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RESEARCH ARTICLE

Effect of germination on ascorbic acid, sodium, tannin and fatty acid profile of quinoa seeds cultivated in eastern Morocco

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Abstract

Seed germination is a simple and effective method for producing germinated seeds with high nutritional quality. These seeds can be incorporated into various foods, providing a healthy alternative to meet the growing demand for nutritious products. This study investigates the effect of germination on ascorbic acid, sodium, condensed tannins and fatty acids in quinoa seeds grown in eastern Morocco. Seeds were germinated under controlled conditions (25 °C, 70 % relative humidity, 16 hr light/8 hr dark) for 24, 48, 72 and 96 hr, then dried, crushed and analysed. Ungerminated seeds served as the control (0 hr). Analyses were performed in triplicate and data were statistically evaluated using one-way ANOVA followed by Tukey's test. Compared to the control, significant variations occurred during germination. Ascorbic acid was enhanced at 72 hr and 96 hr (+5.03 and +4.57 mg/100 g DW, $p < 0.05$). Monounsaturated fatty acids also showed significant improvement at 24 hr and 96 hr ($p < 0.01$), while sodium and condensed tannins showed significant reduction at 96 hr ($p < 0.01$). At 72 hr, a marked decline in saturated fatty acids ($p < 0.05$) was accompanied by a significant improvement of polyunsaturated fatty acids ($p < 0.01$). These findings demonstrate that prolonged germination (> 72 hr) improves quinoa powder's nutritional quality by enhancing ascorbic acid and unsaturated fatty acids while reducing condensed tannins, sodium and saturated fats. Therefore, germinated quinoa powder (> 72 hr) produced in eastern Morocco represents a novel functional ingredient for nutritionally fortified food products, supporting germination as an effective natural method to enhance quinoa's nutritional value and reduce antinutritional compounds.

Keywords: bioactive compounds; *Chenopodium quinoa;* germinated seeds; health benefits; nutrients

Introduction

Although quinoa was introduced to Morocco in 1999 and is increasingly cultivated in the Marrakech-Safi, Casablanca-Settat and eastern regions, particularly in arid and semi-arid areas (1), limited information exists on the nutritional and bioactive composition of quinoa seeds produced in Eastern Morocco, especially under germination.

Globally, quinoa is recognised as a highly nutritious pseudocereal, rich in proteins, unsaturated fatty acids, fibre, vitamins, essential amino acids, minerals and antioxidants (2–4). Its adaptability to adverse conditions allows cultivation in arid, semi-arid and marginal soils, highlighting its relevance for climate-resilient agriculture (5–7).

These characteristics led the United Nations to declare 2013 the International Year of *Chenopodium quinoa* Willd., highlighting its role in food sovereignty and nutrition worldwide (8).

However, quinoa seeds also contain significant amounts of anti-nutritional compounds, such as saponins, phytic and oxalic acids, tannins and trypsin inhibitors, which can limit nutrient assimilation (9, 10).

Several techniques are considered effective for improving the nutritional quality of *C. quinoa* seeds and reducing their antinutrient content. Seed germination is a simple and practical method that activates enzymes, enhances nutrient bioavailability and reduces antinutritional compounds (11, 12).

For instance, previous results showed that germination enhances seed nutrient content while reducing antinutrient content (13). Germination increased the seeds' macromolecule, amino acid and antioxidant content (14). A diet enriched with 10 % germinated quinoa seeds had a beneficial anti-anemic effect on rats (15).

However, there is a clear research gap regarding germination and its effect on underexplored compounds such as ascorbic acid, tannins, sodium and fatty acids in Moroccan quinoa. Quinoa grown in Eastern Morocco is of particular interest, as this region combines arid and semi-arid climatic conditions with the growing demand for functional foods. To date, no study has examined how germination affects these compounds in seeds produced in this region.

Ascorbic acid is an essential antioxidant vitamin supporting the immune system, acting as a cofactor for enzymes and protecting against oxidative stress (16). Tannins are polyphenols with both antioxidant and antinutritional properties, which can reduce nutrient bioavailability (17). Sodium is an essential mineral for fluid balance and cardiovascular health (18), while the balance of fatty acids influences lipid quality and metabolic health (19).

