

# Phenolic Compounds, Antioxidant Capacity, and Protein Content of Three Varieties of Germinated Quinoa (*Chenopodium quinoa* Willd)

## Compuestos fenólicos, capacidad antioxidante y contenido proteico de tres variedades de quinua germinada (*Chenopodium quinoa* Willd)

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### ABSTRACT

Quinoa (*Chenopodium quinoa* Willd) is a pseudocereal with a high nutritional potential and a significant content of bioactive compounds, which is consumed mainly by the inhabitants of the South American Andes. The aim of this study was to evaluate the protein content, total phenols, and antioxidant activity of quinoa grains of the *Salcedo INIA*, *Pasankalla*, and *Negra collana* varieties, germinated for 24 and 48 hours at 35 °C. Organic quinoa grains were grown in the Andahuaylas province in Peru, at an altitude of 3582 m. The protein content was determined through the Kjeldahl method, total phenols, in turn, by spectrophotometry with the Folin-Ciocalteu reagent, and the antioxidant activity of the DPPH type were registered. The data were analyzed through an Analysis of Variance (ANOVA), a Tukey test, and Pearson's correlation at 5% significance. The germinated quinoa grains showed a considerable increase ( $p < 0,05$ ) in their protein content, total phenolic compounds, and antioxidant activity, as well as a strong positive correlation with the size of sprouts during the germination time. Therefore, germinated quinoa could be considered as a promising product for human nutrition and health.

**Keywords:** quinoa, bioactives, antioxidant activity, proteins, germinated quinoa

### RESUMEN

La quinua (*Chenopodium quinoa* Willd) es un pseudocereal con alto potencial nutricional y un contenido significativo de compuestos bioactivos, el cual es consumido principalmente por los habitantes de los Andes sudamericanos. El objetivo del presente estudio fue evaluar el contenido de proteínas, fenoles totales y actividad antioxidante de granos de quinua de las variedades Salcedo INIA, Pasankalla y Negra collana, germinados a 24 y 48 horas y 35 °C. Los granos de quinua orgánica fueron cultivados en la provincia de Andahuaylas, Perú, a 3582 m de altitud. Se determinaron el contenido proteico a través del método Kjeldahl, los fenoles totales por espectrofotometría con el reactivo Folin-Ciocalteu, y la actividad antioxidante del tipo DPPH. Los datos fueron analizados a través de un análisis de la varianza (ANOVA), un test de Tukey y la correlación de Pearson al 5% de significancia. Los granos de quinua desaponificados y germinados presentaron un incremento considerable ( $p < 0,05$ ) en contenido proteico, compuestos fenólicos totales y actividad antioxidante, así como una fuerte correlación positiva con el tamaño de brotes durante el tiempo de germinación. Por lo tanto, la quinua germinada podría considerarse un producto prometedor para la nutrición y salud humana.

**Palabras clave:** quinua, bioactivos, actividad antioxidante, proteínas, quinua germinada

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### Introduction

Quinoa (*Chenopodium quinoa* Wild) is an endemic South American plant typical, domesticated thousands of years ago by the Inca people of the Andes of Peru and Bolivia, as well as parts of Ecuador, Argentina, and Chile. It comes with a diversity of forms, genotypes, and wild progenitors (Navruz-Varli, and Sanlier, 2016), as well as a high resistance and

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adaptability to climatic and edaphic conditions (Jacobsen, Mujica, and Jensen, 2003; Filho *et al.*, 2015).

Quinoa grain is considered to be a pseudocereal with a high nutritional value, mainly due to its high protein content and essential amino acids, which are higher than traditionally consumed cereals (Filho *et al.*, 2015; Dakhili, Abdolalizadeh, Hosseini, Shojaee-Aliabadi, and Mirmoghtadaie, 2019), whose importance is increasingly recognized in food safety, since it considerably replaces and complements the diet, especially for people who rarely consume meat and dairy products (Elsouhaimy, Refaay, and Zaytoun, 2015; Navruz-Varli, and Sanlier, 2016). It is one of the main sources of protein in developing countries (Repo-Carrasco-Valencia, and Serna, 2011).

Quinoa grains have been found to contain numerous phytochemical compounds such as phytosterols, phytoecdysteroids, and phenols, which provide health benefits with their metabolic, cardiovascular, gastrointestinal, and anticarcinogenic aspects (Navruz-Varli and Sanlier, 2016; Vilcacundo and Hernández-Ledesma, 2017), and therefore have a high antioxidant activity, especially associated with phenolic compounds (Contreras-Jiménez, Torres-Vargas, and Rodríguez-García, 2019; Repo-Carrasco-Valencia, 2020), with up to 27 types of free and conjugated phenols (Abderrahim, Huanatico, Segura, Arribas, Gonzalez, Condezo-Hoyos, 2015), mostly phenolic acids (Repo-Carrasco-Valencia, and Serna, 2011; Tang, *et al.*, 2015; Tang *et al.*, 2016).

