was between 1% and 4%, with maximum values corresponding to samples from the central region of the country (Miranda *et al.*, 2012). Moreover, Troisi *et al.* (2013) evaluated eight commercial varieties of quinoa from different regions of South America, demonstrating that the Pink variety from Jujuy province (Argentina) turned out to be the richest variety in saponins with a value of 4.99% while the Real variety from Bolivia presented a minimum level of 0.1%.

### Statistical multivariate analysis

All the parameters determined for the grains of the 14 quinoa lines studied in this work were submitted to a multivariate analysis to select those with the best potential to be cultivated in the region. Therefore, a principal components analysis (PCA) was applied to the data set concerning the nutritional profile and the bioactive and antinutritional contents of the quinoa grains. In this way, a smaller number of artificial variables that will account for most of the variance of the observed variables (called principal components) is developed. Figure 1 shows a biplot graphic with the first two components that resulted from this analysis, which explained 53% of the total variance. The considered parameters are represented as vectors from the origin. The angles of 90° between the two variables indicate that these are not correlated, while the smaller or higher values imply a correlation (positive or negative, respectively). An angle near 0° implies that the variables are strongly positively correlated, while a straight angle indicates a strong negative correlation between them.

Figure 1 shows that the phenolic compound content is positively correlated with the *in vitro* antioxidant activity (assessed by the DPPH method). In addition, the level of the total tocoph-

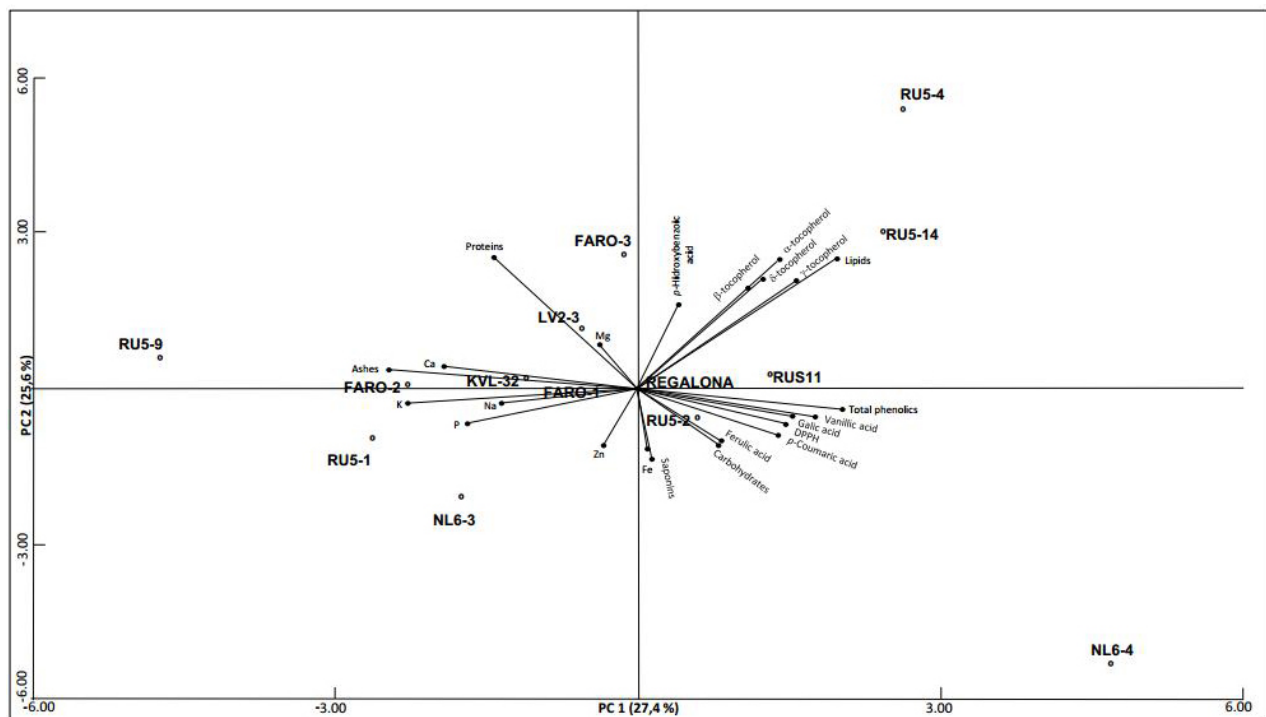


Figure 1. Biplot graphic resulting from the PCA of the parameters values for the grains from the selected quinoa lines.

Line	Proteins	Lipids	Carbohydrates	Mg	Ca	P	Fe	Zn	Na	K
RU5-1	++	+	+++	+	+	+++	-	++	++	++
RU5-2	++	+	+++	+	++	+++	+++	++	+	+
RU5-4	+++	+++	+	+	+	++	+	+	+	+
RU5-9	+++	+	+	+	+++	+++	++	+++	++	+++
RU5-11	++	+	++	+	++	++	+	+	++	+
RU5-14	++	++	+	++	++	+++	-	+	++	+
FARO-1	++	+	++	++	++	+++	+	++	+	++
FARO-2	+++	+	+	++	+++	+++	+	+	+++	++
FARO-3	++	+	+	+++	++	++	+	+	++	++
NL6-3	++	+	+++	+	+	+++	+	+	++	+++
NL6-4	+	++	+++	+	+	+++	+++	+++	++	++
LV2-3	++	++	+	++	++	+++	+	++	++	+++
KVL32-4	++	+	++	++	+++	+++	+++	+++	++	+
REGALONA	++	++	+	++	++	+++	-	++	++	+

+++ High, ++ Medium, + Low, - Scarc or not detected.

