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Effect of Germination Time on the Content of Nutritional and Bioactive Compounds of *Chenopodium quinoa* Wild. Seeds Cultivated in Eastern Morocco

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Germination can be considered an important process for modifying quinoa seeds' nutritional and bioactive compounds. Understanding how germination modifies seed composition is essential to optimize their use in different food preparations and to meet the current trend for a healthy and balanced diet. The present study focuses on the effect of prolonged germination on the composition of quinoa seeds collected from a farm in the eastern Morocco. Seeds were germinated in a growth chamber with a controlled environment for different times (24, 48, 72, 96, and 120 h). At the end of each germination period, the seeds were dried and powdered, and their composition was analyzed. Powder obtained from ungerminated seeds was used as control. The results showed that germination led to a significant enhancement in the content of protein, fiber, total phenolics, and total flavonoids, with the highest increases observed at 96 h by 6.09 g/100 g dry matter (DM), 0.89 g/100 g DM, 50.27 mg/100 g DM, and 73.49 mg/100 g DM, respectively, compared to the control. Tocopherols (α, β, and δ) increased by 1.63, 1.21, and 2.67 µg/g of oil at 24 h, 72 h, and 120 h, respectively, compared to the control. Conversely, carbohydrate, energy, and saponin content decreased significantly relative to the control by 9.43–10.11 g/100 g DM (seeds sprouted for 72–96 h), 20.35 kcal/100 g DM (seeds sprouted for 72 h), and 0.58 g/100 g DM (seeds sprouted for 48 h and 72 h). This suggests that powder from germinated *C. quinoa* seeds subjected to prolonged germination (96 h) could be used as functional ingredients in food formulations, offering high levels of macronutrients, minerals, and bioactive compounds with a reduced saponin content.

Keywords: functional ingredient, germination period, health benefits, nutritional profile, quinoa seeds, tocopherols

INTRODUCTION

Chenopodium quinoa Wild. is an emerging crop worldwide, cultivated for over 7,000 years. It is characterized by its ability to grow in different marginal environments [Choukr-Allah *et al*., 2016], with marked resistance to various abiotic stresses, including salinity, drought, and frost [Jacobsen *et al*., 2003; Nazih *et al*., 2024].

C. quinoa seeds are distinguished by their high nutritional value and bioactive properties [Nowak *et al*., 2016; Vega-Gálvez

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et al., 2010]. They are rich in protein (9.1 to 15.7 g/100 g), lipids (4.0 to 7.6 g/100 g) and crude fiber (7.0 to 14.1 g/100 g), and their average carbohydrate content is 58 g/100 g [Pathan & Siddiqui, 2022]. Quinoa seed proteins contain all the essential amino acids and have a high efficiency ratio. These seeds are also rich in minerals, including potassium, calcium, magnesium, and iron [Pathan & Siddiqui, 2022]; tocopherols (7.29 mg/100 g) [Carciochi *et al*., 2016]; and phenolic compounds (71.7 mg/100 g) with high antioxidant activity [Alvarez-Jubete *et al*., 2010]. Other important phytochemicals of quinoa seeds are saponins with contents between 1.41 and 2.03 g/100 g [Mhada *et al*., 2020]. It should be emphasized, however, that the nutritional and bioactive composition of *C. quinoa* seeds depends on the plant genotype and the environmental conditions in which it grows [Granado-Rodríguez *et al*., 2021a,b]. From a nutritional point of view, regular consumption of quinoa seeds in the diet elicits certain health benefits and can reduce the risk of development of cardiovascular disease, obesity and diabetes [Lan *et al*., 2023]. Quinoa is also a good alternative for patients suffering from celiac disease as it is gluten-free [Alvarez-Jubete *et al*., 2010].

Due to its precious qualities, quinoa, with its multiple uses, certainly has a great future, especially in arid and semi-arid areas severely affected by climate impacts, as is the case in several marginal regions of Morocco. In this context, several studies have shown that germination enhances the nutritional value of quinoa by modifying its composition [Omary *et al*., 2012; Suárez-Estrella *et al*., 2020]. Bhinder *et al*. [2021] showed that germination can modify protein content, starch functionality, and bioactive compound profile, enhance the availability of certain minerals such as copper and zinc, and reduce antinutrient levels, including saponins and phytic acid. Additionally, Ramos-Pacheco *et al*. [2024] observed a notable improvement in proteins, lipids, ash, and fiber and a reduction in carbohydrate contents of quinoa seeds as a result of germination. They also noted increases in phosphorus, iron, manganese, and potassium, as well as in total phenolic and total flavonoid contents and antioxidant capacity. These modifications could be due to the activation of endogenous enzymes, which reduce antinutrients and improve nutrient profile, and antioxidant potential [Darwish *et al*., 2021]. However, most of these studies focused on shorter germination periods (up to 72 h) and did not fully investigate the variations in bioactive compounds, especially tocopherols. Therefore, the current study aims to evaluate the effect of prolonged germination (up to 120 h) on the nutritional and bioactive compound compositions of *C. quinoa* seeds and to determine the optimal germination time that enhances the content of valuable compounds, in order to find alternatives for patients with gluten intolerance or celiac disease and individuals seeking highly nutritious food. In addition, the current study provides new insights into the effect of germination on the contents of macronutrients, minerals, and bioactive compounds of quinoa grown and harvested in the eastern region of Morocco.