A previous study highlighted the effect of germination on macronutrients (proteins, carbohydrates, fibre), phenolic compounds, flavonoids, tocopherols and saponins in quinoa seeds grown in Eastern Morocco (20). The present study complements these findings by focusing on other underexplored compounds, such as ascorbic acid, sodium, tannins and fatty acids, providing novel insights into the nutritional enhancement of quinoa seeds grown in this region.

A 96-hr germination period was chosen based on previous researchers, who reported that prolonged germination enhances antioxidants and reduces antinutritional compounds such as saponins, phytic acid and tannins in quinoa seeds (21).

The objective of the current study is to evaluate the effect of germination on ascorbic acid, sodium, tannins and fatty acids in quinoa seeds grown in Eastern Morocco, highlighting their nutritional enhancement and potential use in functional foods.

This work is aligned with current recommendations for a healthy diet, which emphasise the consumption of nutrient-rich, low-antinutrient and functional foods, as highlighted by the World Health Organization (22).

Materials and Methods

Plant material

Selected *C. quinoa* cv. Titicaca seeds were used for this experiment. These seeds were grown and harvested in 2023 on a farm located in the eastern region of Morocco, in the province of Berkane (34°57'36.65'' N, 2°21'34.73'' W), at an altitude of 106 m. No chemical or organic fertilisers were used on this farm.

Sample preparation

For each germination period, including the control (0 hr of germination - ungerminated seeds), 120 g of quinoa seeds were soaked in 600 mL of distilled water for 24 hr. The seeds (except the control) were then placed in plastic boxes covered with wet filter paper to maintain moisture, three for each sprouting period and placed in a culture chamber (Memmert, Germany) under the following conditions: 25 °C, 70 % relative humidity, a photoperiod of 16 hr light and 8 hr dark, for periods of 24, 48, 72 and 96 hr.

After each germination period, germinated seeds and the control were dried at 40 °C, then crushed and sieved through a 500 µm sieve to collect a homogeneous powder. Powdered samples were frozen until analysis. The standard protocol was used with some adjustments, including longer soaking time (24 hr instead of 4 hr), extended germination time (up to 96 hr instead of 48 hr), lower drying temperature (40 °C instead of 55 °C) and the inclusion of a controlled photoperiod to optimise conditions for quinoa cultivated in Eastern Morocco (23).

Extraction procedures

Analyses focused on ascorbic acid content, sodium content, condensed tannins and fatty acid profile. The resulting data of the fatty acid profile were normalised to 100 % for comparison. A total of three analyses were conducted per treatment (n = 3).

Extraction of ascorbic acid

To determine ascorbic acid, 2 g of powder was diluted in 5 mL of solution A (15 g of metaphosphoric acid (7.5 % m/v) and 40 mL of glacial acetic acid (20 % v/v) were dissolved and made up to 200 mL with distilled water (24).

Extraction of sodium

Sodium was extracted from 2 g of quinoa powder. The samples were incinerated at 650 °C for 4 hr and the resulting ashes were dissolved in 3 mL of hydrochloric acid (25).

Extraction of condensed tannins

To extract condensed tannins, 1 mL of methanol and distilled water mixture (80:20, v/v) was added to 500 mg of the powder, then the mixture was vortexed for 15 min at 1500 rpm and centrifuged for 15 min at 1130 g. The supernatant obtained was transferred to a 10 mL flask. Three successive extractions were performed (26). Samples were spiked with methanol and frozen until analysis.

Extraction of fatty acid profile

Fatty acids content in oil was extracted (Soxhlet with hexane solvent) from quinoa powder and converted to fatty acid methyl esters (FAMEs) (27). The fatty acid composition of the esterified oils was then determined using gas chromatography-mass spectrometry (GC-MS), following the procedure outlined by (28). 1 µL sample prepared in hexane was injected into a GC-MS system (QP2010-MS, Shimadzu GC-2010, Kyoto, Japan) equipped with RTX-5 capillary column (5 % diphenyl, 95 % dimethylpolysiloxane with dimensions of 30 m × 0.25 mm × 0.25 µm). Helium was the carrier gas, at a linear flow of 1.4 mL/min and maintained at a stable pressure of 100 kPa. The oven temperature was set to start at 50 °C for 1 min, then increased at +10 °C/min to 250 °C and held for 1 min.

