RESEARCH PAPER

Quinoa leaf as a nutritional alternative

Alma Vazquez-Luna[1], Vanessa Pimentel Cortés[1], Francisco Fuentes Carmona[2], Rafael Díaz-Sobac[1]

[1]Universidad Veracruzana, Instituto de Ciencias Básicas. Av. Dr. Luis Castelazo s/n, Col. Industrial Animas, Xalapa, C.P. 91192, Veracruz, México.
[2]Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal. Avenida Libertador General Bernardo. O´Higgins 340, Santiago, Chile.

Abstract

A. Vazquez-Luna, V. Pimentel Cortés, F. Fuentes Carmona, R. Díaz-Sobac. 2019. Quinoa leaf as a nutritional alternative. Cien. Inv. Agr. 46(2): 137-143. *Chenopodium quinoa* is an herbaceous plant that possesses green polymorphic leaves. They are traditionally consumed in America and are considered nutritive vegetables. Most vegetables are considered valuable sources of micronutrients, such as mineral, vitamins, carbohydrates and dietetic fiber; however, because they are poor in proteins, they are considered to have no energetic value. The consumption of vegetables generates a satiety sensation and favors the reduction of total calories consumed. Quinoa leaves can be consumed raw when they are ripe or steam cooked; they retain most of their vitamins and minerals. The FAO considers quinoa to be the "perfect food", and it is not only used in common diets, but it is also suitable for the unique diets of those that are vegetarian or high-performance athletes as well as those with celiac disease and diabetes. The objective of this work was to determine the nutritional value of quinoa leaves. For every test, dried and powdered quinoa leaves were used, and the following parameters were determined: total polyphenols, total flavonoids, proteins, carbohydrates, reducing sugars, water content, ash content, and raw fiber, and the flavonoids were determined by HPLC. The results obtained for the polyphenols were 131.8 ± 10.3 mg 100 g^{-1} and 62.07 ± 5.1 mg 100 g^{-1} for flavonoids, and the main compounds were gallic acid, kaempferol and catechin. The content of proteins was $11.8 \pm 0.6\%$, the carbohydrates was 18.3 ± 0.9, the reducing sugars were $3.2 \pm 0.27\%$, the water content was $2.8 \pm 0.9\%$, the ash content was $1.4 \pm 0.14\%$, and the raw fiber content was $43.7 \pm 3.9\%$. Based on the nutritional profile and the content of polyphenols and total flavonoids, quinoa leaves can be considered an alternative for human consumption because they offer interesting potential in nutrients and antioxidant capacity, which is a dietary requirement.

Keywords: Nutrient profile, phytochemicals, quinoa leaf.

Introduction

Quinoa is a dicotyledonous annual plant that is usually herbaceous and reaches a height of 0.2 to 2.5 meters. The plants can present different colors from green to purple to red and intermediate colors between purple and red (Gandarillas, 1968). The principal stem can be dendritic depending on the ecotype, crop, seeding density and the environmental conditions in which they are cultivated. There is a circular section near the

root, and this transforms to an angular region at the height of the branches and leaves. Dendritic properties are common among cultivars from the inter-Andeans valleys of Bolivia and South Peru. Indeed, variations in this simple property are observed in a small number of cultivars from the altiplane and in a large number of cultivars from the center and north of Peru and Ecuador. (Gandarillas, 1968; Tapia, 1990).

Quinoa leaves are considered nutritious vegetables, and based on their dry weight, they present a better profile than grains. The protein content in their fresh leaves is greater than that in spinach (2.86%), chard (1.82%) and broccoli (2.98%). The nutritional properties of quinoa leaves is due to their high mineral and vitamin contents, with 100 grams of leaves possessing 410 mg of magnesium. This content meets the recommended quantity for men (400–420 mg) and surpasses the amount recommended for adult women (310–320 mg), which is why quinoa should be a key part of the diet (Nutri-Facts, 2014).