MATERIALS AND METHODS

Plant material

To conduct this study, fresh-matured *C. quinoa* seeds of the certified Titicaca cultivar were harvested from a farm in Berkane province in the eastern region of Morocco. Titicaca is a Danish quinoa variety developed by the University of Copenhagen. It is known for its early maturity, tolerance to abiotic stress, and adaptability to marginal soils. Its fruit is an achene containing round seeds about 2 mm in diameter.

Germination of *C. quinoa* seeds

The process of germination of quinoa seeds was carried out according to the methodology of Aguilar *et al*. [2019], with slight modifications. A total of 720 g of *C. quinoa* seeds (120 g *per* germination time, including the control) were soaked in 3.6 L of distilled water (600 mL *per* batch) for 24 h. After soaking, the seeds were placed in plastic boxes (three boxes for each germination time) with filter paper wetted with distilled water to keep moisture conversing. The boxes were then transferred to a growth chamber (Memmert GmbH, Schwabach, Germany), where the temperature, relative humidity, and light/dark cycle were 25°C, 70%, and 16/8 h respectively. The soaked seeds (control, germination time – 0 h) and seeds after 24, 48, 72, 96 and 120 h of germination were dried in a forced-air oven (Pol-Eko Aparatura, Wodzisław Śląski, Poland) at 40°C for 24 h to inhibit the activity of hydrolytic enzymes such as amylases, proteases and phytases without significantly altering heat-sensitive nutrients [Guardianelli *et al*., 2022], then crushed with a mill and sifted with a 500 µm sieve to obtain the powders, which were stored at −20°C until analysis of their nutrient and bioactive compound profiles.

Water activity measurement

Water activity (a_w) of powders from sprouted quinoa seeds was measured using an AW meter (Steroglass, Perugia, Italy). Calibration was performed with pure water of a_w=1. Samples weighing 1 g were used for the a_w reading [Ligarda-Samanez *et al*., 2022].

Dry matter determination

The dry matter content of powdered quinoa seeds after germination and drying was determined using the AOAC International method 934.01 [AOAC, 2005]. The samples (1 g) were heated at 105°C until their mass reached a constant value.

Protein content determination

To determine the total nitrogen content of the sprouted quinoa seeds by the Kjeldahl method [AFNOR, 2002], 1 g of powder was weighed and placed in a mineralization tube containing 2 g of catalyst (K_2SO_4, $CuSO_4 \times 5H_2O$, Se), 10 mL of 30% hydrogen peroxide and 20 mL of 98% sulfuric acid. The heating protocol involved a progressive temperature: 45 min at 190°C, 45 min at 290°C until carbonization, and 3 h at 420°C until a lipid liquid appears. After mineralization, 50 mL of distilled water and 80 mL of 40% sodium hydroxide were added for distillation, followed by collection in 50 mL of 4% boric acid and titration with 0.2 N sulfuric acid. A blank sample was treated in the same way for each series. The total nitrogen content (N, %) was calculated using Equation (1):

$$N\ (\%) = \frac{(V - V_0) \times 14 \times N_A \times 100}{E \times 1{,}000} \quad (1)$$

where: V is volume of H_2SO_4 used for the sample titration in mL, V_0 is volume of H_2SO_4 used for the blank titration in mL, N_A is acid solution normality, and E is sample weight in g.

Protein content was obtained by multiplying N×6.25 [Nascimento *et al*., 2014; Zhou *et al*., 2023] and expressed in g/100 g of dry matter (DM) of sprouted seeds.

Lipid content determination

A Soxhlet extractor (Gerhardt, Konigswinter, Germany) with *n*-hexane as a solvent was used for lipid determination according to the AOAC International method 920.39 [AOAC, 1990]. The procedure consisted of placing 40 g of sprouted quinoa seed powder in a cellulose cartridge. The cartridge was then placed in a ground flask containing 150 mL of *n*-hexane. The flask was fitted with a heating mantle and heated for 6 h. The solvent was then evaporated by distillation using a rotary evaporator (Hahn Vapor, Gimpo, South Korea). Finally, the flask containing the resulting lipids was weighed. The lipid content of sprouted seeds was expressed in g/100 g DM.

Crude fiber determination

The crude fiber was determined according to the AOAC International method 988.15 [AOAC, 1990] using a Fibertech system (VELP Scientifica, Usmate Velate, Italy). The sprouted quinoa seed powder was decarbonated and degreased with 100 mL of 1.25 N sulfuric acid and 100 mL of 1.25 N sodium hydroxide solutions. The residue obtained was then separated by filtration through a glass filter. The crucibles containing the residue were then washed and dried in an oven at 103°C for 12 h to obtain the dry weight (Dw) and then incinerated in a muffle furnace at 550°C for 5 h to obtain the ash weight (Aw). The crude fiber content (CF) was calculated according to the Equation (2) and expressed in g/100 g DM of sprouted seeds:

$$CF = \frac{(T + Dw) - (T + Aw)}{Sw} \times 100 \quad (2)$$

where: T is tare weight and Sw is sample weight.

Ash content determination

The ash content was determined by the AOAC International method 942.05 [AOAC, 2005]. The powders weighing 1 g were incinerated in a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at 650°C for 8 h. The ashed samples were weighed, and ash content of sprouted seeds was expressed in g/100 g DM.

