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## Optimization of the extraction of antioxidant compounds from quinoa (*Chenopodium quinoa* Willd.)

Optimización de la extracción de compuestos antioxidantes a partir de quinua (*Chenopodium quinoa* Willd.)

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### ABSTRACT

Argentina contains a great biodiversity of natural foods such as quinoa that can be included in the human diet because of their nutritional characteristics and content of bioactive compounds. Among other properties, these bioactive have an antioxidant capacity that protects biomolecules from oxidant damage. Bioactive compounds contribute beneficially to diverse antimicrobial, anti-inflammatory and anti-carcinogenic physiological activities. The objective of this study was to optimize the parameters for the extraction of antioxidant compounds from quinoa: drying temperature of the grain, liquid/solid ratio (L/S), and ethanol concentration in the extraction solvent, based on an experimental design of three variables at three levels. A face-centered central composite design was used. The proposed levels were 40°C, 60°C and 80°C; 20:1, 30:1 and 40:1, and 30%, 50%, and 70% v/v of ethanol. Antioxidant capacity was determined by the capture of the DPPH free radical. The values obtained were from 16.3 mg to 161.5 mg of equivalent trolox (ET) 100 g<sup>-1</sup> of quinoa. The maximum antioxidant capacity was obtained for the L/S ratio of 28:1, and the drying temperature of the grain was 58°C and 39% v/v of ethanol in the extraction solvent. The ethanol concentration was the most influential variable in the antioxidant compound extraction.

**Key words:** hydroalcoholic extraction, bioactive compounds, face-centered central composite design.

### RESUMEN

Argentina posee una gran biodiversidad de alimentos tales como la quinua que pueden ser incluidos en la dieta humana por sus características nutricionales y su contenido de compuestos bioactivos. Estos compuestos, entre otras propiedades, presentan una capacidad antioxidante que protege a las biomoléculas frente al daño oxidativo. Los compuestos bioactivos contribuyen benéficamente a diversas actividades fisiológicas antimicrobianas, antiinflamatorias y anticancerígenas. El objetivo de este estudio fue optimizar los parámetros para la extracción de compuestos con capacidad antioxidante de la quinua: temperatura de secado del grano, relación líquido/sólido (L/S) y concentración de etanol en el solvente de extracción, basado en un diseño experimental de tres variables a tres niveles. Se usó un diseño central compuesto centrado en las caras. Los niveles propuestos fueron 40°C, 60°C y 80°C; 20:1, 30:1 y 40:1, y 30%, 50%, 70% v/v de etanol. La capacidad antioxidante se determinó mediante la captura del radical libre DPPH. Se obtuvieron valores desde 16.3 a 161.5 mg de trolox equivalente (TE) 100 g<sup>-1</sup> de quinua. La máxima capacidad antioxidante se obtuvo para la relación L/S de 28:1, 58°C de temperatura de secado de grano y 39% v/v de etanol en el solvente de extracción. La variable de mayor influencia fue la concentración de etanol en el solvente.

**Palabras clave:** extracción hidroalcohólica, compuestos bioactivos, diseño central compuesto centrado en las caras.

### Introduction

Quinoa is an annual plant from the Andean region of South America, cultivated from Colombia to Argentina and Chile. In recent years, there has been a progressive increase in quinoa crops, especially in Bolivia, Peru, and Ecuador, which have been the main producers (FAO-ALADI, 2014).

Quinoa exhibits multiple abilities to adapt itself to solar radiation, temperature, water availability, and atmospheric CO<sub>2</sub> concentration, allowing its cultivation in different

agroecological zones (Zurita-Silva *et al.*, 2014; Melo, 2016; Reguera *et al.*, 2018). It is a plant with additional notable agronomic adaptations to different adverse weather conditions like drought, high salinity, and frosts (Ruiz *et al.*, 2014; Ruiz *et al.*, 2016).

Quinoa is considered a pseudocereal because of its particular composition, making it a grain of special interest as a human food. Its grains are exceedingly nutritious (López *et al.*, 2011; Padrón Pereira *et al.*, 2015). Quinoa seeds are rich in lipids, carbohydrates, polyphenols, fiber, and proteins

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(Padrón-Pereira *et al.*, 2015; Tang *et al.*, 2015; Fischer *et al.*, 2017; Vidaurre-Ruiz *et al.*, 2017; Jaikishun *et al.*, 2019). The protein contents range between 12% and 16% depending on the quinoa cultivar. They have high nutritional value because of the presence of essential amino acids such as leucine and isoleucine. These seeds contain proteins without gluten, and they are currently appearing in diets free of this component (Álvarez-Jubete *et al.*, 2009).

