Original article[1]

ORAC-based assessment of antioxidant properties in *Chenopodium quinoa* Willd. and *Chenopodium pallidicaule* Aellen

Eric Maldonado, José A. Bravo, Patricia Mollinedo*

Instituto de Investigaciones Químicas IIQ-*Alimentos-NatProd*, Universidad Mayor de San Andrés UMSA, Av. Villazón N° 1995, La Paz, Bolivia

Keys: *ORAC, antioxidants, quinoa, cañahua, antioxidant capacity, free radicals*; **Claves:** *ORAC, antioxidantes, quinua, cañahua, capacidad antioxidante, radicales libres.*

ABSTRACT

Quinoa (*Chenopodium quinoa*) and cañahua (*Chenopodium pallidicaule*) are pseudocereals native to the Andes, known for their exceptional nutritional profile and rich content of bioactive substances, particularly antioxidants. To measure their antioxidant potential, we have employed the ORAC (Oxygen Radical Absorbance Capacity) method, a standard approach for assessing antioxidant levels in foods. The findings revealed notable antioxidant activity in both grains, reinforcing their value as natural sources of antioxidants. These results underscore the dietary significance of quinoa and cañahua and suggest promising uses in both the food and pharmaceutical sectors.

RESUMEN

Evaluación por el método ORAC, de las propiedades antioxidantes en Chenopodium quinoa Willd. y Chenopodium pallidicaule Aellen. La quinua (*Chenopodium quinoa*) y la cañahua (*Chenopodium pallidicaule*) son pseudocereales andinos reconocidos por su alto valor nutricional y contenido de compuestos bioactivos, incluyendo antioxidantes. El método ORAC (Capacidad de Absorción de Radicales de Oxígeno) es una técnica ampliamente utilizada para cuantificar la capacidad antioxidante de alimentos. Este estudio evaluó la actividad antioxidante de extractos de quinua y cañahua mediante ORAC, encontrando valores significativos que respaldan su potencial como fuentes de antioxidantes naturales. Los resultados destacan la importancia de estos granos en la dieta y su posible aplicación en la industria alimentaria y farmacéutica.

Revista Boliviana de Química, 2025, 42, 74-80
ISSN 0250-5460, Rev. Bol. Quim. *Paper edition*
ISSN 2078-3949, Rev. boliv. quim. *e-edition*, *May-Nov*
30 noviembre 2025, https://doi.org/10.53287/qcii6321bs33d

https://bolivianchemistryjournaliiq.umsa.bo

[1]Received May 12, 2025, accepted October 1, 2025, published November 30, 2025. *Mail to: pattymollinedo@gmail.com

INTRODUCTION

Quinoa (*Chenopodium quinoa*), a pseudocereal native to the Andes, is grown in the highland's regions of Ecuador, Peru, Bolivia, Chile, and Argentina. Its seeds have served as a staple food since the era of the Inca civilization, with a legacy stretching back over 5,000 years in pre-Columbian history

Quinoa (Chenopodium quinoa Willd.), belonging to the Chenopodiaceae family, has historically been considered a sacred food by Andean civilizations and used for traditional therapeutic purposes. It is an annual species that exhibits significant morphological variability, reaching heights between 1 and 3.5 meters. Its terminal inflorescence, called a panicle, can measure between 15 and 70 cm and produce up to 200 grams of seed per unit. The seeds exhibit a wide chromatic diversity—including white, brown, yellow, gray, pink, red, and black shades—and are classified according to their diameter into three categories: large (2.2–2.6 mm), medium (1.8–2.1 mm), and small (less than 1.8 mm), which can influence their agronomic performance and industrial applications[1].

Quinoa is notable for its outstanding nutritional value and its contribution to health, largely due to bioactive components like polyphenols, which are known for their antioxidant effects. As a result, quinoa oil has attracted increasing attention for its potential applications in the food sector and its role in reducing the risk of conditions such as heart disease, diabetes, and cancer[1] [2] [3]. Additionally, its anti-inflammatory qualities and distinctive coloration make it a promising ingredient for use in dietary supplements[3] [4].