Carbohydrate content estimation

Carbohydrate content of sprouted quinoa seeds was estimated by difference according to Equation (3) [Abedin *et al*., 2022]:

$$\text{Carbohydrates} = 100 - \text{contents of (moisture + protein + + lipid + ash + crude fiber)} \quad (3)$$

The results were expressed in g/100 g DM of sprouted seeds.

Energy value estimation

The energy value of the sprouted quinoa seed powders was calculated using Equation (4) [FAO, 2003]:

$$\text{Energy value} = (\text{carbohydrate content} \times 4) + (\text{protein content} \times 4) + (\text{lipid content} \times 9) \quad (4)$$

Results were expressed in kcal/100 g DM.

Mineral composition analysis

The method described by Granado-Rodríguez *et al*. [2021b], with some modifications, was used to determine the content of K, Mg, Ca, Fe, Zn, Cu, and Mn in sprouted quinoa seeds. Powders weighing 2 g were placed in a muffle furnace at a temperature of 650°C for 4 h. The ash formed was dissolved with 3 mL of concentrated hydrochloric acid (37%) and heated in a boiling water bath until the ash was completely dissolved. The volume was made up of 100 mL with pure water, and the solution was analyzed using an atomic absorption spectrophotometer (PerkinElmer, Waltham, MA, USA). The results were expressed in g/kg DM of sprouted seeds.

Determination of total phenolic content and total flavonoid content

The powders were extracted according to the method proposed by Ollivier *et al*. [2004]. The sample was weighed (500 mg) and added to 3 mL of a mixture of methanol and distilled water (80:20, *v/v*) in Eppendorf tubes. The tubes were vortexed at 1,500 rpm for 15 min and then centrifuged at 1,130×*g* for 15 min. The supernatant was collected into a 10 mL flask, and the extraction was repeated three times. Finally, the samples were spiked with methanol and stored in a freezer at −20°C until analysis.

The total phenolic content was determined with a Folin-Ciocalteu reagent according the method described by Joy Ujiroghene *et al*. [2019], with slight modifications. The extract (2 mL) was mixed with 5 mL of 10% Na_2CO_3, 1 mL of the Folin-Ciocalteu reagent, and 5 mL of distilled water. This mixture was incubated in the dark for 30 min. Absorbance readings were then taken using a spectrophotometer (PG Instruments Ltd, Lutterworth, United Kingdom) at a wavelength of 750 nm. The results were expressed as mg gallic acid equivalent (GAE) *per* 100 g DM of sprouted seeds.

The total flavonoid content was quantified using the aluminum chloride colorimetric method described by Suárez-Estrella *et al.* [2020], with minor modifications. Volume of 1 mL of 2% $AlCl_3$ was added to 1 mL of the extract. After shaking, the mixture was incubated for 10 min. Its absorbance was then

read with a spectrophotometer (PG Instruments Ltd) at a wavelength of 430 nm. The results were expressed as mg quercetin equivalent (QE) *per* 100 g DM of sprouted seeds.

Determination of saponin content

The method used to extract saponins from sprouted quinoa seed powders was the one described by Rafik *et al*. [2021], which involved a Soxhlet extraction with *n*-hexane, repeated three times, to delipidate the powder. Next, 5 g of the delipidated powder was mixed with 50 mL of ethanol and stirred for 30 min. The mixture was then filtered, and the filtrate collected was made up to 50 mL with ethanol. Then, 2 mL of a reagent (mixture of glacial acetic acid and concentrated sulfuric acid (1:1, *v/v*), was added to 250 µL of the extract, and the mixture was vortexed and incubated at 60°C for 30 min in a water bath (Bunzen, Madrid, Spain) [Torrez Irigoyen & Giner, 2018]. After incubation, the mixture was cooled in an ice bath. The absorbance was measured using a spectrophotometer (PG Instruments Ltd) at 527 nm. Saponin (CAS No. 8047-15-2), purchased in Solvachim (Casablanca, Morocco), was used as a reference. The results were expressed as g saponin standard equivalent *per* 100 g DM of sprouted seeds.

Tocopherol analysis

To determine tocopherols, 200 mg of oil, extracted from quinoa powder by solid-liquid extraction (Soxhlet) and previously filtered with a 0.45 µm filter, was dissolved in 1 mL of methanol. The mixture was then vortexed at 1,500 rpm for 2 min. Finally, the sample was injected into a column connected to a high-performance liquid chromatography (HPLC) system (Agilent Technologies, Waldbronn, Germany) with fluorescence detector (FLD) for tocopherol analysis. The chromatographic separation was performed on a Poroshell 120 EC-C18 column (4.6×150 mm, 4 µm; Agilent Technologies) in an isocratic elution mode. The mobile phase was a mixture of acetonitrile and methanol (50:50, *v/v*) (both solvents of HPLC grade), with a flow rate of 1 mL/min and an injection volume of 20 µL. Detection was performed at 290 nm for excitation and 330 nm for emission. A calibration curve was plotted using α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol standards. The results were expressed as µg/g of oil.