Because of its large agricultural and nutritional potential, interest in quinoa has increased in recent years, and it has become an alternative to diets in the Andean Region. Currently, it is considered a "star" product in the world because of its nutritional and medical properties. Quinoa includes a variety of species, and it is different from cereals because it has all of the required amino acids; it is also the only vegetable-derived food that can replace animal protein. In this sense, the increases in production and exportation are largely due to these qualities (Ayala, 2013).

At present, providing a platform for the development of new food products in which wheat semolina has been replaced with pseudocereals to increase their nutritional value or improve their digestibility, to meet the demands of the proportion of the population with food intolerances such as gluten intolerance is of great importance (Navruz-Varli and Sanler, 2016). Significant advances have been reported in the development and evaluation of pastas prepared with a mix of wheat semolina, amaranth, chickpeas, beans, corn and quinoa made in different countries. Quinoa can be used in different types of preparations; it can be steamed, toasted, or prepared by a method similar to popcorn. Additionally, the seeds can be macerated and converted to flour for use in many products. They can be ingested in by themselves or in potages, soups, oatmeal, cereals, and candies and even in sushi (Sánchez, 2012). Because of this diversity in the applications of quinoa, the objective of this work was to obtain and characterize quinoa leaves to evaluate their nutritional composition as a food alternative.

Materials and Methods

Materials

For every method, dried quinoa leaves were used. The leaves were provided by the Universidad Pontificia Católica de Chile through the agreement established with Dr. Francisco Fuentes.

Determination of proteins

Biuret Method

Twenty milliliters of distilled water was mixed with 1 g of quinoa leaf, and the solution was diluted to 50 mL with 20% sodium sulfate solution. The solution was left to stand for 10 min, and then 2 mL of this solution was transferred to tubes and mixed with 2 mL of sodium sulfate and 8 mL of Biuret reagent. The solution was left to stand in the dark for 30 min, and the absorbance was then read at 540 nm. For quantification, a standard curve was prepared using bovine albumin.

Carbohydrates

A solution was prepared with 1 mg of test material in 10 mL of distilled water. Six tubes were

prepared, and to each tube was added 1 mL of the aqueous solution of the test compound, 0.6 mL of 5% phenol solution and 3.6 mL of sulfuric acid. After 30 min, the absorbance at 480 nm was measured. For the blank, the same procedure was used, but the test compound was replaced with water. A standard curve was prepared with glucose, and it was treated the same way as the test material (NMX-F-312-1978).

Reducing sugar determination

The method was a modified version of that described in the NMX-F-006-1983. Briefly, 25 mL of solution A was combined with 25 mL of solution B in a 500 mL Erlenmeyer flask, this was put on a universal stand equipped with a heat plate and a burette with glucose standard for titration. The solution was brought to reflux and then titrated one drop at a time. After the first color change, the titration was stopped, and 0.5 mL of methylene blue was added, and the solution was brought back to reflux. Titration was continued until an orange copper color was observed, and the cuprous acid precipitated out of the solution. The same procedure was used to test l g of quinoa leaves in 100 mL of distilled water.

Water content

The watch crystals were dried to a constant weight for 2 hours in an oven, and then, 1 g of the sample was placed intoevery. The watch crystals were placed back in the oven for approximately 4 h to determine their percentage of water content (Nielsen, 2003).

Ash content in food

In a melting pot dried to a constant weight, 3 g of the test material was burned in direct fire until ash was obtained without additional smoke formation. The melting pot was transferred to an oven at 100 °C for 8 h to calcine the sample. It was then cooled and weighed (NMX-F-066-S-1978).

Ash content %= (P-p) x 100/M

P= Melting pot mass with ashes in grams

p= Mass of the empty melting pot

M= Mass of the test material in grams

Determination of raw fiber

It was determined by the official method described by the A.O.A.C (1996), which consisted of submitting the dry and defatted sample to acid hydrolysis and then to alkaline hydrolysis. The fiber content in the sample was gravimetrically calculated following calcination.