Quinoa grains are consumed in different forms by the inhabitants of the American Andes: in soups, extruded, instant breakfasts, flour, as a constituent of cookies, flour, bread, tortillas, among others (Bhargava, Shukla, and Ohri, 2006). This is due to their content of fiber, starch, and sugars such as maltose, D-galactose and D-ribose, fructose and glucose (Zhu, 2018; Piñuel *et al.*, 2019), which makes them ideal even for the production of fermented beverages such as beer and chicha (FAO, 2011).

The digestibility of quinoa protein is a limiting factor in their use in food (Elsouhaimy *et al.*, 2015), but it improves considerably when subjected to germination, fermentation, and thermal treatments, thus increasing the bioavailability of its amino acids (Graf *et al.*, 2014; Navruz-Varli and Sanlier, 2016; Nickel, Spanier, Botelho, Gularte, and Helbig, 2016).

The germination process allows obtaining grains with high biological activity after enzymatic hydrolysis, thus enabling the accumulation of bioactive compounds such as polyphenols (Gan, Wang, Lui, Wu, and Corke, 2016; Televiciute *et al.*, 2020), which improves antioxidant activity (Banchuen, Thammarutwasik, Ooraikul, Wuttijumnong, and Sirivongpaisal, 2009; Sani, Iqbal, Chan, and Ismail, 2012). Thus, the germination process makes quinoa a more functional food (Dávila, Sangronis, and Granito, 2003; Graf *et al.*, 2014; Mariod and Salama, 2020), which implies a new way of sell a nutraceutical food, with good acceptance, especially in people who cannot consume food from animal sources (Piñuel *et al.*, 2019).

The aim of this research was to evaluate the effect of the germination of quinoa (*Chenopodium quinoa* Willd) of the *Salcedo INIA*, *Pasankalla*, and *Negra collana* varieties on phenolic compounds, antioxidant capacity, and protein content.

## Materials and methods

### Vegetable material

Organic quinoa grains (grown without the addition of pesticides and synthetic fertilizers) of the *Salcedo INIA*, *Pasankalla*, and *Negra collana* varieties were provided by the Machu Picchu agrarian cooperative in the province of Andahuaylas, Perú. These grains were cultivated during the vegetative period of 2017-2018, in fields located at 13° 39'26" S, 73° 17'32" W, and 3 682 m of altitude.

### Obtaining germinated quinoa

Quinoa grains were washed with abundant treated water (pH 7.5), generating friction between grains to eliminate saponin. The washing continued until an evident absence of foam was reached. Then, they were subjected to a humidity between 43% and 45%, and packed in a wet gauze at 35 °C, for periods of 24 and 48 h, in order to cause germination, and the size of the sprout was measured with a Vernier. The sprouts were taken to a horizontal dryer at 60 °C, until constant humidity was reached, and then they were ground to 250 microns in an Agate mortar.

### Protein quantification

The nitrogen content was determined through AOAC method 984.13 (AOAC, 2016). The protein content was calculated by  $N \times 6.25$ , and the results were expressed as percentage on dry basis (d.b).

### Total phenol quantification

The spectrophotometric method used by Ah-Hen *et al.* (2012) was followed with some modifications. 1.0 g of sample was taken (germinated quinoa ground) and mixed with 10 ml of acidified methanol with 1% HCl. The homogenate was stored for 24 hours at 4 °C in darkness. Then, it was centrifuged for 15 min at 7000 rpm. An aliquot of the supernatant was taken in a test tube, thus obtaining a germinated quinoa extract, which was stored in a dark refrigerator.

100 µL of the extract were taken and caused to react under agitation with 750 µL of the reagent 1.0 N and 750 µL of sodium carbonate (60 g/L). The mixture was stored for 30 minutes in darkness at room temperature. In the same way, a blank was prepared using distilled water, which served to calibrate the spectrophotometer. Then, the absorbance was read at 755 nm in a spectrophotometer, model T80+ from PG Instruments.

The total phenolic compounds (TP) were estimated from a standard curve made with an aqueous gallic acid solution

( $R^2 = 0,96$ ). The results were expressed as the mg gallic acid equivalent (GAE) per 100 g sample on dry basis (d.b).