Statistical analysis

Three replicates were performed for each treatment (germination time) and three samples (one from each replicate) were analyzed by each method. Means and standard deviations were calculated. One-way analysis of variance (ANOVA) was used to determine the effect of germination time (factor) on quinoa composition using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). The post hoc Tukey's test was used to compare means and determine significant differences between samples. Differences were considered as significant at $p \leq 0.05$. The graph was generated using Excel software for Microsoft 365 MSO, version 2405 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Effect of germination time on water activity

Water activity of powders obtained from ungerminated and germinated quinoa seeds varied between 0.22 and 0.27, with insignificant ($p>0.05$) differences between powders from germinated and control seeds as well as between powder from seeds sprouted for different times (**Figure 1**). This slight change in a_w indicated that the powders did not present a risk of proliferation of microorganisms [Pellegrini *et al*., 2018] and can be used as a safe ingredient. Our results were consistent with those reported by Ramos-Pacheco *et al*. [2024], who found values of $a_w<0.3$ in all germinated and ungerminated quinoa seed powders.

Effect of germination time on macronutrient content

Protein content

The protein content of sprouted quinoa seeds varied between 15.73 and 23.64 g/100 g DM (**Table 1**). Compared to the control (17.55 g/100 g DM), a significant ($p \leq 0.05$) decrease was observed in the seeds sprouted for 24 h. However, an important increase

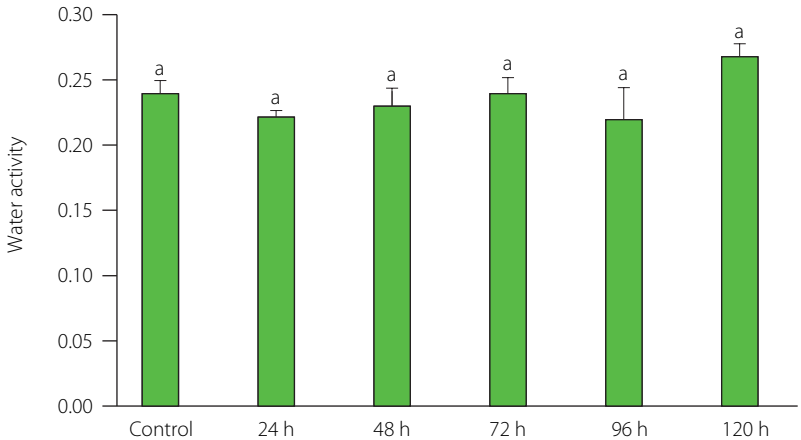

Figure 1. Water activity of powders from quinoa seeds sprouted at different times. Results are shown as means and standard deviation. The same letter above bars indicates no significant differences between means according to Tukey's test ($p>0.05$).

Table 1. Macronutrient content and energy value of quinoa seeds sprouted for different times.

Germination time	Protein (g/100 g DM)	Lipids (g/100 g DM)	Carbohydrates (g/100 g DM)	Crude fiber (g/100 g DM)	Energy value (kcal/100 g DM)
0 h (Control)	17.55±0.07^{c}	3.87±1.09^{ab}	67.23±0.14^{b}	4.49±0.01^{c}	373.0±12.1^{a}
24 h	15.73±0.43^{d}	3.38±0.32^{b}	68.46±0.62^{a}	4.88±0.39^{bc}	366.6±2.3^{ab}
48 h	19.61±0.50^{b}	4.54±0.38^{ab}	62.65±0.83^{c}	4.99±0.50^{ab}	368.1±1.9^{a}
72 h	19.89±0.72^{b}	5.05±0.51^{a}	57.80±0.04^{d}	4.98±0.49^{ab}	352.7±0.3^{b}
96 h	23.64±0.18^{a}	5.02±0.39^{a}	57.12±0.39^{d}	5.38±0.89^{a}	366.1±1.5^{ab}
120 h	20.39±0.33^{b}	3.65±1.21^{ab}	62.74±0.61^{c}	4.98±0.49^{ab}	363.9±13.5^{ab}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter.

(p≤0.05) was observed in the seeds sprouted for 48–120 h, with the highest protein content determined in the seeds germinated for 96 h (increase of 6.09 g/100 g DM compared to the control). This remarkable augmentation could be explained by the synthesis of new amino acids and the loss of carbohydrates through seed respiration during germination [Bertazzo *et al*., 2011]. In addition, Pilco-Quesada *et al*. [2020] explained the augmentation in protein content during germination by biological synthesis and the mobilization of nutrient reserves. Bewley *et al.* [2013] reported that during the first three days of germination, amino acid content increased, leading to an increase in protein content. In the same context, Jimenez *et al*. [2019] estimated that 2 to 3 days after imbibition, proteolytic enzymes hydrolyzed proteins into peptides and amino acids, increasing the bioavailability of nutrients. Our study results are close to those of Aguilar *et al*. [2019], who observed that *C. quinoa* seeds (Negra Collana variety) displayed a maximum increase of 8% in protein content after 48 h of germination. Similarly, Thakur *et al*. [2021] noted a considerable improvement in protein content from 14.94% (control) to 17.88% after 72 h of germination of quinoa seeds. Pilco-Quesada *et al*. [2020] also observed an enhancement in protein from 9.6 to 26.0% after 72 h of germination. Guardianelli *et al*. [2022] also confirmed a positive correlation between germination time and protein content.