Polyphenol determination

The polyphenols were determined using the Folin Ciocalteu method, and the determinations were made in triplicate. Gallic acid was used as a standard, and a standard curve was made starting with a solution with a concentration of 50 mg mL^{-1}. Three grams of quinoa leaves were washed in 20 mL of hexane to remove the chlorophyll and then soaked in 10% methanol for 18 h. Then, 200 µL of 2 N Folin Ciocalteu reagent was added, and the solution was agitated in a vortex mixer for 3 min (Fisher Scientific G560 USA). Later, 2 mL of 7% Na_2CO_3 and 2.6 mL of distilled water were added. The mixture was incubated for an hour at room temperature. The absorbance was measured to 750 nm using a UV-Vis spectrophotometer (Agilent, 8453), and the results are reported as milligrams equivalents of gallic acid (mg EAG) per 100 g of test material (Vázquez-Luna et al., 2011).

Flavonoid determination

A 250-µL aliquot of the methanolic extract was mixed with 1250 µL of distilled water and 75 µL of 5% $NaNO_2$. The mixture was homogenized and then 150 µL of 10% $AlCl_3$ was added, and the results are expressed as mg of quercetin/100 g of test material (Vázquez-Luna *et al.*, 2011).

Quantification by liquid chromatography

For the determination of these photosynthetic pigments, the solution of the test material was filtered through a nylon filter (pore size 0.2 µm and a diameter of 25 mm, ADVANTEC MFS) to eliminate impurities in the extract that could damage the column, and 20 µL of the filtered solution was injected. The flow rate was controlled by a quaternary peristaltic pump (0.6 mL min^{-1}), the column was maintained at room temperature, and the eluate was monitored with a detection wavelength of 290 mm using a UV-visible detector. The wavelength was chosen based on the absorption spectra of the test compounds and standard solutions using a UV/Visible spectrophotometer (Agilent, 8453), and the intensities, separations and peak resolutions indicated that this was a good detection method. An efficient division of the components was achieved by shortening the run time through the use of a solvent gradient. Solvent A: 2% acetic acid in distilled water, and solvent B: acetonitrile. Solvents A and B were mixed as follows: a linear gradient up to 100% Solvent A over 10 min, followed by 15 min of isocratic elution with Solvent B. Between every injection of the test material, the column was re-equilibrated for a minimum of 10 min to eliminate the residual effects of the mixture with acetic acid (Skoog and Leary, 2001). A calibration curve was prepared with the following standards: gallic acid (Sigma Aldrich), catechin (Sigma Aldrich), rutin (Sigma Aldrich), morin (Fluka Analytical) and kaempferol (Sigma Aldrich). To validate the method, standardized and experimental tests were performed to confirm the precision, selectivity, limit of detection, limit of quantification and linearity.

Results and Discussion

The obtained results are presented in Table 1. The highest reported value was from the proteins; the content was higher than that in spinach (2.86%), chard (1.82%) and broccoli (2.98%), the most common vegetables in daily diets (Nutri-facts, 2014). Their importance is related to the quality of their proteins because they are comparable to those of albumin and globulin, and they have a balanced composition of the essential amino acids. Some authors mention that quinoa has an amino acid composition similar to that of the milk protein casein (Mujica and Jacobsen, 2006). The reducing sugar contents were low, which is considered desirable for this type of product, making digestion easier, so it can be used in foods with a low glycemic index, which are necessary for people with diabetes (Hernández, 2015).

Table 1. Nutritional values obtained.

