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Evaluation and Comparative Study of the Nutritional Profile and Antioxidant Potential of New Quinoa Varieties

 \mathbf{G} abriela Silvina Razzeto 1 , María Angelina Uñates 1 , José Elías Rojas Moreno 1 , **Romina Viviana Lucero López² , Elba Graciela Aguilar1 , Héctor Sturniolo1 and Nora Lilian Escudero1***

1 Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, 5700, San Luis, Argentina. ² School of Health, National University of Villa Mercedes, Villa Mercedes, 5730, San Luis, Argentina.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Quinoa (*Chenopodium quinoa*) is an ancient crop known for its high nutritive potential. The goal of the present work is to study the nutritional composition, identify some antinutritional factors and antioxidant compounds, and evaluate their antioxidant activity in four advanced lines of quinoa seeds obtained in experimental plots.

Methodology: For this purpose, proteins, total lipids, fiber, moisture, ash and carbohydrates, as well as fatty acid composition and mineral content, were determined in whole meal flours of these advanced lines. The presence of trypsin inhibitors, saponins, nitrates, oxalates and phytate was also evaluated, as well as total phenols and antioxidant activity.

Results: These new quinoa varieties have good nutritional properties, with high protein content in comparison to cereals. In this work, the analysis of proximate and mineral profile of quinoa showed that this pseudocereal has a similar profile but significantly higher than rice, a traditional cereal. Quinoa is a rich source of magnesium, iron, manganese, copper and molybdenum, which are

elements that are deficient in almost all gluten-free cereals. The tests performed on the evaluated antinutrient compounds resulted within the acceptable values for human consumption. The seed extract showed a total phenol content between 43.42 ± 1.35 and 25.82 ± 1.47 mg of gallic acid equivalent/100 g dry weight $(P= .05)$. The antioxidant activities were estimated by DPPH, β carotene and nitric oxide scavenging activity. The results of the methanolic extract were, in average, 88.95 for %DPPH, 26.56 for %β carotene, and varied between 85.82 ± 8.32 to 22.20 ± 1.80 for %NO (*P*= .05).

Conclusion: Therefore, it can be concluded that the new quinoa lines obtained in the central-west region of Argentina, which present agronomic advantages, are safe for human consumption and beneficial due to the content of nutrients and bioactive compounds that exert protection against many diseases.

Keywords: Quinoa; nutrient; antinutrient; phenols; antioxidant activity.

1. INTRODUCTION

Recent studies have highlighted the need of improving the nutritional quality of cereal-based gluten-free products. There are many gluten-free grains, such as amaranth, quinoa and buckwheat pseudocereals, which are characterized by an excellent nutritional profile [1].

Quinoa (*Chenopodium quinoa*) is a native pseudocereal in the Andean region. The cultivation of the plant goes back to at least 5000 years, being the main food of the entire Inca Empire. Currently, quinoa is under an expansion process, due to that it represents a great potential for global food security. Quinoa belongs to the genus *Chenopodium* of the *Chenopodiaceae* family, and is widely distributed worldwide, with around 250 species. Furthermore, it has demonstrated to be a strategic crop due its wide genetic diversity, which allows it to adapt to diverse agro-climatic and soil conditions [2].

The grain is the most consumed part, and represents an excellent resource of macronutrients, in particular proteins with high content of essential amino acids, thus, differentiating themselves from traditional cereals. In addition, it represents a good source of micronutrients, such as vitamins and minerals [3].

Many studies have demonstrated that quinoa seeds present a significant polyphenols content (such as flavonoids and phenolic acids) with antioxidant capacity [4,5], and other bioactive compounds with beneficial health effects.

Another characteristic of this grain is the presence of antinutritional factors such as saponins, proteases inhibitors and lectins [6]. These compounds can be responsible of affecting proteins digestibility and nutrients availability; nevertheless, they are attributed with bioactive properties.

Quinoa, along with amaranth and buckwheat, is recommended by the World Gastroenterology Organization in celiac patient's diets, given that they are gluten-free cereals [7]. Alvarez-Jubete et al. [1] also recommend the use of quinoa and amaranths as possible healthy ingredients to enrich the nutritional value of gluten-free baked goods.

The United Nations General Assembly declared the year 2013 as the "International Year of Quinoa", in recognition to its elevated nutritional quality, genetic diversity and potential role in poverty eradication [8]. In addition, in 2011, the FAO classified quinoa as one of the promising crops that can contribute to food security in the $21st$ century [9].