Analysis

Ascorbic acid determination

The titration was performed with a mixture of diphenylamine (0.05 % m/v) and sodium carbonate (0.042 % m/v) prepared in 100 mL of distilled water. Three blanks were prepared by replacing the 2 g of powder with 2 mL of solution A. Additionally, three controls were prepared using 2 mL of an ascorbic acid solution (1 mg/mL) as a standard reference. Ascorbic acid, expressed in mg/100 g dry weight (DW) was calculated using the following formula:

$$\text{Ascorbic acid} = (V - V_0) \times \left(\frac{2}{Vc}\right) \times 100 \qquad \text{(Eq. 1)}$$

Where: V is the volume (mL) used to titrate the sample, V_0 is the volume (mL) used to titrate the blank, 2 mg ascorbic acid equivalent to 1 mL ascorbic acid standard solution and Vc is the volume (mL) used to titrate the control.

Sodium determination

Sodium content was measured using a flame photometer (Sherwood Model 360, UK) and expressed in mg/100 g DW.

Calibration was performed using sodium chloride (NaCl) stock solution (1 g/L), from which standard solutions of 5, 10, 15, 20, 25, 30, 40 and 100 mg/L were prepared. Pure distilled water was used as the blank.

Condensed tannins determination

To determine condensed tannins, 1500 µL of 4 % vanillin (prepared in pure water (w/v)) was added to an extract of 50 µL. After vortexing, 750 µL of concentrated HCl was added and the mixture was incubated at 25 °C for 20 min (29).

Quantification was performed using catechin standards (0.1–0.6 mg/mL, stock 1 mg/mL). Pure distilled water served as the blank and a control was prepared without extract. Absorbance was read at 550 nm using a spectrophotometer (PG Instruments Ltd, UK) and results were expressed as milligrams of catechin equivalents per 100 g DW.

Fatty acid profile determination

Samples were analysed in split injection mode, with a split ratio between 50 : 1 and 80 : 1. The GC-MS operated in scan acquisition mode. Compound identification was carried out by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST 147) database. Data acquisition and processing were performed using LabSolutions software, version 2.5. The software calculated the area under each peak, corresponding to the relative abundance of each fatty acid methyl ester. Fatty acid composition was expressed as a percentage of total fatty acids.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) software (IBM Corp, USA) was used to analyse data. Normality was assessed using the Shapiro-Wilk test and variance homogeneity by Levene's test. As the assumptions were met, no data transformation was required. One-way analysis of variance (ANOVA) was used to assess the effect of germination time on the measured parameters. Tukey's test was used for multiple comparisons, with post hoc analysis to determine significant differences. The difference was significant at $p < 0.05$ and highly significant at $p < 0.01$. Results are presented as means and standard deviations.

Results and Discussion

Ascorbic acid

The ascorbic acid content of ungerminated and germinated quinoa seeds ranged from 4.85 ± 0.87 to 12.80 ± 1.31 mg/100 g DW (Fig. 1). In comparison with the control (7.77 ± 0.44 mg/100 g DW), an increase ($p < 0.05$) was noted in germinated seeds for 72 hr and 96 hr, with relative increases of 65 % and 59 % respectively (Error bars indicate standard deviation (n = 3). According to Tukey test, different letters above bars represent significant differences ($p < 0.05$), while the same letters indicate no significant difference).

This pronounced enhancement in ascorbic acid could be due to de novo synthesis during germination, as most ungerminated seeds contain little to no vitamin C (30). This process involves the reactivation of enzymes in the L-galactose pathway, particularly L-galactono-1,4-lactone dehydrogenase (GLDH), which catalyses the final step of ascorbic acid biosynthesis and increases to support seedling development.

A 65 % increase in ascorbic acid after 72 hr of germination makes germinated seeds a richer source of this antioxidant, enhancing their nutritional and functional value by protecting against oxidative stress, supporting immunity and modulating inflammation (31, 32).