The cañihua or cañahua (*Chenopodium pallidicaule*) is native to the Andean highlands of Peru and Bolivia, and is characterized by its ability to adapt to extreme climatic conditions, such as frosts, droughts and very low temperatures. It is considered one of the most cold-resistant grain crops, tolerating down to -3 ºC without compromising its performance. Its grain contains between 15 and 19% protein, and like quinoa and kiwicha, it provides a significant amount of sulfur amino acids. It has a high-quality amino acid profile, notable for its richness in lysine, isoleucine and tryptophan. This protein composition, together with approximately 60% carbohydrates and 8% vegetable oils, makes kañiwa a highly nutritious food[2]. Both quinoa and kañiwa have a considerable lipid content, and their oils are rich in unsaturated fatty acids and tocopherols[4].

Determination of Oxygen Radical Absorption Capacity (OARC)

The ORAC method[5] evaluates the ability of antioxidants present in a sample to counteract peroxyl radicals, which are generated from the thermal decomposition of the compound azobis(2-methylpropionamidine) dichlorate (AAPH) and cause the oxidation of fluorescein, which acts as a substrate. Trolox, a soluble derivative of vitamin E known as 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, was used as the antioxidant standard (Figure 1).

The samples, trolox, AAPH, and fluorescein were diluted in previously prepared phosphate-buffered saline (75 mM; pH 7.4). The reference calibration curve was run daily with different concentrations of trolox (0.2–2 nmol). The fluorescein stock solution (1.17 mM) was prepared daily with the same buffer and stored in the dark at 4°C. The reaction was performed in black polyester multiwell plates with a final volume of 200 µl as follows: 20 µl of the sample/calibration line, 120 µl of fluorescein, and 60 µl of APPH. The resulting mixture was incubated for 10 minutes. The resulting fluorescence was then measured continuously for 95 minutes at 37°C using a fluorimeter (FLUOstar OPTIMA, Labtech, UK) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. Taking the normalized curves into account, the area under the curve (AUC) of fluorescence decrease was quantified for each well using the following formula:

$$AUC = \left(0{,}5 + \left(\sum_{i=1}^{i=31} \frac{fi}{f1}\right)\right) * CT$$

Where:
i = number of cycles
f = fluorescence units
CT = time of each cycle in minutes. In this case, CT = 2

The clean area under the curve (clean AUC) is found by the difference between the AUC of the sample and the AUC of the blank. This clean AUC (y) is plotted in front of the Trolox concentration as standard (X), obtaining the linear regression line.
From this, the equivalent moles of Trolox (ET) per liter of sample are calculated