Argentina has great diversity of foods that protect biomolecules from oxidative damage by different mechanisms. There are two fundamental strategies to protect the organism from free radicals: the enzymatic and non-enzymatic (endogenous and exogenous) (Wu, 2015). The endogenous strategy requires external support, but exogenous antioxidants are recommended as they can capture free radicals from oxygen and chelate transition metals. Exogenous antioxidants can generate reactive oxygen species (ROS) by the Fenton/Haber-Weiss reaction in its free ionic state. This phytochemical benefit is present in quinoa, but it varies in different cultivars of the same plant (Tang *et al.*, 2015).

In addition to being incorporated into the daily diet, concentrate extracts can be considered as possible sources of antioxidant compounds that can be used for the enrichment of other products with antioxidant compounds. The extraction process is regulated by different variables like extraction temperature, the nature of the extraction solvent, and its concentration, treatment time, and the state of aggregation of the substrate. Engineering aims to develop different optimal processes to meet the variable values and costs.

In this research, the optimal values of the pretreatment parameters for the extraction of antioxidant compounds were studied. These were the drying temperature of quinoa grains, liquid/solid ratio, and the ethanol concentration in the extraction solvent.

## Materials and methods

Quinoa was provided by the Quinoa Real company, whose crops are located in Yavi, Jujuy, Argentina (22°7'47" S, 65°27'44" W). The cultivar used was Real Blanca. Quinoa was preserved in glass flasks of 5 L with hermetic covers and stored in the dark until used. The trials were carried out in a uniform batch material.

### Obtaining antioxidant compounds

Following the wet de-saponification treatment of quinoa, the grains were dried in a forced convection laboratory

dryer (Tecnodalvo Model CHC/F/I, Argentina) until reaching a moisture content of 12%. This process was required to avoid the deterioration caused by microorganisms. De-saponified and dried quinoa grains were ground in a mill (IKA, Germany) to pass through a 40-mesh sieve.

The flour obtained was added to a mixture of water and ethanol for 1 h in an orbital shaker (OS-20 Orbital Shaker, Boeco Germany, Germany) at 25°C. This solution was filtered and conserved at  $4 \pm 1^\circ\text{C}$  in a caramel-colored container, until subsequent tests. Water-ethanol mixtures were used as extractants because of their efficiency, selectivity, low cost, low toxicity, and ease of removal.

### Antioxidant capacity determination

The compound 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a stable radical that has an intense violet color and absorbs at 517 nm. The method proposed by Brand-Williams *et al.* (1995) evaluates the antioxidant capacity by measuring the reduction of the absorbance. It translates into a DPPH concentration decrease because of the scavenging effect.

One ml of the quinoa water-ethanol solution was reacted with 5 ml of 100  $\mu\text{M}$  DPPH solution prepared at the moment of use. The mixture was conserved at room temperature in the dark for 50 min. Then, absorbance at 517 nm was recorded by using a UV-1800 spectrophotometer (UV-1800 Spectrophotometer, Shimadzu, Japan). The results were expressed as Equivalent Trolox (ET) 100  $\text{g}^{-1}$  quinoa on dry bases (d.b.). A calibration curve was obtained using a Trolox standard solution.

The linear equation was as follows:

$$y = -108.6x + 103 \quad (1)$$

with  $R^2 = 0.996$ , where  $y = \text{mg de ET/L of solution}$  and  $x = \text{absorbance}$ .

### Experimental design and statistical analysis

The optimization of the extraction process of compounds with antioxidant capacity was performed based on an experimental design of three variables and three levels; therefore, a face-centered central composite design was used. The parameters and their levels were as follow: grain drying temperature (T) of 40°C, 60°C and 80°C, liquid/solid extractant ratio (L/S): 20:1, 30:1 and 40:1, and ethanol concentration in the solvent (% Et): 30%, 50%, and 70% v/v.

Table 1 shows codified values of the levels (variables) used in the experimental design and their corresponding real values for the entry factors.

**TABLE 1.** Relationship among codified variables and real values of independent variables.

	Temperature T (°C)	Ethanol concentration % Et	Liquid/solid ratio L/S
Level -1	40	30	20
Level 0	60	50	30
Level +1	80	70	40

Eighteen experiences in a group of 8 were required to check the two-level design (-1, 1) to the three factors ( $2^k$ ,  $k = 3$ ); Four replicates were used on the central point to evaluate the pure error, and the remaining six trials to test faced-centered values (star points).