### Antioxidant capacity quantification

The method of Brand-Williams, Cuvelier, and Berset, (1995) was followed with some modifications. 5,0 g of sample (germinated quinoa ground) were weighed, and 20 ml of 80% methanol were added and mixed for 15 min at 800 rpm. The extract was stored for 24 hours at 4 °C in darkness. Then, it was centrifuged at 3000 rpm for 20 minutes, taking an aliquot of the supernatant (extract) and placed in the darkness at 4 °C lined with aluminum foil.

150  $\mu$ L of the extract were taken and 2850  $\mu$ L of diluted DPPH solution (24 mg/100 ml methanol) were added. Identically, a blank was prepared with 150  $\mu$ L of 80% methanol to obtain a correction factor due to the dilution. The mixture was left to react in darkness for 30 min at 20 °C and then taken to the spectrophotometer; the absorbance was read at 515 nm.

The antioxidant capacity was calculated using a standard Trolox curve (0,5 mM) and expressed as the  $\mu$ mol Trolox equivalent (TE)/g sample on dry basis (d.b.).

### Statistic analysis

The data were collected in triplicate, and an analysis of variance (ANOVA) and Tukey test were applied. Correlations between variables were determined by the Pearson correlation coefficient ( $R_s$ ) at 5% significance.

## Results and discussion

### Sprout size

In Figure 1, the size of the sprouts during germination can be observed. After 24 h, sprouts of around 6,4 mm are seen for the *Salcedo INIA* and *Pasankalla* varieties, and 8,8 mm for the *Negra collana* varieties, respectively; after 48 h of germination, these increased significantly ( $p < 0,05$ ) in 175%, 232%, and 214% for *Salcedo INIA*, *Pasankalla*, and *Negra collana*, respectively (Figure 2). Similar results were reported by Bravo, Reyna, Gómez, and Huapaya (2013) for the Blanca de Junín variety. The difference in sprout size between varieties is due to genotypic differences (Piñuel *et al.*, 2019) and by grain color (Televičiutė *et al.*, 2020), as well as composition (sugars, proteins, minerals, lipids, among others) (Han *et al.*, 2019; Piñuel *et al.*, 2019).

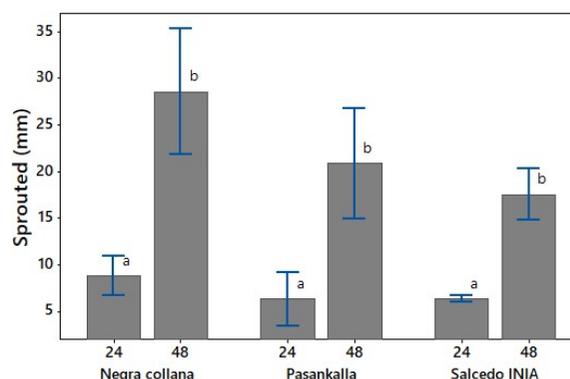
### Protein content

The protein contents of the ungerminated quinoa grains were between 13,73% and 15,61% (Table 1), with *Negra collana* being the variety that reported the highest content  $15,61 \pm 0,23\%$ . These results were similar to those reported by Pereira *et al.*, (2019), and Dakhili *et al.*, (2019).

On the other hand, it was observed that the protein content in the germinated quinoa increased significantly ( $p < 0,05$ ) for the *Salcedo INIA* and *Pasankalla* varieties, unlike the *Negra*



**Figure 1.** Quinoa varieties germinated at 24 and 48 h. **Source:** Authors



**Figure 2.** Sprout size of quinoa varieties germinated at 24 and 48 h. **Source:** Authors

**Table 1.** Protein percentage variation in germinated quinoa

Time (h)	Salcedo INIA	Increase (%)	Pasankalla	Increase (%)	Negra collana	Increase (%)
0	13,73 $\pm$ 0,17a		14,71 $\pm$ 0,17a		15,61 $\pm$ 0,23a	
24	14,75 $\pm$ 0,23b	7,5	15,25 $\pm$ 0,18b	3,7	15,70 $\pm$ 0,24a	0,6
48	15,18 $\pm$ 0,23b	10,6	15,60 $\pm$ 0,23b	6,1	16,13 $\pm$ 0,17a	3,4

Evaluated through a Tukey test at 5% significance.

**Source:** Authors

*collana* ( $p > 0,05$ ). Likewise, it was observed that *Salcedo INIA* reported the greatest increase in protein content, from 13,73% to 14,75% in 24 hours (7,5%), and it increased to 15,18% in 48 hours, which represented 10,6%. On the other hand, *Pasankalla* and *Negra collana* reported a 6,1% and 4,8% increase from the initial value after 48 hours of germination, respectively. The same behavior was observed by Chaparro, Pismag, and Elizalde (2010), Bravo *et al.* (2013), and Piñuel *et al.* (2019).