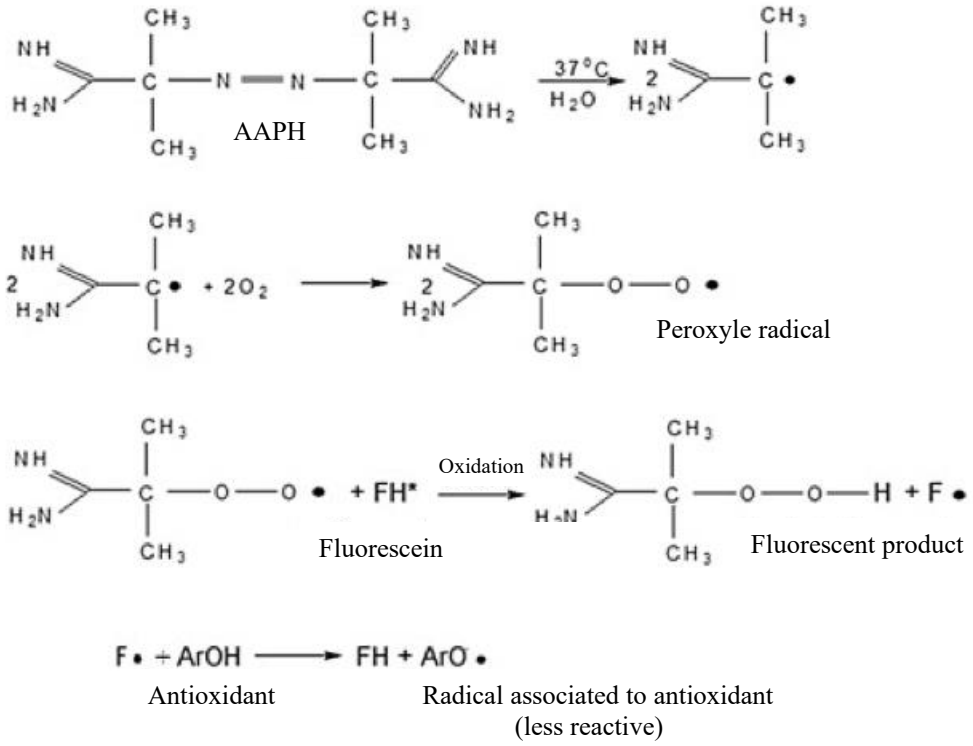

Figure 1. Representative scheme of the oxidation process of fluorescein by peroxyl radicals and neutralization by antioxidant compounds[6 7 8 9].

$$ORAC = \frac{AUC_{AH}}{AUC_{Trolox}} * \frac{[Trolox]}{[AH]}$$

Where:
AUC AH: Area under the curve in the presence of antioxidant.
AUC Trolox: Area under the Trolox curve.
[Trolox]: Trolox concentration.
[AH]: Antioxidant concentration.
Fluorescence values were obtained as the average of at least three independent assays for each sample run in triplicate. Results were expressed as µmol of Trolox equivalents (TE)/mg of protein.

EXPERIMENTAL

Plant Material

Quinoa samples were collected in the Chojñakota community, La Paz department in April 2022. Kañawa was collected in San Andrés de Machaca community, La Paz department, May 2022.

Materials and Methods

AAPH [2,20-Azo-bis(2-amidinopropane) dihydrochloride] used as a source of peroxyl radicals, Trolox (6-Hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), sodium fluoresceinate, BMCD (β-methyl cyclodextrin) and gallic acid were obtained from Sigma Chemical Co. (St Louis, MO). Absorbance measurements were performed on a Cytation Biotek TM3 UV-Vis spectrophotometer from BioTek® Instruments, Inc.

Materials

50 ml, 100 ml, and 250 ml beakers.
10 ml, 25 ml, 50 ml, 100 ml, and 250 ml volumetric flasks.
Spatula
Test tubes
Test tube rack
2-20 µL, 20-200 µL, and 100-1000 µL micropipettes.
Analytical balance
Costar UV microplate
Biotek plate reader

Reagents

Na_2HPO_4•12H2O
NaH_2PO_4•2H2O
1µM Fluorescein
6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid Trolox $C_{14}H_{18}O_4$
2,2-azobis(2-amidinopropane) dihydrochloride AAPH $C_8H_{20}Cl_2N_6$

Reagent Preparation

• 10 mM phosphate buffer solution: Weigh 0.276 g of Na_2HPO_4•12H2O and 0.035 g of NaH_2PO_4•2H_2O, dissolve them in distilled water, and dilute to 100 ml.
• 1µM Fluorescein: Initially prepare a 1 mM fluorescein solution by weighing 3.76 mg of fluorescein and bringing it to a volume of 10 ml with phosphate buffer solution. Then, make a 1µM dilution.
• 250 mM AAPH Solution: Weigh 678 mg of AAPH and make up to 10 ml with phosphate buffer solution.
• 2000 µM Trolox Solution: Weigh 5 mg of Trolox and make up to 10 ml with phosphate buffer solution. The solution can be heated for easier dissolution.

Samples' Preparation

If the sample has a high fat content, the first step is to defat it.
Make dilutions in distilled water of 1:10 and/or 1:100.

Methods, Determination of Antioxidant Capacity

-Make dilutions for the Trolox curve: 5, 12, 5, 25, 50, 75, and 100 µl of the above solution. Bring the volume to 1 ml with distilled water. Make the curve from 10 to 200 µg/ml.
-Add the following components to each well: 150 µl of fluorescein and 25 µl of the Trolox dilution.
-In parallel, make an assay blank containing only: 150 µl of fluorescein and 25 µl of phosphate buffer.
-Incubate for 30 minutes at 37°C.
-Add 25 µl of the 250 mM AAPH solution to each well.
-The intensity of fluorescein is measured every 2 min over a period of 2 hours with an excitation and emission wavelength of 485 and 520 nm respectively.