These observations are consistent with early results, which reported that prolonged germination (> 96 hr) significantly improved ascorbic acid in five quinoa varieties (33). In turn, previous results showed a 5.68 mg/100 g enhancement after 72 hr of germination, which is slightly higher than 5.03 mg/100 g, possibly due to cultivar, germination conditions or methods (34). Collectively, these findings highlight quinoa powder as a natural dietary source of vitamin C, offering a practical option for daily supplementation.

Sodium content

The sodium content of germinated and ungerminated quinoa seeds varied from 24.14 ± 3.37 to 45.18 ± 4.98 mg/100 g DW (Fig. 2) (Error bars indicate standard deviation (n = 3). According to Tukey's test, different letters above bars represent significant differences ($p < 0.05$), while the same letters indicate no significant difference).

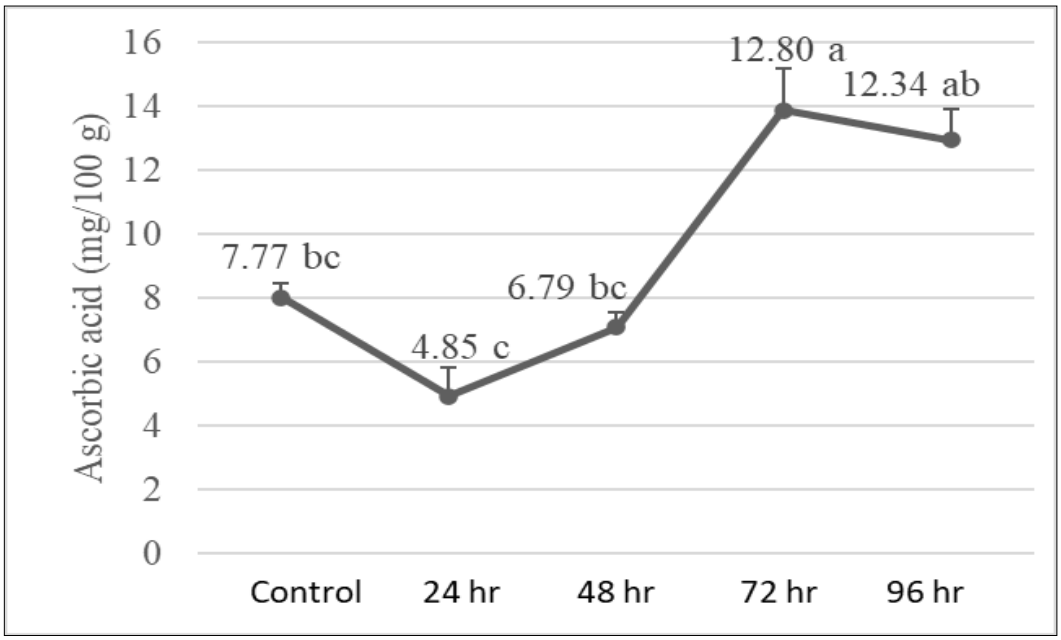


Fig. 1. Effect of germination on ascorbic acid content in quinoa powder (mg/100 g dry weight (DW)).