Determination	Obtained results[†]
Proteins (%)	14.77 ± 0.6
Carbohydrates (%)	8.04 ± 0.8
Reducing sugars (%)	3.05 ± 0.27
Humidity (%)	2.8 ± 0.9
Ash content (%)	3.54 ± 0.21
Raw fiber (%)	43.7 ± 3.9
Polyphenols (mg $100g^{-1}$)	131.8 ± 10.3
Flavonoids (mg $100g^{-1}$)	62.07 ± 5.1

[†]Represents the average ± standard deviation of three repetitions

In contrast, the humidity and ash data are within the parameters set by NMX-F-006-1983 and NMX-F-066-S-1978. They established the importance of humidity in products, and levels of these constituents above the desired, could favor the growth of microorganisms that could damage the food texture. The value of raw fiber is suitable for this type of

food since its main function in food is to facilitate digestion, produce a feeling of satiety, regulate cholesterol levels and stimulate the development of bacterial flora (Rayas and Romero, 2008).

Table 1 also shows that the polyphenols were 131.08 ± 10.3 mg 100 g^{-1} and flavonoids 62.07 ± 5.1 mg 100 g^{-1}. These values are above those reported in the literature as the recommended daily servings, indicating that quinoa can serve as a natural antioxidants in organisms, which is required for the prevention of chronic and degenerative diseases (Martínez-Flores *et al.*, 2002).

Flavonoid quantification based on liquid chromatography

Figure 2 shows the HPLC chromatograms obtained from the standards of the most abundant compounds. The presence of gallic acid, catechin, kaempferol and morin, which have retention times of 8.893, 19.263, 10.964 and 11.476, was observed. Flavonoids and polyphenols in general have been the focus of many studies due to their potential beneficial effects on human health, and many vegetables and fruits are considered functional foods because of their contents of these compounds (Cvejic *et al.*, 2018).

Phenolic compounds have very important biological properties and offer benefits to human health by controlling and preventing cardiovascular diseases, neurodegenerative diseases and cancers. Table 2 shows the results of the concentrations of different flavonoids in quinoa leaves. The most abundant flavonoid was catechin, followed by morin with a similar concentration, leaving kaempferol and gallic acid with the lowest concentrations. However, Stewart *et al.* (2000) reported that 0.03 mg 100 g^{-1} of kaempferol daily is sufficient for the prevention of cancers. It has been reported that the antioxidant and antiradical activities of flavonoids are positively correlated with the number of hydroxyl groups on their aromatic ring, which can scavenge peroxide (Sroka and Cisowski, 2003).

Table 2. Flavonoid content in quinoa leaves.

Flavonoid	Concentration mg 100 g^{-1} MS[†]
Gallic acid	3.71 ± 0.32
Catechin	45.58 ± 3.63
Kaempferol	4.74 ± 0.49
Morin	39.05 ± 1.31

[†]Represents the average ± standard deviation of three repetitions
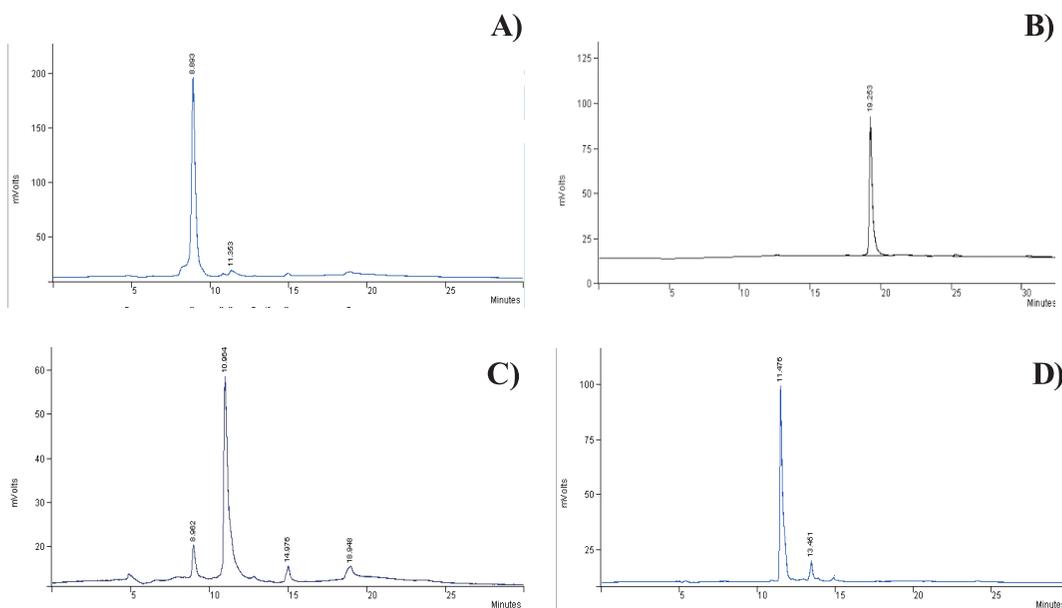