The goal of the present work is to characterize the nutritional composition, identify some antinutritional factors and antioxidant compounds, and evaluate their antioxidant activity in four advanced lines of quinoa seeds obtained in experimental plots, which is important considering that the new varieties studied in this work have agronomic advantages that improve the crop and the resistance towards some pathogens.

2. MATERIALS AND METHODS

2.1 Sample and Reagents

Work was performed on seeds of advanced lines (LAQ) of *Chenopodium quinoa* (LAQc/31, LAQb/41, LAQf/104 and LAQp/16), from experimental crops of the Faculty of Agronomy and Veterinary of the National University of Río Cuarto, Cordoba, Argentina (2016 vintage). The

dry seeds were ground and sieved, obtaining a beige-color whole flour, which was conserved in a hermetically sealed container, protected from light, and stored at 4ºC. All reagents were of analytical grade. The analyses were performed in triplicate, and the mean value of dry matter was obtained.

2.2 Proximate Chemical Composition

The determination of proteins, lipids, dietary fiber, moisture and ashes, was performed according to the methodology proposed by the AOAC [10]. The carbohydrates content was calculated by difference.

2.3 Minerals

Mineral elements quantification was performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The standards and reagents used were of spectroscopic grade. The procedure was carried out following the methodology used by Aguilar et al. [11].

2.4 Fatty Acids

Fatty acids were determined as methyl esters by gas chromatography [12,13]. For their analysis, the chromatographic method was applied in a Varian chromatograph *(Berkeley, NC)* with a 10% SP-2330 packed column and flame ionization detector. Standard solutions of fatty acids were acquired from Sigma (St. Louis, MO).

2.5 Antinutrients

The determined antinutrients were: antitryptic factors [14], saponins [15], nitrates [16], oxalates [17] and phytates [18].

2.6 Total Phenol

The extraction of total phenols was performed from flour using a 1.2 mol/L HCl, 50% methanol:water solution. The sample was heated at 90°C for 3 h, and then cooled and diluted with methanol [19]. The supernatant was used for the determination of phenols and antioxidant activity. The determination of total phenols was performed using Folin Ciocalteu reagent with gallic acid as standard. The absorbance was measured at 750 nm (UV–vis Beckman DK-2ª). The results were expressed as mg/100 g of dry weight of gallic acid equivalent [20].

2.7 Antioxidant Activity

The DPPH scavenging assay is related to the sample capacity to inhibit the action of free radicals generated by a 0.004% methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [21]. In this case, the absorbance was measured at 517 nm.

The β-carotene scavenging assay involves measuring β-carotene bleaching at 470 nm, resulting from the β-carotene oxidation by linoleic acid degradation products at 50°C [22]. The absorbance at 470 nm was taken at time zero (t = 0), and then measured at 15 min intervals until the color of β-carotene disappeared in the control tube (t = 60 min). A mixture prepared without $β$ carotene served as blank.

Nitric oxide scavenging activity uses sodium nitroprusside in 0.02 mol/L phosphate buffer (pH 7.4) to generate nitric oxide (NO), which interacts with oxygen to produce stable nitrite ions. These ions can be estimated by using Griess reagent at 542 nm [23].

In all cases, buthylated hydroxytoluene (BHT) was used as positive control, and the results were expressed as percentage (%) of radical scavenging activity (RSA).

2.8 Statistical Analysis

Results were expressed as mean ± standard deviation. Statistical differences were tested by variance analysis ANOVA, and the means were compared using the Tukey test. Probabilities of 0.05 or less indicate significant difference [24].

3. RESULTS AND DISCUSSION

The presence of macronutrients, minerals, antinutrients and bioactive compounds such as polyphenols, was studied in seeds of advanced lines of *Chenopodium quinoa*: LAQc/31, LAQb/41, LAQf/104 and LAQp/16.

Several studies have confirmed that quinoa has high-quality protein in terms of digestibility and nutritional balance, presenting a high biological value due to the balanced composition of essential amino acids similar to casein, the milk protein [2]. Specifically, quinoa proteins are rich in lysine (6.2 g / 100 g of protein) and threonine (4.8 g / 100 g of protein), which are usually the limiting amino acids in conventional cereals [25].