Linear interaction and quadratic coefficients were evaluated by an ANOVA ( $P < 0.05$ ). F-test and probability (p) were determined to analyze the significant statistical contribution of all the terms. The results of the experimental design were processed by applying multiple regression analyses. The model's goodness of fit was checked by the coefficient of determination ( $R^2$ ) and the significance of the model terms was established with a confidence level of 95%. The process conditions were optimized by response-surface graphics. All the analyses were performed using the free version of the Minitab 18 software (Minitab, Pennsylvania, USA).

## Results and discussion

The values of the antioxidant capacity obtained for the different test conditions are shown in Table 2.

The antioxidant capacity values were coincident with the range published by Vollmannová *et al.* (2013), who evaluates the antioxidant capacity of five cultivars of quinoa by the DPPH method. They report values between 65.08 mg and 310.36 mg ET 100 g<sup>-1</sup> quinoa. Valencia *et al.* (2017), using 24 Peruvian quinoa accessions by the DPPH method achieve values between 121.66 mg and 299.28 mg ET 100 g<sup>-1</sup> of quinoa. It would be possible that the antioxidant capacity differs between plant varieties and among the parts of the same plant and the maturation phase of each plant organ (Sawa *et al.*, 1999). Factors such as soil characteristics, climatic conditions, and storage conditions could modify

**TABLE 2.** Antioxidant capacity response values.

Temperature T °C	Ethanol concentration % Et	Liquid/solid ratio L/S	Antioxidant capacity mg equivalent Trolox 100 g <sup>-1</sup> quinoa
40	30	20	107.4 ± 1.9
80	30	20	101.8 ± 0.2
40	70	20	70.8 ± 0.7
80	70	20	63.3 ± 0.5
40	30	40	105.1 ± 4.8
80	30	40	97.0 ± 5.3
40	70	40	16.3 ± 1.1
80	70	40	25.0 ± 0.6
60	50	30	156.3 ± 7.3
60	50	30	158.9 ± 0.3
60	50	30	157.5 ± 5.2
60	50	30	161.5 ± 3.3
40	50	30	100.9 ± 4.3
80	50	30	61.7 ± 3.2
60	30	30	104.1 ± 0.3
60	70	30	51.7 ± 3.8
60	50	20	102.9 ± 3.1
60	50	40	30.9 ± 0.7

the content of antioxidant capacity compounds (Naczka & Shahidi, 2006).

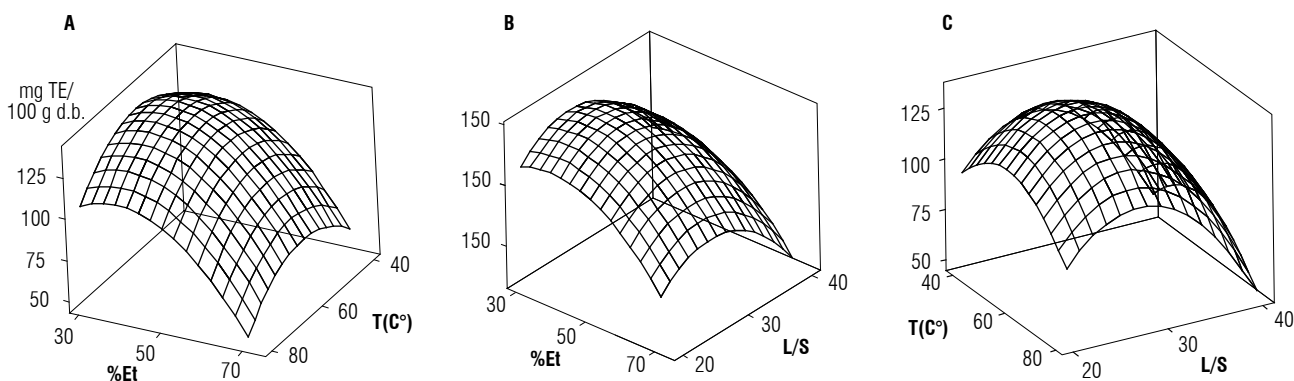
A quadratic equation (Eq. 2) was obtained by the software. It showed the effects of each factor, and its interactions according to the antioxidant capacity.

$$\begin{aligned} \text{mg TE } 100 \text{ g}^{-1} \text{ d.b.} = & -425.5 + 6.48 T(^{\circ}\text{C}) & (2) \\ & + 5.03 \% \text{Et} + 20.40 \text{ L/S} - 0.056 \\ & 2 T(^{\circ}\text{C}) * T(^{\circ}\text{C}) - 0.0647 \% \text{Et} * \% \\ & \text{Et} - 0.3687 \text{ L/S} * \text{L/S} \end{aligned}$$

The statistical analysis (Tab. 3) showed that the percentage of ethanol and the L/S ratio had a significant effect ( $P < 0.05$ ) on the antioxidant capacity as well as the quadratic interaction of temperature, percentage of ethanol, and the L/S ratio. A second-degree polynomial model could be used to represent the relationship between the selected parameters. The percentage of ethanol in the solvent showed the most influence.