RESULTS AND DISCUSSION

Table 1. Results obtained after manipulations executed after the Experimental Section

Sample	Trolox µmol/g b.s. sample	Trolox µg/g b.s. sample	% inhibition
Quinoa grain A	87.40	21851.09	21.41
Quinoa grain C	74.59	18647.18	10.61
Quinoa grain D	115.84	28960.28	16.16
Mix red black	62.44	15611.13	9.08
Quinoa Challapata	53.39	13347.10	7.88
Organic quinoa grain	209.40	52349.83	30.78
Red quinoa	151.46	37864.45	21.17
Cañahua Proimpa 2	20.78	5195.46	30.53
Cañahua Proimpa 9	11.71	2926.61	17.01
Cañahua Proimpa 10	14.36	3689.36	20.30
Janqu Qañawa	2.56	640.08	13.52
Kispiña Juyra	3.27	816.44	15.56

Quinoa exhibited greater antioxidant capacity than cañahua, possibly due to its higher content of phenolic compounds[11]. Figure 2 shows the fluorescence decay curves in the ORAC assay at different Trolox concentrations.

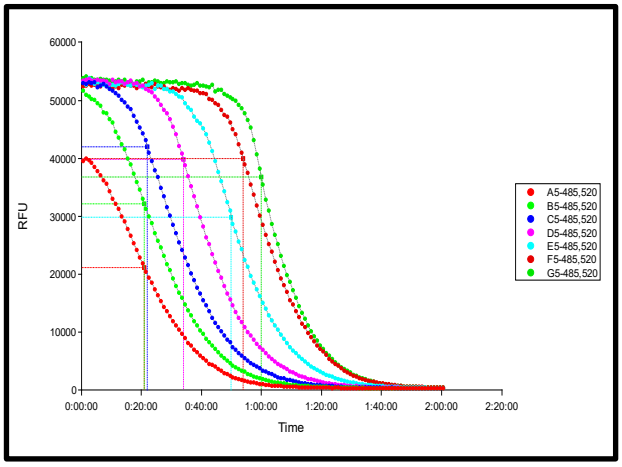

Figure 2.

In Figure 3, the fluorescein decay curves in the ORAC assay against different quinoa samples were determined.

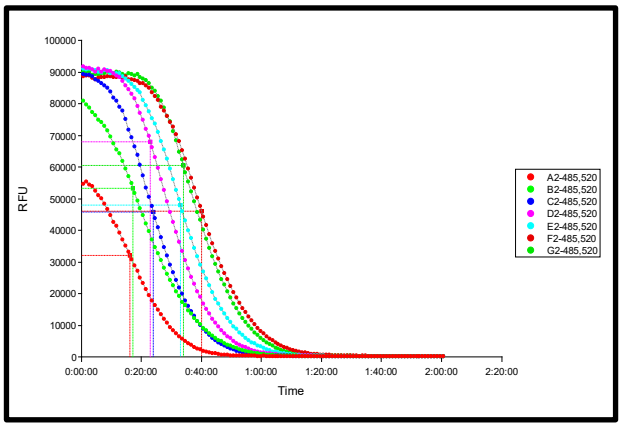

Figure 3.

In Figure 4, the fluorescein decay curves were determined in the ORAC assay against different cañahua samples.

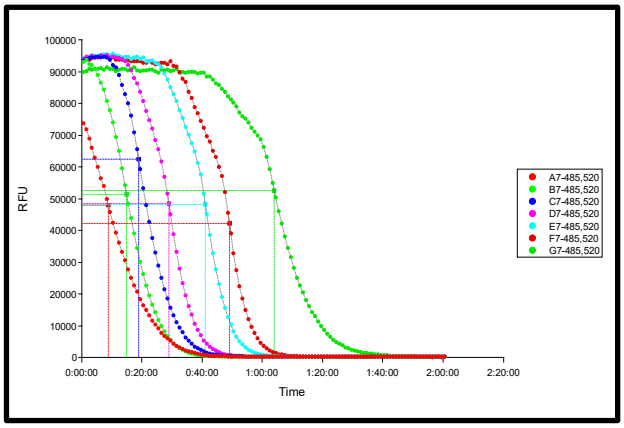

Figure 4.