Figure 2. Chromatograms obtained from different standards: A) gallic acid, B) catechin, C) kaempferol and D) morin.

Conclusions

The obtained results from the analytical determinations of Andean quinoa leaves showed that it has important nutritional value as a vegetable. The highest values in this research were the contents of proteins, which could make quinoa leaves an attractive alternative to meat for many consumers. On the other hand, the obtained values of phenolic compounds indicate that quinoa leaves can serve as natural antioxidants, making them a functional food.

Resumen

A. Vazquez-Luna, V. Pimentel Cortés, F. Fuentes Carmona, y R. Díaz-Sobac. 2019. La hoja de quinoa como alternativa nutricional. Cien. Inv. Agr. 46(2): 137-143. *Chenopodium quinoa* es una planta herbácea que posee hojas polimórficas verdes. Se consumen tradicionalmente en América y se consideran vegetales nutritivos. La mayoría de los vegetales se consideran fuentes valiosas de micronutrientes, como minerales, vitaminas, carbohidratos y fibra dietética; sin embargo, debido a que son pobres en proteínas, se considera que no tienen valor energético. El consumo de hortalizas genera una sensación de saciedad y favorece la reducción de las calorías totales consumidas. Las hojas de quinoa se pueden consumir crudas cuando están maduras o cocidas al vapor, conservan la mayor parte de sus vitaminas y minerales. La FAO considera que la quinoa es el "alimento perfecto", y no solo se usa en dietas comunes, sino que también es adecuada para las dietas únicas de aquellos que son vegetarianos o atletas de alto rendimiento, así como aquellos con enfermedad celíaca ó con diabetes. El objetivo de este trabajo fue determinar el valor nutrimental de las hojas de quinoa. Para cada prueba, se utilizaron hojas de quinoa secas y en polvo, determinando los siguientes parámetros: polifenoles totales, flavonoides totales, proteínas, carbohidratos, azúcares reductores, contenido de agua, contenido de cenizas y fibra cruda, y los flavonoides se determinaron por HPLC. Los resultados obtenidos para los polifenoles fueron 131.8 ± 10.3 mg 100 g^{-1} y 62.07 ± 5.1 mg 100 g^{-1} para flavonoides, y los compuestos principales fueron ácido gálico, kaempferol y catequina. El contenido de proteínas fue de $11.8 \pm 0.6\%$, carbohidratos 18.3 ± 0.9, azúcares reductores $3.2 \pm 0.27\%$, humedad $2.8 \pm 0.9\%$, cenizas $1.4 \pm 0.14\%$ y el contenido de fibra cruda de $43.7 \pm 3.9\%$. Según el perfil nutrimental y el contenido de polifenoles y flavonoides totales, las hojas de quinoa pueden considerarse una alternativa para el consumo humano porque ofrecen un potencial interesante en nutrientes y capacidad antioxidante, lo cual actualmente representa un requisito dietético.

Palabras clave: Fitoquímicos, hoja de quinoa, perfil de nutrientes.

References

Ayala, F.J.F. 2013. Desarrollo de estrategias de posicionamiento. Caso: Producto Quinua. Revista Perspectivas 32:39–56.