** Calculated as: 100 - (moisture + protein + fat + ash); LAQc/31, LAQb/41, LAQf/104, LAQp/16: advanced lines of Chenopodium quinoa; Results are expressed as mean ± standard deviation from three replicates; Values that do not share letters in common are significantly different by the Tukey's test (P= .05)*

Table 2. Proximate chemical composition (g/100 g) of quinoa grains, compared to uncooked quinoa, uncooked amaranth grain, and unenriched and uncooked rice white, in the USDA nutrient database

USDA nutrient database (U.S. Department of Agriculture, Agricultural Research Service, 2013)

Table 3. Fatty acids composition (mg/g total lipid) of quinoa grains

LAQc/31, LAQb/41, LAQf/104, LAQp/16: advanced lines of Chenopodium quinoa

Results are expressed as mean ± standard deviation from three replicates.

Values that do not share letters in common are significantly different by the Tukey's test (P= .05)

Table 4. Fatty acid composition (mg/g total lipid) of quinoa grains, compared to uncooked quinoa, uncooked amaranth grain, and uncooked and unenriched white rice, in the USDA nutrient database

USDA nutrient database (U.S. Department of Agriculture, Agricultural Research Service, 2013)

Table 5. Minerals (mg/100 g) of quinoa grains

LAQc/31, LAQb/41, LAQf/104, LAQp/16: advanced lines of Chenopodium quinoa; Results are expressed as mean ± standard deviation for analysis in three replicates; Values that do not share letters in common are significantly different by Tukey's test (P= .05); ULOQ: upper limit of quantification; LOD: limit of detection

Table 6. Minerals (mg/100 g) of quinoa grains compared to uncooked quinoa, uncooked amaranth grain, and uncooked and unenriched white rice, in the USDA nutrient database

USDA nutrient database (U.S. Department of Agriculture, Agricultural Research Service, 2013); ULOQ: upper limit of quantification; LOD: limit of detection

Table 7. Macronutrients and elements contribution of quinoa according to the Dietary Reference Intakes (DRIs)

DRI adapted from the Food and Nutrition Board, Institute of Medicine, National Academies suggested indispensable nutrients and elements requirements for adults (Life Stage Group: males, 35-50 years) https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx

Table 8. Antinutrient contents of quinoa grains

LAQc/31, LAQb/41, LAQf/104, LAQp/16: advanced lines of Chenopodium quinoa; Results are expressed as mean±standard deviation for analysis in five replicates; Values that do not share letters in common are significantly different by the Tukey's test (P= 0.05)

LAQc/31, LAQb/41, LAQf/104, LAQp/16: advanced lines of Chenopodium quinoa; Results expressed as mean ± standard deviation for analysis in three replicates; Values that do not share letters in common are significantly different by the Tukey's test (P= .05)

In addition, these pseudocereals are characterized by having a high lipid content with respect to common cereals, with a high proportion of unsaturated fatty acids. Linoleic acid is the most abundant fatty acid in quinoa (50%), followed by oleic acid (25%) [1].

It is known that the consumption of natural foods rich in dietary fiber is beneficial for maintaining a good health [26]. Studies have demonstrated that pseudocereals like quinoa present fiber levels comparable to the ones found in common cereals [1].

Table 1 shows the proximate chemical composition of the quinoa lines under study. The highest protein content found was of 17.29 \pm 0.37 g/100 g (LAQb/41), 30% higher than the line

that presented the lowest value (LAQc/31). The highest fat content was of 9.48 ± 0.75 g/100 g (LAQb/41), being 24% higher than for LAQp/16. The highest carbohydrate content was of 68.77 \pm 0.31 g/100 g (LAQf/104), being 11% higher than for LAQb/41. The highest crude fiber content was of 3.17 ± 0.03 g/100 g (LAQf/104), being 26% higher than for LAQp/16. The values and variations found between lines are also in agreement with the ones published by other authors [1,27,28]. It is noteworthy that the content of quinoa nutrients varies significantly between lines. In the available literature, there is no clear foundation for such differences. Some possible explanations are attributed to the interaction of several factors, such as the crop genetics, the analytical methods used for the determination, and the environmental conditions [26].

Compared in Table 2 are the average values of the proximate chemical composition of quinoa, amaranth and rice, provided by the composition tables of the United States Department of Agriculture database, 2013 [29].

Water content was lower in LAQ with respect to the compared grains. In LAQ, the total protein values are within the range informed for pseudocereals (quinoa and amaranth), and two times higher than traditional cereals such as rice. The total lipids results informed for LAQ were slightly higher with respect to pseudocereals, and 14 times higher with respect to rice. Carbohydrate data are within the range informed for pseudocereals and slightly lower than rice. The USDA nutrient database did not informed ashes nor crude fiber.