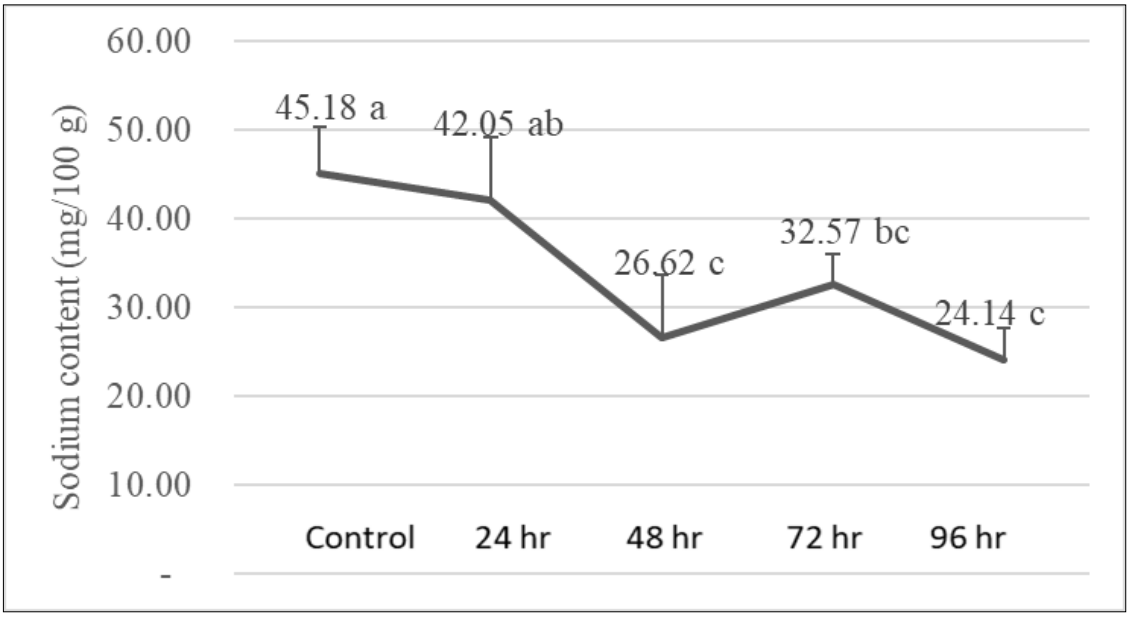


Fig. 2. Effect of germination on sodium content in quinoa powder (mg/100 g dry weight (DW)).

Seeds germinated for 96 hr had a lower sodium content (24.14 ± 3.37 mg/100 g DW), with a relative decrease of 47 % compared to the control group (45.18 ± 4.98 mg/100 g DW) ($p < 0.01$).

This considerable reduction in sodium may result from its release (leaching) during hydrolysis of organic complexes and redistribution to growing embryonic tissues, as mineral mobilisation is a key process in germination. Sodium also acts as a cofactor in enzymatic processes during germination (35). Additionally, the breakdown of oxalic acid by activated oxalate oxidase limits sodium oxalate complexes, further contributing to lower sodium content (36). A 47 % reduction in sodium content improves dietary suitability and supports cardiovascular health, consistent with recommendations for adults to consume less than 2000 mg of sodium per day (22).

Similar observations were previously reported by researchers who observed a significant decrease in red quinoa during germination (18–48 hr) compared to the control (91 mg/100 g), which had a higher sodium content (91mg/100 g) than our quinoa, likely due to varietal differences and growing conditions (37).

Condensed tannins

The condensed tannins of germinated and ungerminated quinoa seeds varied from 10.12 ± 1.41 to 24.43 ± 2.82 mg/100 g DW (Fig. 3) (Error bars indicate standard deviation (n = 3). According to Tukey's test, different letters above bars represent significant differences ($p < 0.05$), while the same letters indicate no significant difference). Seeds germinated for 96 hr had a lower condensed tannin content (10.12 ± 1.41 mg/100 g DW), showing a relative decline of 55 % compared to the control (22.52 ± 0.47 mg/100 g DW) ($p < 0.01$).

Our results show that both germinated and ungerminated quinoa seeds contain low levels of condensed tannins, with germination at 96 hr contributing to a significant further reduction in this compound. This decline is likely due to leaching during soaking in water and enzymatic activity during germination, which promotes tannin binding to proteins and carbohydrates (15, 38, 39).

Our results concur with previous studies reporting reductions in tannin content during germination (40), although our values are lower than those reported, who found levels between 28 and 43 mg/100 g depending on the quinoa variety (41).