Our findings, in accordance with literature data, suggest that germination is an effective strategy for enhancing the protein content of quinoa, making it a promising approach for improving the nutritional value of quinoa-based food products.

Lipid content

The lipid content of sprouted seeds ranged from 3.38 to 5.05 g/100 g DM, with insignificant (p>0.05) variation observed between germinated and control seeds (**Table 1**). However, the continuous significant (p≤0.05) increase in lipid content was found from 24 to 72 h of processing. This minor uptick in lipid content could be attributed to the abundance of fatty acids released from triglycerides or phospholipids by lipolytic enzymes during germination [Obizoba & Atii, 1991]. On the other hand, during germination, seeds use fatty acids to produce sucrose *via* gluconeogenesis, which is an energy source necessary to complete growth [Lan *et al*., 2023; Nelson *et al*. 2013].

Our study results do not agree with those reported by Obizoba & Atii [1991], who showed an improvement in lipid content in seeds germinated for 96 h, with a value of 5.7% compared to 3.1% for ungerminated sorghum seeds. Darwish *et al*. [2021] also found a 0.8% increase for seeds germinated for 72 h compared to ungerminated quinoa seeds.

Crude fiber content

The crude fiber content of sprouted seeds varied between 4.88 and 5.38 g/100 g DM (Table 1). Compared to the control (4.49 g/100 g DM), a considerable increase (p≤0.05) was observed in the seeds sprouted for 48–120 h. However, it should be noted that the crude fiber content did not differ significantly (p>0.05) between seeds germinated for 48, 72, 96 and 120 h. This significant increase compared to the control could be explained by the abundance of fiber in the early stages of germination [Guardianelli *et al*., 2022]. Pilco-Quesada *et al.* [2020] justified this change in fiber content by the loss of other nutritional components. This augmentation is consistent with the findings of Darwish *et al*. [2021], who highlighted an increase in the crude fiber of 1.77 g/100 g in *C. quinoa* seeds germinated for 72 h compared to the ungerminated seeds. Furthermore, Thakur *et al*. [2021] found that crude fiber content increased significantly with germination time.

Carbohydrate content

The carbohydrate content of sprouted seeds varied between 57.12 and 68.46 g/100 g DM (**Table 1**). Compared to the control (67.23 g/100 g DM), a remarkable increase (p≤0.05) was observed in the seeds sprouted for 24 h. Nevertheless, a noticeably lower (p≤0.05) carbohydrate content was noted in the seeds sprouted for 48 h to 120 h. The maximum reduction compared to the control was 9.43–10.11 g/100 g DM in the seeds germinated for 72–96 h. This substantial decrease could be attributed to the decomposition of complex carbohydrates into simple carbohydrates by enzymes activated during germination [Nelson *et al*., 2013] to provide the energy required for new plants [Ferreira

Table 2. Ash content and mineral composition of quinoa seeds sprouted for different times.

Germination time	Ash (g/100 g DM)	Iron (g/kg DM)	Manganese (g/kg DM)	Calcium (g/kg DM)	Copper (g/kg DM)	Zinc (g/kg DM)	Potassium (g/kg DM)
0 h (Control)	3.87±0.02^{d}	56.8±0.8^{a}	21.24±0.11^{a}	1,244±140^{ab}	2.51±0.13^{a}	23.04±0.93^{a}	2,526±86^{a}
24 h	5.73±0.12^{a}	59.8±15.2^{a}	19.86±2.38^{a}	1,059±196^{abc}	2.72±0.49^{a}	21.74±3.32^{ab}	2,144±6^{b}
48 h	4.02±0.08^{cd}	47.8±5.6^{a}	14.11±0.16^{bc}	762±22^{c}	2.25±0.40^{a}	18.63±0.63^{ab}	1,670±5^{c}
72 h	4.80±0.42^{b}	67.3±1.9^{a}	16.32±0.86^{ab}	1,002±262^{bc}	2.76±0.58^{a}	22.01±0.48^{a}	1,512±167^{c}
96 h	4.32±0.21^{c}	57.6±19.4^{a}	12.47±2.11^{bc}	1,421±136^{a}	2.07±0.80^{a}	16.33±5.12^{b}	438±53^{d}
120 h	3.68±0.07^{d}	56.9±4.2^{a}	9.41±1.74^{c}	944±185^{bc}	2.06±0.11^{a}	18.04±1.41^{ab}	559±123^{d}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter.

et al., 2009]. Guardianelli *et al*. [2022] explained this reduction by the structure of starch, which facilitates its hydrolysis by endogenous amylolytic enzymes. Likewise, Elkhalifa & Bernhardt [2010] signaled that α-amylase was synthesized at low concentrations during the first hours of germination. As germination progressed, the concentration of α-amylase increased, breaking down starch into glucose.

Our results are in line with those reported by Pilco-Quesada *et al*. [2020], who observed a reduction of 8.1% in carbohydrates in the seeds germinated for 72 h compared with the control. In addition, Ramos-Pacheco *et al*. [2024] observed a non-significant decrease in carbohydrate content in seeds sprouted for 0 to 72 h. Guardianelli *et al*. [2022] reported that the content of starch, the main component of carbohydrates, decreased with germination time (≥24 h), especially in red *C. quinoa*.

To illustrate, the lower carbohydrate content observed in germinated quinoa seeds could be beneficial to health by helping to prevent diseases induced by high carbohydrate consumption, such as diabetes and cardiovascular disease.