Cvejic, H.J., G. Russo, J. Godos, N. Mimica-Dukie, N. Simin, A. Bjelica, and G. Grosso. 2018. 3 - Beneficial effects of polyphenols on chronic diseases and ageing. In Polyphenols: Proper-ties, Recovery and Applications. Edit: Charis M. Galanakis. Elsevier, pp. 69–102.

Gandarillas, H. 1968. Caracteres botánicos más importantes para la clasificación de la quinua. In: Universidad Nacional Técnica del Altiplano (Ed). Anales de la Primera convención de Quenopodiáceas quinua - cañahua. Puno, Perú. pp 41–49.

Hernández, J.R. 2015. La quinua, una opción para la nutrición del paciente con diabetes mellitus. Qui-

noa, an option for feeding of the diabetes mellitus patient. Revista Cubana de Endocrinología 26(3):304–312.

Martínez-Flores, S., J. González-Gallego, J.M. Culebras, and M.J. Tuñón. 2002. Los flavonoides: propiedades y acciones antioxidantes. Nutrición Hospitalaria, 17(6):271–278.

Mujica, A., and S.E. Jacobsen. 2006. La quinua (*Chenopodium quinoa* Willd.) y sus parientes silvestres. In Botánica Económica de los Andes Centrales. Editores: M. Moraes R., B. Øllgaard, L. P. Kvist, F. Borchsenius and H. Balslev Universidad Mayor de San Andrés, La Paz, pp. 449–457.

Navruz-Varli, S., and N. Sanlıer. 2016. Nutritional and health benefits of quinoa (*Chenopodium quinoa* Willd.). Journal of Cereal Science 69:371–376. doi: 10.1016/j.jcs.2016.05.004.

Nielsen, S.S. (Ed), 2003. Food Analysis Laboratory Manual; Kluwer Academic/Plenum Publishers, New York.

NMX-F-312-1978. Determinación de reductores directos y totales. Normas mexicanas. Dirección General de Normas.

NMX-F-006-1983. Alimentos. Galletas. Normas mexicanas. Dirección General de Normas.

NMX-F-066-S-1978. Determinación de cenizas en alimentos. Normas mexicanas. Dirección General de Normas.

Nutri-facts. "s. f." Magnesio. Consultando el 9 de julio de 2014. Disponible en ww.nutri-facts.org.

Rayas, D.P., and B.A.L. Romero. 2008. Fibra a base de frutas, vegetales y cereales: función de salud. Fiber from fruits, vegetables and cereals: A function of health. Revista Mexicana de Agronegocios, 23(12):613–621.

Sánchez, K.A. 2012. Observations regarding consumption of Peruvian native grains (quinoa, amaranth and kañiwa), weight status, and perceptions of potential risk factors, warning signs and symptoms of type 2 diabetes among Peruvian adults: a case study [Doctoral dissertation]. University of Maryland.

Skoog, D.A., and J.J. Leary. 2001. Análisis instrumental. McGraw-Hill. España, p. 935. 4ª edición.

Sroka, Z., and W. Cisowski. 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Fd and Chem Toxic 41(6):735–758.

Stewart, A.J., S. Bozonnet, W. Mullen, G.I. Jenkins, M.E.J. Lean, and A. Crozier. 2000. Occurrence of Flavonols in Tomatoes and Tomato-Based Products. J. Agric. Food Chem. 48(7):26632669.

Tapia, M. 1990. Cultivos Andinos sub-explotados y su aporte a la alimentación. Instituto Nacional de Investigación Agraria y Agroindustrial INIAA – FAO, Oficina para América Latina y El Caribe, Santiago de Chile.

Vázquez-Luna, A., F. Rivera-Cabrera, L.J. Perez-Flores, and R. Díaz-Sobac. 2011. Effect of Rootstock on Mango Fruit Susceptibility to Infestation by Anastrepha obliqua. A. J. Econ. Entomol. 104(6):1991-1998. DOI: doi.org/10.1603/EC11223