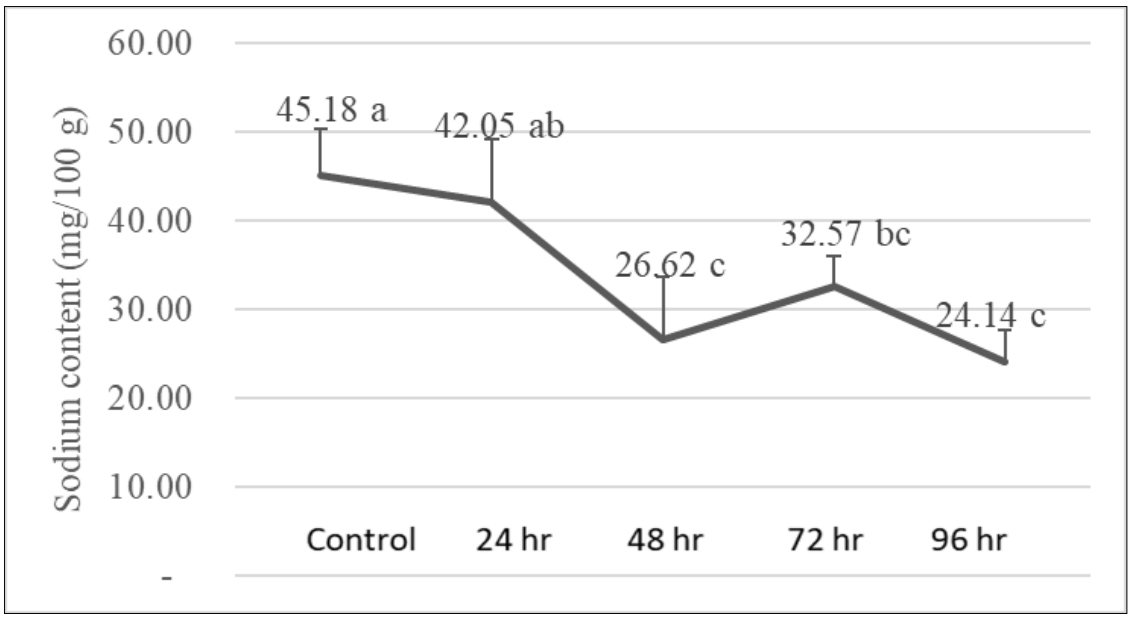


Fig. 3. Effect of germination on condensed tannin content in quinoa powder (mg/100 g dry weight (DW)).

Tannins have both antioxidant and anti-nutritional properties (17). While hydrolysable tannins can reduce iron bioavailability, condensed tannins, which are more common in the diet, have a less pronounced effect on iron absorption, even at high concentrations (160 mg/100 g) (42).

Therefore, a 55 % reduction in tannins induced by germination enhances quinoa's nutritional bioavailability, improving its suitability for human consumption. Nevertheless, it remains important to maintain a balanced diet and regulate the intake of anti -nutrients, particularly tannins and phytates, to limit their effect on iron absorption.

Further research is needed to assess the impact of consuming germinated quinoa seeds on iron bioavailability and the absorption of other nutrients over time.

Fatty acid

Table 1 shows the content of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in ungerminated and germinated quinoa seeds, with the predominant fatty acid identified for each class. The results showed the presence of palmitic acid as the major SFA (17.65 ± 2.67 %), oleic acid as the predominant MUFA (24.11 ± 1.68 %) and linoleic acid as the main PUFA (58.22 ± 9.01 %).

Compared to the control (17.81 ± 2.09 % and 23.74 ± 1.03 %), a relative decrease of 18 % in palmitic acid was observed during 72 hr of germination ($p < 0.05$), while oleic acid rose significantly by 29 % and 18 % during 24 hr and 96 hr of germination, respectively ($p < 0.01$). Linoleic acid increased notably during 72 hr of germination, with a relative increase of 17 % compared to the control (58.43 ± 9.74 %) ($p < 0.01$).

These divergent trends in fatty acid profile reflect dynamic metabolic adjustments during germination. Desaturase enzymes convert MUFA into PUFA, while lipases mobilise fatty acids for energy and biosynthesis. Some fatty acids are synthesised or desaturated earlier, whereas others are consumed or modified later in the germination process (38, 43).