During the germination process, the enzymatic systems are activated, mobilizing reserve proteins located in the cotyledons of the quinoa grain. In the same way, changes in the amino acid composition occur due to enzymatic activity (Gan *et al.*, 2016; Televičiutė *et al.*, 2020), which

allows the production of peptides of intermediate molecular weight due biological activity (Torres, Cova, and Valera *et al.*, 2018; Banchuen *et al.*, 2009). This also happens with the mobilization of nitrogen stored in the quinoa grain, which allows a significant increase in protein, as observed by El-Safy, Mukhtar, and Salem (2013), Graf *et al.* (2014), and Li, *et al.* (2014). However, this depends on the humidity and temperature of the environment.

### Total phenol content

Polyphenolic compounds are beneficial for human health due to their antioxidant potential, which reduces the risk of cardiovascular diseases, neurodegenerative disorders, and diabetes (Gawlik-Dziki *et al.*, 2013; Navruz-Varli and Sanlier, 2016; Vilcacundo, and Hernández-Ledesma, 2017; Anngeli *et al.*, 2020).

The TP for the quinoa grains was between 159,69 and 198,23 mg GAE/100 g on dry basis (Table 2). These values are similar to those reported by Abderrahim *et al.* (2015), Nickel *et al.* (2016), and Saad-Allah and Youssef (2018). The differences in the total phenol content are due to growing conditions such as soil type (Nsimba, Kikuzaki, and Konishi, 2008; Liberal, Calhelha, Pereira, and Adega, 2016; Huang, Qin, Shi, and Wen, 2017), as well as the variety, since the colored varieties have a higher TP, as reported by Tang *et al.* (2015), Abderrahim *et al.* (2015), and Han *et al.* (2019). *Pasankalla* (red coloration) reported the highest content, followed by the *Negra collana* variety (shiny black coloration).

**Table 2.** TP variation (mg GAE/100 g d.b) in germinated quinoa

Time (h)	Salcedo INIA	Increase (%)	Pasankalla	Increase (%)	Negra collana	Increase (%)
0	172,56 ± 0,51a		198,23 ± 1,46a		159,69 ± 4,89a	
24	278,27 ± 3,80b	61,3	307,64 ± 3,66b	55,2	233,78 ± 4,29b	46,4
48	308,82 ± 3,93c	79,0	417,75 ± 1,78c	110,7	403,30 ± 4,74c	152,6

Evaluated through a Tukey test at 5% significance.

**Source:** Authors

It was observed that, after 24 h of germination, the TP for the *Salcedo INIA* variety had an increase of 61,3% and, 79,0% after 48 h. The *Pasankalla* variety increased to 55,2% and 110,7% after 24 and 48 h, respectively (Table 2). It could also be observed that *Negra collana* reported the highest increase (152,6% after 48 h), which is due to its genotypic differences; since this variety is darker, it releases a greater amount of phenolic compounds due to the leaching effect during germination (Televičiutė *et al.*, 2020; Piñuel *et al.*, 2019).

The TP increase during the germination process of quinoa has been observed by Alvarez-Jubete, Wijngaard, Arendt, and Gallagher (2010), Filho *et al.* (2015), Gan *et al.* (2016), and Piñuel *et al.* (2019). It is due to the fact that quinoa grains suffer biotic and abiotic stress during germination, which induces the generation mainly of oxygen-reactive species (ROS). These compounds are vital to protecting the grains during germination (Shulaev, Cortes, Miller, and Mittler,

2008; Televičiutė *et al.*, 2020) and for the biochemical and physiological functions of the sprouts, releasing aglycones due to enzymatic activity, which translates into an increase in phenols (Shetty, 2004; Sani *et al.*, 2012). Another critical aspect is the germination temperature, which was 35 °C for this research. The recommended standard is between 35 and 45 °C (Televičiutė *et al.*, 2020).

Thus, germination is used as a strategy to increase the presence of bioactive compounds such as phenols, anthocyanins, flavonoids and others in legumes, cereals, and pseudocereals such as quinoa (Mbithi, Van, Rodríguez, and Huyghebaert, 2001; Fernandez-Orozco *et al.*, 2008; Sarvajeet, and Narendra, 2010; Oghbaei, and Prakash, 2017; Televičiutė *et al.*, 2020).