Table 3 shows the LAQs fatty acid composition. These grains have a relatively high amount of unsaturated fatty acids. They present a high content of omega 6 linoleic acid, 54.30 mg/g in average, followed by omega 3 linolenic acid, 5.55 mg/g in average.

In Table 4, it can be observed that the linoleic acid composition of LAQ is almost double the informed by the USDA for quinoa and amaranth pseudocereal, and 28 times higher than in rice.

The linolenic acid composition of LAQ is double the informed for quinoa, 13 times higher than in amaranth and much higher than in rice. These results follow the tendency reported in the literature [2,30,1].

Presented in Table 5 are the results of the main and trace essential minerals of nutritional interest, and of chemical elements, such as zinc and copper, with antioxidant activity. It is noteworthy the levels $(mg/100 g)$ of magnesium (204.36) , calcium (54.76) , iron (4.51) , (204.36), calcium (54.76), iron manganese (2.66), copper (0.56), molybdenum (0.03) and zinc (2.24). Potassium and phosphorus contents were above the upper limit of quantification. The data obtained for the minerals of LAQ are adequate for grains, being in the order of the reported in the literature for quinoa [31,28]. Calcium, magnesium and iron are minerals that are deficient in gluten-free products; pseudocereals such as quinoa are usually a good source of these elements and other important minerals [1]. However, it can be observed that the mineral values vary significantly, and this can be explained by the same factors that affect the nutritional composition of vegetable foods such as cultivation and climatic conditions, as well as the analytical determination methods.

In Table 6, it can be observed that the pseudocereals studied by the USDA, have 7 times more potassium and phosphorus than rice. According to this database, magnesium in the LAQs was in the order reported for quinoa and amaranth, being 9 times higher than rice. Calcium was in the order of quinoa, 3 times lower than amaranth and 5 times higher than rice. Iron presented a tendency similar to calcium. Manganese, copper and zinc were similar in the three pseudocereals, presenting an amount of almost 3 times higher than rice. All analyzed samples are considered as of low-sodium content according to the publications of the WHO [32].

In general, it is known that pseudocereals cover higher nutritional demands than traditional cereals. In Table 7, it can be observed the contribution of quinoa to the intake of
macronutrients and chemical elements. macronutrients and chemical elements. According to the international nutritional requirements, the LAQs provide, for every 100 g of intake, 26% of the required daily proteins for adults, which is a high content compared to cereals, and 50% of carbohydrates.

The mineral results show that quinoa presents a significant content of elements that are considered essential for human nutrition, providing 50% or more of the daily required magnesium, iron, copper and molybdenum, and more than 100% of manganese. It is noteworthy the contribution of more than 30% of linoleic and linolenic acids.

These results confirm that a diet balanced in proteins, fats and minerals, could be obtained from quinoa and other Andean cereals such as amaranth, as a significant part in gluten-free diets.

In order to optimize the information on the nutritional potential of these seeds, it is important to identify the antinutritional factors that interfere in the metabolic processes or nutrient bioavailability, and thus affect the consumer's health.

The antinutrients quantified in this study are presented in Table 8. Proteases inhibiting factors inhibit the action of digestive enzymes, in particular trypsin and chymotrypsin, causing a digestibility decrease in the protein; however, many studies suggest that these compounds can have a beneficial effect through their anticarcinogenic action [33]. The results of the antitryptic factors determination are, in average, around 0.72 UTI/mg for the lines studied, being below the maximum acceptable value for foods, which according to the FAQ is of 5 UTI/mg. Furthermore, this thermolabile component can be reduced through different food preparation processes involving a thermal treatment.

Even though saponins can affect zinc and iron absorption, many studies indicate that they have a wide range of biological activities and beneficial effects, such as their hypocholesteremic and hypoglycemic actions, among others [34]. According to the FAO, the maximum allowed saponin content is of 0.11% [9]. The saponin content reported for quinoa varies between 0.1 and 5% [30]. The culture lines under study presented an average saponin content of 0.43 g/100 g or 0.43%. The quinoa grain pericarp is the one containing the saponins, giving it a bitter taste, and it has to be removed for the grain to be consumed. A dry-heat toasting process is used by some companies to remove the shell. It has to be considered that the quinoa grain is preferentially consumed cooked.