Energy value

The energy value varied between 352.7 and 373.0 kcal/100 g DM (**Table 1**). The sprouted seeds tended to have lower energy value than the control, but a significant (p≤0.05) difference was found only between the seeds germinated for 72 h and ungerminated seeds with a decrease of 20.4 kcal/100 g DM. This decrease could be attributed to the low carbohydrate content. Our study results are consistent with those of Thakur *et al*. [2021], who found a 16.4 kcal/100 g reduction in quinoa seeds germinated for 72 h compared to the control.

Effect of germination time on ash content and mineral composition

The ash content fluctuated between 3.68 and 5.73 g/100 g DM with 3.87 g/100 g DM of the control seeds (**Table 2**). However, ash content of the ungerminated seeds did not differ significantly (p>0.05) from that of the seeds sprouted for 48 and 120 h. A noticeably higher (p≤0.05) ash content was determined in the seeds sprouted for 24, 72 and 96 h, with the highest increase compared to the control of 1.86 g/100 g DM noted in the seeds germinated for 24 h. This important enhancement was previously pointed out by Rao & Deosthale [1983] who reported that the high ash content in seeds germinated for 48 to 96 h may result from the treatment method used (reduction of humidity during germination), which concentrates the minerals. In contrast, the decrease in ash content may be due to the soaking and/or the transfer of minerals to the radicles during germination, where they act as co-enzymes in carbohydrate and protein catalysis [Bewley *et al*., 2013]. These radicles are then removed during the drying process.

The mineral composition of germinated quinoa seed varied with germination time. Manganese content varied between 9.41 and 21.24 mg/kg DM. Compared to the control, a marked decline (p≤0.05) was observed in the seeds germinated for 48, 96 and 120 h. The highest potassium content was found in ungerminated seeds (2,526 mg/kg DM) and it decreased significantly (p≤0.05) in the samples for subsequent germination times to a value of 438–559 mg/kg DM determined in the seeds germinated for 96–120 h. Calcium content ranged from 762 to 1,421 mg/kg DM. Relative to the control, a notable reduction of 482 mg/kg DM was observed in the seeds germinated for 48 h (p≤0.05). Regarding zinc, a significant difference (p≤0.05) was only found between the control (23.04 mg/kg DM) and the seeds sprouted for 96 h (16.33 mg/kg DM). No significant differences (p>0.05) were found between the seeds from different germination times with respect to iron and copper contents.

The important decline in the content of certain minerals could be explained by the germination method adopted and the use of distilled water, which allows the minerals to leach out [Bewley *et al*., 2013]. Furthermore, Kajla *et al*. [2017] explained that changes in mineral content during germination are due to the hydrolysis of organic complexes, releasing minerals that act as enzymatic co-factors and support macromolecules catalysis to provide the energy needed for germination. Our study results are close to those of Darwish

Table 3. Total phenolic content, total flavonoid content, and saponin content of quinoa seeds sprouted for different times.

Germination time	Total phenolic content (mg GAE/100 g DM)	Total flavonoid content (mg QE/100 g DM)	Saponin content (g/100 g DM)
0 h (Control)	83.5±4.6^{d}	25.6±0.8^{d}	1.06±0.01^{a}
24 h	92.4±2.6^{cd}	36.6±1.6^{c}	0.99±0.02^{ab}
48 h	97.1±4.1^{c}	36.9±1.5^{c}	0.48±0.04^{b}
72 h	119.6±1.5^{b}	73.5±7.9^{b}	0.48±0.02^{b}
96 h	133.8±7.1^{a}	99.1±1.6^{a}	1.42±0.01^{a}
120 h	94.8±6.7^{cd}	42.3±2.4^{c}	1.22±0.02^{a}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05). DM, dry matter; GAE, gallic acid equivalent; QE, quercetin equivalent.

et al. [2021], who observed a 39.43% enhancement in calcium content, in *C. quinoa* seeds. In addition, Bhinder *et al*. [2021] reported a decrease in manganese, zinc, and potassium with increasing germination time in quinoa seeds.

Effect of germination time on bioactive compound content

Total phenolic and total flavonoid contents

The total phenolic content of ungerminated and germinated quinoa seeds fluctuated between 83.5 and 133.8 mg GAE/100 g DM (**Table 3**). The highest value was found in the seeds germinated for 96 h, which was 50.3 mg GAE/100 g DM higher than that determined in the control. A marked augmentation (p≤0.05) was also observed in the seeds germinated for 48 and 72 h relative to the control. Regarding the total flavonoid content, the control seeds had the lowest content (25.6 mg QE/100 g DM). This value gradually increased with the extension of germination time up to 96 h and reached 99.1 mg QE/100 g DM. The total flavonoid content of the seeds germinated for 120 h was higher (p≤0.05) compared to the control, but did not differ significantly (p>0.05) from those of the seeds germinated for 24 and 48 h. The remarkable increases in total phenolic and total flavonoid contents could be attributed to the release of phenolic compounds from the cell walls [Alvarez-Jubete *et al*., 2010]. This increase is one of many metabolic changes that occur during seed germination, mainly due to the increased activity of the hydrolytic action of esterases and glucosidases on non-extractable phenolic compounds [Kim *et al*., 2016]. Kim *et al*. [2016] also noted that soaking seeds in water could activate enzymes, such as phenylalanine ammonia-lyase, which catalyzes the main phenylpropanoid reactions and, therefore, the formation of secondary metabolites.