Fatty acid profiling showed that quinoa seeds remain rich in MUFA and PUFA and low in SFA, suggesting improved lipid quality. These changes enhance nutritional quality by supporting cardiovascular health and providing essential fatty acids. A 17 % increase in PUFA makes germinated seeds a better dietary fat source. Omega-6, the most abundant fatty acid in quinoa seeds, is essential and must be obtained from the diet (40–42). It provides hypocholesterolemic benefits, although excessive intake could be pro-inflammatory (44). An 18 % decrease in SFA further contributes to reduced risk of type 2 diabetes, cardiovascular disease and metabolic syndrome (45).

Our results partially agree with previous studies, which show that germination decreases SFA and increases PUFA, although variations in MUFA have also been reported. For instance, germination for 48 hr led to a 1.6 % decrease in oleic acid and a 0.8 % increase in linoleic acid, compared to the control (37). Another study observed a decrease in MUFA and an increase in PUFA after the same duration (39). At 72 hr, a reduction in palmitic acid and an increase in linoleic acid were reported (46). Similarly, a study that analysed different lipid fractions were analysed (nonpolar lipids, glycolipids and phospholipids), showed that 72 hr of germination led to an increase in oleic acid and a decrease in linoleic acid (47). These variations could be primarily attributed to differences in the cultivation environment of the studied quinoa varieties.

Finally, Table 2 showed changes in bioactive compounds observed in the current study and those reported in previous research, highlighting key similarities and differences.

Conclusion

Germination led to significant modifications in quinoa seed composition. Specifically, germination for 72 hr optimally enhances ascorbic acid and polyunsaturated fatty acids while reducing saturated fatty acids, whereas germination for 96 hr optimally reduces sodium and tannins and enhances monounsaturated fatty acids. Therefore, powders obtained from germinated quinoa seeds represent a novel functional ingredient for developing nutritionally fortified food products.

Table 1. Effect of germination on fatty acid content in quinoa powder (%)

Germination time	Saturated fatty acid (Palmitic acid, C16:0)	Monounsaturated fatty acid (Oleic acid, C18:1)	Polyunsaturated fatty acid (Linoleic acid, C18:2)
0 hr (Control)	17.81 ± 2.09 a	23.74 ± 1.03 b	58.43 ± 9.74 b
24 hr	18.12 ± 2.55 a	30.62 ± 0.95 a	51.24 ± 2.38 d
48 hr	19.62 ± 1.77 a	21.23 ± 3.55 b	59.13 ± 9.11 b
72 hr	14.62 ± 2.48 b	17.00 ± 1.97 c	68.37 ± 7.71 a
96 hr	18.09 ± 4.60 a	27.95 ± 0.91 a	53.95 ± 16.11 c

Values are presented as mean ± SD (n = 3). According to Tukey's test, means followed by different letters differ significantly ($p < 0.05$), while the same letters indicate no significant effect.

Table 2. Comparative changes in bioactive compounds in germinated quinoa seeds compared to control: current study vs. previous reports

Compound	Trend in current study	Trend in previous study	Reference
Ascorbic acid (mg/100 g)	↑ 72 hr (+5.03), ↑ 96 hr (+4.57)	↑ 96 hr (+13.75)	(33)
Sodium (mg/100 g)	↓ 96 hr (-21.04)	↓ 24 hr (-41.10)	(37)
Condensed tannins (mg/100 g)	↓ 96 hr (-12.40)	↓ 72 hr (-13.00)	(40)
SFA (% palmitic acid)	↓ 72 hr (-3.19)	↓ 72 hr (-0.21)	(46)
MUFA (% oleic acid)	↑ 24 hr (+6.88), ↑ 96 hr (+4.21)	↑ 72 hr (+0.11)	
PUFA (% linoleic acid)	↑ 72 hr (+9.94)	↑ 24 hr (+3.94)	(39)

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

However, this study is limited to a single quinoa variety and the absence of sensory, bioavailability and shelf-life evaluations. Future research should therefore explore multiple varieties, assess consumer acceptability and nutrient bioavailability and evaluate the microbiological quality and potential incorporation into functional foods.

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Authors' contributions

AN conceived and designed the study and wrote the manuscript. LE contributed to the methodology and carried out the experiments. NH participated in the interpretation of the results. KA conducted statistical analyses. AM participated in the coordination and critically revised the manuscript. MB supervised the overall project. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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