Nitrates can be reduced to nitrites by the intestinal flora, and cause the transportation of hemoglobin into methemoglobin. In addition, nitrites can react with amines, forming carcinogenic nitrosamines [35]. The FAO/OMS have determined, as nitrates acceptable daily intake (ADI), a value of $0 - 3.7$ mg/kg of body weight, equivalent to 222 mg of nitrate for a 60 kg adult [3]. Thus, the nitrate contents in the studied quinoa seeds can be considered as nontoxic (0.45 mg/100 g). On the other hand, it is known that cooking foods in boiling water can be an efficient way of reducing the seeds antinutritional effects, mainly from nitrates and oxalates.

Oxalates are present in plants as sodium oxalate, and are mainly accumulated in the leaves. This compound is soluble and combines with calcium and magnesium in the bloodstream, resulting in insoluble salts. These salts, when present in large amounts in human tissues, can provoke damages by oxidation and glutathione depletion, and can also generate cascade inflammation by an immunological effect, and the formation of kidney stones [36]. According to some authors, the oxalate in the whole grains is higher in comparison to refined products, which suggests that oxalic acid is also in the outer layers of cereals. The maximum allowed content of oxalic acid, according to the FAO, is of 0.10% [9]. The oxalate values reported in this study (1247.27 mg/100 g, equivalent to 1.25%), could be reduced by cooking methods to which quinoa can be subjected.

Phytic acid is present in most cereals and legumes, and in some fruits and vegetables. The phytic acid antinutritional action is mainly due to its capacity to form complexes with essential minerals, which decreases their absorption and bioavailability; it can also interact with basic residues of proteins forming complexes, interfering with enzymatic reactions at the digestive level [37]. In addition, phytic acid beneficial health effects have been informed, such as lipid-lowering and antioxidant actions [38]. In quinoa seeds, a phytic acid concentration of 1180 mg/100 g has been informed in five quinoa varieties [30]. In the studied samples, the phytic acid values are much lower than the reported in the literature, 0.267 mg/100 g, being a food with a very low content of this antinutrient.

The antinutrient values informed for LAQ are consistent with diverse author [39], who concluded that the antinutrients present in quinoa, in particular saponins, do not pose a significant risk for health. It is noteworthy that differences can be observed between species in the values informed, which can be attributed to genetic differences of the plant, cultivation area, seeding year, among others.

Quinoa also contains bioactive compounds that influence on the cell activity and physiological mechanisms, with beneficial health effects, such as phenols. Polyphenols are secondary metabolites of plants; they include several antioxidant compounds and are usually considered involved in the defense against chronic human diseases, including cancer and cardiovascular diseases [40]. The total phenols content and the antioxidant activity evaluated by the DPPH, β-carotene and Nitric Oxide methods, are shown in Table 9.

The highest phenolic content (mg gallic acid/100 g) was observed in LAQp/16 (43.42 ± 1.35) and LAQb/41 (43.01 \pm 1.50), followed by LAQc /31 (38.45 ± 0.55) and LAQf/104 (25.82 ± 1.47). Based on these results and the consulted literature [41], it is deduced that the studied seeds have an adequate level of phenolic compounds, and that the variations between lines could be attributed to genetic factors and other agrotechnical aspects.

The free-radical scavenging capacity analyzed by DPPH was in the order of 88.95%, and the inhibition of fatty acids oxidation evaluated by βcarotene bleaching was of 26.56%, without showing significant differences. The NO inhibition presented the highest value for LAQc /31 (85.82 \pm 8.32%), followed by LAQb/41 (41.60 \pm 2.01%), LAQp/16 (28.82 ± 1.93) and LAQf/104 (22.20±1.80). These results agree with the informed by other authors, and are similar to other vegetable species considered as with significant antioxidant activity [5].

4. CONCLUSION

Quinoa is currently recognized by its high nutritional value compared to traditional cereals, and for being a gluten-free food similar to amaranths.

The new quinoa lines evaluated in this work are rich in nutrients, such as proteins, essential fatty acids.

They are a significant source of calcium, magnesium and iron, which are minerals deficient in gluten-free food products.

The evaluated antinutrients do not pose a risk for human health.

It also presents natural bioactive compounds with significant antioxidant activity such as phenols.

Based on the results presented in this work, these quinoa lines are recommended as an important functional food in the diet of celiac patients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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