Our results concur with those found by Thakur *et al*. [2021], who observed a significant enhancement of 34.4% in the total phenolic content in quinoa seeds germinated for 72 h. A similar study by Alvarez-Jubete *et al*. [2010] determined an increase in the total phenolic content by 147.2% in quinoa seeds after 82 h of germination. In turn, Ramos-Pacheco *et al*. [2024] observed that contents of total phenolics and total flavonoids increased with increased germination time, especially in white *C. quinoa*. Therefore, germination can be an efficient means to improve the antioxidant properties of *C. quinoa* seeds.

It is important to note that the initial antioxidant composition of the seeds and their germination response can be influenced by the year of cultivation, the variety, genetic factors, and their interaction [Aguilar *et al*., 2019; Granado-Rodríguez *et al*., 2021a]. In addition, the region where the quinoa is grown and harvested, climatic conditions, the quality of the soil and the water used for irrigation, as well as farming practices determine the content of these compounds in the plant.

Saponin content

The saponin content of the ungerminated seeds was 1.06 g/100 g DM, and that of the germinated seeds varied between 0.48 and 1.42 g/100 g DM (**Table 3**). A significant (p≤0.05) decrease was observed compared to the control in the seeds germinated for 48 and 72 h.

Saponins are mainly found in the pericarp of *C. quinoa* seeds [Suárez-Estrella *et al*., 2020; Yadav *et al*., 2023], which contains about 86% of these secondary metabolites [Ruiz *et al*., 2017]. Considered as anti-nutrients [Granado-Rodríguez, *et al*., 2021b], saponins form insoluble complexes with certain minerals and vitamins, thereby reducing their intestinal absorption [Ruales & Nair, 1993; Zhou *et al*., 2023].

Saponin content in quinoa seeds depends on the variety [Granado-Rodríguez *et al*. 2021a; Mora-Ocación *et al*., 2022] and cultivation conditions such as rainfall, which can reduce saponin content [Lim *et al*., 2020]. The Titicaca, used in our study, is a bitter variety, with a saponin content above the 0.12% threshold set by the Codex Alimentarius [2019] as an acceptable limit to avoid bitterness. Therefore, the seeds of this variety require prior treatment before consumption, such as mechanical processing (polishing or sieving) and/or washing with water [Zhou *et al*., 2023]. However, mechanical treatments can negatively affect the nutritional profile of quinoa [Casalvara *et al*., 2024; Gómez-Caravaca *et al*., 2014], which facilitates treatment by washing or the use of alternative processing, such as germination, to remove saponins [Lan et al., 2024].

Our study results support those of Mhada *et al.* [2020], who found a saponin content of 2.03% in raw quinoa of the Titicaca variety. This level was reduced to 0.07% after polishing and processing into semolina. Furthermore, Nickel *et al*. [2016] reported a saponin content of 3.33% in raw quinoa, which became 2.75% after soaking for 15 min under a stream of running water. Similarly, Chaudhary *et al*. [2024] showed that soaking quinoa seeds in water for 24 and 48 h reduced the saponin content by 0.4% and 0.7%, respectively, compared to 1.9% saponin in raw quinoa. These reductions could be explained by the solubility of saponins in water [Bhinder *et al*., 2021]. Regarding germination, Suárez-Estrella *et al*. [2021] observed a reduction in saponin content from 0.40% to 0.05% in seeds germinated for 72 h. Similarly,

Table 4. Tocopherol content of oil from quinoa seeds sprouted for different times.

Germination time	α-Tocopherol (µg/g oil)	β-Tocopherol (µg/g oil)	γ-Tocopherol (µg/g oil)	δ-Tocopherol (µg/g oil)
0 h (Control)	4.82±0.80^{b}	2.93±0.18^{b}	1.43±0.61^{a}	1.38±0.19^{d}
24 h	6.45±0.68^{a}	2.25±0.15^{cb}	1.16±0.03^{ab}	2.83±0.18^{b}
48 h	3.24±0.04^{d}	1.14±0.18^{d}	0.97±0.02^{ab}	2.52±0.14^{c}
72 h	4.65±0.01^{bc}	4.14±0.01^{a}	0.79±0.01^{b}	1.35±0.01^{d}
96 h	3.25±0.11^{cd}	1.37±0.01^{d}	1.05±0.01^{ab}	2.97±0.04^{b}
120 h	3.54±0.03^{bcd}	1.90±0.01^{cd}	1.20±0.01^{ab}	4.05±0.03^{a}

Results are shown as mean ± standard deviation (n=3). According to Tukey's test, means with different letters in column differ significantly (p≤0.05).

Bhinder *et al*. [2021] reported that germination reduced saponin content in white and black quinoa after 96 h. Moreover, Darwish *et al*. [2021] found a 60% reduction in saponins in quinoa seeds germinated for 72 h.