The antioxidant capacity of quinoa and cañahua was determined in grains from 7 samples of quinoa and 5 samples of cañahua (Table 1).

These values vary in quinoa between 53.39 and 209.40 while in cañahua these values are 2.56 and 20.78 expressed as Trolox µmol/g b.s./sample.

The antioxidant capacity of quinoa and cañahua grains was evaluated using the ORAC (Oxygen Radical Absorbance Capacity) method, expressed in Trolox units (µmol/g d.b. and µg/g d.b.) and percentage inhibition. The results show significant differences between samples, reflecting the variability in antioxidant capacity depending on the type of grain and its origin.

In the case of quinoa, the sample with the highest antioxidant capacity was organic quinoa grain, with a value of 209.40 µmol/g d.s. (52349.83 µg/g d.s.) and an inhibition percentage of 30.78% (Table 1). This result highlights the importance of organic production systems in preserving antioxidant capacity, which is consistent with previous studies indicating that less aggressive agricultural methods can better preserve bioactive compounds.

Quinoa grain D (115.84 µmol Trolox/g) also showed remarkable activity, possibly due to its genetic profile or cultivation conditions. In contrast, Challapata quinoa showed the lowest value, with 53.39 µmol/g d.s. (13347.10 µg/g d.s.) and an inhibition percentage of 7.88%. Variability in antioxidant capacity can be influenced by factors such as genetic variety, cultivation conditions, soil, climate or post-harvest processing[12] (Table 1).

Regarding the cañahua samples, the highest antioxidant capacity was observed in cañahua Proimpa 2, with 20.78 µmol/g d.w. (5195.46 µg/g d.w.) and an inhibition percentage of 30.53%. This highlights the antioxidant potential of cañahua as a functional food, which has been supported by studies highlighting its high content of polyphenols and flavonoids, while Janqu Cañawa and kispiña Juyra presented the lowest values (2.56 and 3.27 µmol Trolox/g, respectively). This variability may be due to differences in the content of bioactive compounds such as phenolic acids and anthocyanins, which are influenced by genetic and environmental factors[13] (Table 1).

It is noteworthy that, despite their lower absolute values, some cañahua samples (such as Proimpa 2) achieved a similar or higher inhibition percentage than certain quinoa varieties (e.g., 30.53% vs. 30.78% in organic quinoa). This suggests that cañahua may have a unique antioxidant profile, effective in specific oxidative stress systems (Table 1).

The overall comparison between the two grains suggests that quinoa exhibits greater antioxidant capacity compared to cañahua, especially in organic samples and specific varieties. This could be due to intrinsic differences in chemical composition, such as the content of phenolic compounds and flavonoids, which are the main contributors to antioxidant capacity.

Finally, it is important to consider that the results obtained may be influenced by the analysis method and experimental conditions, so it is essential to conduct additional studies evaluating other varieties and processing techniques to obtain a more complete picture.

CONCLUSIONS

The results obtained demonstrate that quinoa has a significantly higher antioxidant capacity than cañahua, especially in the case of organic quinoa, highlighting the positive impact of less aggressive cultivation methods on the preservation of bioactive compounds. The variability observed between samples of the same grain suggests that both cultivation conditions and genetic characteristics play a crucial role in antioxidant capacity.

Furthermore, the antioxidant potential of cañahua, although lower than that of quinoa, highlights its value as a functional food due to its polyphenol and flavonoid content, as indicated by several previous studies. However, the difference in antioxidant capacity may be associated with the chemical composition and genetic variability between varieties.

It is recommended to continue researching the influence of agronomic and genetic factors on the antioxidant capacity of these grains, as well as to evaluate the impact of processing methods on the preservation of their bioactive compounds.

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