Each level corresponds to a value that belongs to a previously defined numerical range for each nutrient (not shown).

Table 10. Nutrient levels qualitative comparison of the grains from the selected quinoa lines.

erol isomers was logically correlated with the total lipid content. Similarly, the overall content of the mineral elements analyzed correlated positively with the ash values.

Regarding the distribution of the selected lines in the biplot graph, the first component (PC 1) allowed us to differentiate those with the highest ash, mineral, and protein contents (RU5-

Line	Saponins	TPC	<i>in vitro</i> antioxidant activity (DPPH)	Total tocopherol
RU5-1	+++	+	+	+
RU5-2	++	+++	++	+
RU5-4	+	+++	+	+++
RU5-9	+	+	+	+
RU5-11	++	+++	++	++
RU5-14	-	+++	++	++
FARO-1	+	+	++	+
FARO-2	++	++	++	+
FARO-3	+++	++	+	++
NLG-3	+++	+++	+	-
NLG-4	+++	+++	+++	+
LV2-3	+	+++	+	+
KVL32-4	+	+	+	+
REGALONA	++	++	+	+

+++ High, ++ Medium, + Low, - Scarce or not detected.

Each level corresponds to a value that belongs to a previously defined numerical range for each parameter (not shown).

**Table 11.** Saponin, total phenolic compounds, *in vitro* antioxidant activity, and total tocopherol levels qualitative comparison of grains from the selected quinoa lines.

9, RU5-1, FARO-2, and NL6-3) from those that presented higher content of lipids and phenolic compounds with *in vitro* antioxidant activity. The second component (PC 2) clearly showed a distinction between those lines rich in lipids and tocopherols (RU5-14 and RU5-4) from those with a higher content of phenolic compounds and higher *in vitro* antioxidant activity (NL6-4 and RU5-2).

Finally, the data shown in tables 10 and 11 facilitate selecting those quinoa lines with the higher potential of improvement in terms of specific compositional characteristics, either by classical breeding or molecular biology techniques.

## CONCLUSIONS

This study determined the chemical parameters related to the macro and micronutrient composition, *in vitro* antioxidant estimation, and phenolic acids and saponin contents of the grain of 14 selected quinoa lines adapted to the central region of Buenos Aires province. The statistical analysis of the data set showed that it is possible to differentiate and choose quinoa grain samples with specific compositional features. In particular, RU5-4 stood out for its high content of proteins, lipids, and tocopherols, while RU5-9 showed a high total mineral content. Considering particular mineral compositions, FARO-3 presented the highest magnesium level, and KVL32-4 exhibited maximum values of calcium and iron among all the quinoa grain samples studied. Regarding phosphorus and zinc contents, LV2-3 and NL6-4 were the richest in these elements, respectively. On the other hand, maximum sodium and potassium levels corresponded to FARO-2 and RU5-9, respectively. All the quinoa lines presented grains with high levels of saponin-type compounds, with values above the upper limit permissible for human consumption. This result indicates that any of the quinoa

grains studied should be submitted to a saponin elimination process before its commercialization or consumption.

On the other hand, the total phenolic compound contents of the quinoa grains resulted in higher values than those mentioned in the reviewed bibliography for this species in all the lines analyzed. Notably, NL6-4 resulted in the richest of these compounds, with high levels of gallic and ferulic acids, which could be correlated with the greater *in vitro* antioxidant capacity evaluated by the DPPH method.

The multivariate statistical analysis did not allow identifying a quinoa line among the 14 selected that can be considered ideal, considering the analyzed chemical parameters of the grains. However, it led to the identification of those with specific characteristics to be cultivated in the region. In this way, considering the composition of macronutrients, there is no preferred line. However, taking into account the lower content of saponins, the higher contents of lipids (including tocopherols), RU5-14 and, especially RU5-4, could be considered the most promising for cultivation in the region. Otherwise, if the objective is to select quinoa lines with high levels of phenolic compounds in their grains, RU5-2 and NL6-4 should be selected for harvesting.

In conclusion, the results evidenced differences between the grains from the 14 evaluated quinoa lines concerning the composition of nutrients, bioactive compounds, and antinutrient substances. These results could be considered for a quinoa improvement program to develop varieties with good agronomic performance and specific nutritional and functional characteristics.

Finally, it would be interesting to determine in prospective studies the content of vitamins (mainly folic acid, which is scarce in cereals) to select quinoa accessions with a better nutritional profile. Furthermore, determining the dietary fiber

content in quinoa lines could help to select those with a higher potential to confer beneficial physiological effects.

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