As a result, it can be concluded that germination, particularly for 48 and 72 h, was an effective technique for partially reducing the saponin content of quinoa seeds, with a reduction of 0.58 g/100 g DM, *i.e.*, a reduction of 54.72%. However, this technique is still not sufficient to completely remove the bitterness. According to Koziol [1991], bitterness is perceived by humans at saponin levels above 0.11%, which necessitates the use of additional techniques to reduce this bitterness, such as prolonged soaking, high-pressure washing, mechanical polishing and boiling of the seeds, to obtain a less bitter powder suitable for various food preparations.

Tocopherol content

The highest α-tocopherol content was determined in oil from the seeds sprouted for 24 h (6.45 µg/g oil) (**Table 4**). It was significantly (p≤0.05) increased by 1.63 µg/g oil compared to the control. Germination beyond 24 h notably (p≤0.05) reduced α-tocopherol content to 3.24–4.65 µg/g oil. In turn, the β-tocopherol content fluctuated between 1.14 and 4.14 µg/g oil, with noticeable differences (p≤0.05) observed in the seeds germinated for 48, 72, 96, and 120 h relative to the control. However, an increase (by 1.21 µg/g oil) was found only in the oil from seeds sprouted for 72 h. Samples analyzed at 48, 96 and 120 h had lower β-tocopherol content than the control. γ-Tocopherol content varied between 0.79 and 1.43 µg/g oil. Oil from the ungerminated seeds had significantly (p≤0.05) higher γ-tocopherol content than the oil from seeds sprouted for 72 h. Differences between the remaining samples were insignificant (p>0.05). Concerning δ-tocopherol, its content ranged from 1.35 to 4.05 µg/g oil. Compared to the control, an important increase was detected in the seeds germinated for 24, 48, 96, and 120 h (p≤0.05). with the highest augmentation of 2.67 µg/g oil found in the seeds germinated for 120 h.

α-Tocopherol, which is the most active form of vitamin E [Žilić *et al*., 2014], was the predominant tocopherol throughout the germination period in our study. This finding is consistent with those reported by Pachari Vera *et al*. [2019], who showed that yellow quinoa varieties had higher α-tocopherol levels compared to other forms. In addition, Tarasevičienė *et al*. [2019] reported an increase in α-tocopherol and a decrease in γ-tocopherol contents during germination in edible seeds such as wheat, radish, sunflower, lentil, and amaranth germinated for 24, 72 and 120 h. In turn, Granda *et al*. [2018] demonstrated that the quinoa varieties Amarilla de Marangani and Titicaca had high levels of α-tocopherol and low levels of γ-tocopherol, while δ-tocopherol content of 3.72 mg/kg and 4.59 mg/kg was found in the quinoa varieties Blanca Dulce and Black Quinoa, respectively. However, these results do not agree with those of Žilić *et al*. [2014], who reported γ-tocopherol as the most abundant tocopherol in quinoa seeds, followed by α-tocopherol, with small amounts of β- and δ-tocopherols, as also reported by Carciochi *et al*. [2016]. These dissimilarities could have been due to varietal differences [Tang *et al*., 2016], seed color [Granda *et al*., 2018], germination and extraction methods, as well as climatic and edaphic growing conditions.

Tocopherols play an important role during germination by scavenging free radicals and preventing lipid peroxidation to protect young seedlings from oxidative stress [Yang *et al*., 2018]. In addition, they have been suggested to elicit many health benefits. In particular, γ-tocopherol inhibits inflammation and the proliferation of prostate and colon cancer [Balakrishnan & Schneider, 2023]. Furthermore, Devaraj *et al*. [2008] highlighted the combined ameliorative effect of α- and γ-tocopherol on oxidative stress in patients with metabolic syndrome. These two tocopherols can readily quench free radicals [Balakrishnan & Schneider, 2023]. Likewise, tocopherols have been linked to the prevention of chronic diseases, disorders, and certain types of cancer [Ryynänen *et al*., 2004]. They are also known to regulate gene expression, signal transduction, and cellular functions [Shahidi & De Camargo, 2016]. In addition, Tarasevičienė *et al*. [2019] reported their role in reducing the production of thromboxane, a cytokine with vasoconstrictive effects, which could affect cardiovascular health. Finally, Rizvi's review [2014] supported and described the various benefits of tocopherols, such

as antioxidant protection, prevention of atherosclerosis, and reduction of prostaglandin levels.

CONCLUSIONS

Germination of quinoa seeds resulted in a remarkable change in the compositions of macronutrients, minerals, and bioactive compounds. Significant improvements in the contents of protein, crude fiber, total phenolics, and total flavonoids were observed in the seeds germinated for 96 h, indicating that prolonged germination was effective. A substantial decrease in carbohydrates was observed from 72–96 h of germination. The seeds germinated for 24 h had high levels of α-tocopherol and ash, while saponin content was reduced after 48 and 72 h of germination. In addition, the energy value of the seeds was reduced in those germinated for 72 h.

In this respect, the powder obtained from germinated quinoa seeds, especially for 96 h, has the potential to be used as a valuable ingredient in various food products *e.g.* in the production of savory snacks, offering new and innovative opportunities. In addition, it will be essential to raise consumer awareness of the benefits of powders obtained from germinated quinoa seeds. However, the development of additional processes to further reduce saponin levels is necessary to ensure their safe use and positive impact on public health.

These results are very promising for selecting quinoa varieties with the best nutritional properties to be grown in the semi-arid climate of eastern Morocco and for new cultivation environments.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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