**TABLE 3.** Antioxidant capacity values of extracts of quinoa seeds for different conditions of drying temperature (°C), percentage of ethanol (% Et) and L/S ratio.

Source of variation	T	%Et	L/S	T(°C) x T(°C)	%Et*%Et	L/S*L/S	Model
P-value	0.321	0.000	0.006	0.041	0.022	0.003	0.000
Model summary	S	15.6537	R <sup>2</sup>	93.26%	R <sup>2</sup> (adj)	88.54%	



**FIGURE 1.** Antioxidant capacity response surface, expressed as mg Trolox equivalent of 100 g<sup>-1</sup> of quinoa, according to the process variables: drying grain temperature (T), % of ethanol, and liquid/solid ratio (L/S). A) Setting values of the L/S ratio = 30:1; B) Setting values of T = 60°C; C) Setting values of % Et = 50 % v/v.

Figure 1 shows the behavior of the antioxidant capacity according to the three studied variables. When setting the L/S ratio (Fig. 1A), the antioxidant capacity increased at temperature values near 60°C. Maintaining a constant temperature, the antioxidant capacity was the maximum when alcohol concentration in the solvent was closed to 40% (Fig. 1B). Setting the ethanol concentration (Fig. 1C), the highest antioxidant capacity value was obtained when the L/S ratio was near 30:1, and it was lower for 20:1 and 40:1.

The values of process variables that maximize the antioxidant capacity response were 58°C grain drying temperature, 39% v/v ethanol concentration in the extractant, and a L/S ratio of 28:1.

Miranda *et al.* (2010) evaluate the impact of different drying temperatures on nutritional properties, the content of total phenolic compounds, and the antioxidant capacity of quinoa grains. They find that drying temperatures between 60°C and 80°C lead to degradation of total phenolic compounds. Vidaurre-Ruiz *et al.* (2017) find that phenolic compounds, flavonoids and betalamic pigments of Negra Collana, and Pasankalla varieties of quinoa decrease significantly after the drying process, while other compounds with higher antioxidant capacity are formed.

The optimum L/S ratio used in the extraction will depend on two basic considerations: the maximum content of the solvent is limited in practice by the consequences of the dilution of the substrates of interest and the performance of the extract according to the compounds of interest is directly related to the content of the solid from the starting material. The optimum value will result from the implementation of these considerations (Carciochi, 2014).

Regarding the solvent, the increase of the antioxidant capacity for extractant mixtures with 39% of ethanol indicates a higher presence of bioactive compounds of hydrophilic nature in quinoa (Repo-Carrasco-Valencia & Serna 2011; Stikic *et al.*, 2012; Abderrahim *et al.*, 2015; Fischer *et al.*, 2017; Valencia *et al.*, 2017). Since we propose that the extractant can be used in the food industry, the use of non-toxic solvents is crucial. Particularly, water-ethanol mixtures are most commonly used to obtain antioxidant extracts, due to their efficiency, selectivity, low cost, low toxicity and easy removal (Silva *et al.*, 2007; Wang *et al.*, 2008; Zhang *et al.*, 2011; Gong *et al.*, 2012). By changing the proportion of water and ethanol, solvent polarities change, modifying the nature of the extract, according to the different solubility of phenolic compounds present in the original matrix (Galvan d'Alessandro *et al.*, 2012).

## Conclusions

In this preliminary study, optimal values of grain drying temperature, ethanol percentage in the extractant mixture, and the L/S ratio to the extraction process of antioxidant compounds from quinoa were established (*Chenopodium quinoa* Willd.). The optimal processing values were a L/S ratio of 28:1, 58°C and 39% v/v of ethanol in the extraction solvent. The ethanol concentration was the most influential variable in the extraction of the antioxidant compound. Further research and a consideration of other processing variables, such as temperature and time of extraction, need to be carried out.

## Author's contributions

JL conducted the investigation and formal analysis and prepared the initial draft. HL developed the methodology, provided the study materials, reviewed and edited the

manuscript, and oversaw the research activity planning and execution. MCC reviewed and edited the manuscript, managed and coordinated the research activity planning and execution, and acquired the financial support for the project.

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