### Antioxidant capacity (AA)

AA is an aspect that evaluates the ability of a compound to reduce the impact of ROS, and quinoa grains are an excellent source of antioxidants, being superior to many cereals, pseudocereals, and legumes (Pasko *et al.*, 2009; Tang and Tsao, 2017).

In Table 3, it is observed that the ungerminated quinoa grains reported antioxidant activity between 3,18 to 5,65 μmol TE/g d.b. The *Pasankalla* variety reported the highest antioxidant activity, followed *Negra collana*. This behavior is common for colored quinoa grains (Tang *et al.*, 2014; Abderrahim *et al.*, 2015; Escribano *et al.*, 2017; Mariod and Salama, 2020). The results were similar to those reported by other authors (Pasko *et al.*, 2009; Tang *et al.*, 2015; Nickel *et al.*, 2016; Repo-Carrasco-Valencia, 2000). However, the variation in antioxidant activity is due to genetic, agrotechnological, and environmental factors (Nsimba *et al.*, 2008).

**Table 3.** A variation (μmol TE/g d.b.) in germinated quinoa

Time (h)	Salcedo INIA	Increase (%)	Pasankalla	Increase (%)	Negra collana	Increase (%)
0	3,18 ± 0,05a		5,65 ± 0,04a		4,96 ± 0,04a	
24	3,81 ± 0,05b	19,8	6,66 ± 0,02b	17,8	5,65 ± 0,04b	13,9
48	5,40 ± 0,13c	69,4	6,82 ± 0,01c	20,8	5,90 ± 0,06c	19,0

Evaluated through a Tukey test at 5% significance.

**Source:** Authors

It was observed that, during germination, the AA of the grains of the quinoa varieties showed a significant difference ( $p < 0,05$ ). The *Salcedo INIA* variety reported the greatest increase (19,8% and 69,4% after 24 and 48 h of germination, respectively), while the colored varieties, *Pasankalla* and *Negra collana*, reported increases of 20,8 and 19,0% after 48 h, respectively (Table 3). This behavior is common in quinoa grains that germinate under light or darkness (Pasko *et al.*, 2009; Filho *et al.*, 2015; Piñuel *et al.*, 2019); or when subjected to some biotechnological modifications (Nickel *et al.*, 2016; Mariod and Salama, 2020).

The increase in antioxidant activity is due to the response of the seed to the physiological and biochemical changes to which they are subjected at the beginning of germination, so

germination is used as a strategy to increase this capacity (Banchuen *et al.*, 2009; Sani *et al.*, 2012; Filho *et al.*, 2015; Torres *et al.*, 2018).

### Correlation between variables

It was observed that the size of shoots evinced a high positive correlation with proteins, total phenols, and antioxidant activity ( $R_s > 0,81$ ) (Table 4), which indicates that germination time causes these variables to increase considerably. Likewise, it was observed that the germination process of the three varieties of quinoa showed a high correlation between the study variables.

The protein content reported high correlation with the total phenols, with values above 0,80, as well as with the antioxidant capacity. This indicates that quinoa grains, during the stress that occurs during germination, produce phenols and proteins in parallel to condition the sprouts of the grains (Li *et al.*, 2014; Televičiūtė *et al.*, 2020).

**Table 4.** Correlation of study variables

	SS	P	TP
Salcedo INIA			
P	0,90		
TP	0,90	0,96	
AA	0,99	0,84	0,86
Pasankalla			
P	0,87		
TP	0,96	0,91	
AA	0,81	0,89	0,93
Negra collana			
P	0,82		
TP	1,00	0,80	
AA	0,88	0,63	0,88

Sprout size (SS), Proteins (P), Total phenols (TP), Antioxidant activity (AA).

**Source:** Authors

In the same way, the TP shows a high positive correlation with the AA for the three quinoa varieties ( $> 0,86$ ), which suggests that the phenol content is a good indicator of the antioxidant activity (Pasko *et al.*, 2009; Tang *et al.*, 2014; Contreras-Jiménez *et al.*, 2019), thus making germinated quinoa highly bioaccessible and bioavailable to human health (Tang *et al.*, 2015; Navruz-Varli, and Nevin Sanlier, 2016; Vilcacundo and Hernández-Ledesma, 2017).

### Conclusions

The study showed that unsaponified quinoa grains of the *Salcedo INIA*, *Pasankalla*, and *Negra collana* varieties, subjected to germinations of 24 and 48 hours at 35 °C, increased their content of proteins and total phenolic compounds, as well as their antioxidant activity, presenting a strong positive correlation between them.

The results allow us to confirm that germinated quinoa is a promising product for human nutrition and health.

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