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Original Research

Effect of Calcium Chloride Treatment on the Accumulation of Calcium and Phenolics and the Estimation of Antioxidant Capacity in Germinated Quinoa

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Abstract

Background: Calcium (Ca^{2+}) is an essential mineral element for the human body and plays an important regulatory role in plant growth. Germination is one of the biofortification methods for whole grains, enhancing the nutritional value and functional properties of cereals and significantly reducing antinutritional factors. During evolution, grains evolved to synthesize polyphenolic compounds as a defense mechanism against environmental stresses and for self-protection. However, there is a lack of research on calcium and polyphenol accumulation, as well as on antioxidant capacity, during quinoa germination in the presence of exogenous calcium chloride ($CaCl_2$). **Methods:** This study systematically analyzed the accumulation of calcium and phenolics and evaluated the antioxidant capacity in germinated quinoa treated with exogenous $CaCl_2$ at concentrations up to 60 mM. **Results:** The sprout lengths of quinoa in the 30–60 mM $CaCl_2$ groups were significantly shorter ($p < 0.05$) than those of the control group (0 mM $CaCl_2$). In the 40 mM treatment group, the nutrient reference value (NRV) was over 30% after 24 hours, and in the 50–60 mM treatment group, the NRV also exceeded 30%, meeting the standard of being rich in calcium. However, the total phenolic content (TPC) of calcium-rich germinated quinoa at 24 h in the 60 mM group was 7.65% lower compared with the control group. This indicates that high Ca^{2+} levels can reduce TPC in sprouted quinoa while enhancing calcium bioavailability. In the control group (0 mM), the phytic acid (PA) content after 24 h of germination was 2.45 ± 0.15 mg/g DW. The PA content and PA/Ca ratio in the exogenous calcium treatment groups were significantly lower compared with the control group ($p < 0.05$). The PA/Ca ratio in the 40 mM group (24 h) was significantly reduced by 97.33% compared with the control group, indicating that $CaCl_2$ treatment can mitigate the inhibitory effect of PA on calcium absorption in germinated quinoa. Moreover, the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity in the exogenous $CaCl_2$ groups was significantly increased compared with the control group ($p < 0.05$). **Conclusions:** This study provides useful data for the development of calcium-biofortified germinated quinoa with improved mineral bioavailability and antioxidant capacity.

Keywords: *Chenopodium quinoa*; germination; calcium chloride; phenols; phytic acid; antioxidants; biological availability

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an annual dicotyledonous pseudocereal native to the Andean region. Long-term consumption of quinoa contributes to the prevention and management of various chronic diseases, including obesity, diabetes, cancer, cardiovascular disorders, and coeliac disease, while improving overall health status [1]. The Food and Agriculture Organization (FAO) has classified quinoa as a nutrient-dense pseudocereal [2].

Calcium (Ca^{2+}) is an essential mineral element in plants, serving as a critical regulatory factor throughout plant growth and development. It acts as a key intracellular second messenger [3], playing a pivotal role in modulating plant growth, development, and responses to abiotic stresses such as salinity, drought, and oxidative conditions [4]. Calcium is an essential nutrient required for structural development and signal transmission in both plants and animals. In humans, inadequate calcium intake is associated with various diseases, including osteoporosis, cardiovascular and cerebrovascular diseases, and cancer. The fortification of trace elements such as calcium in seeds can be achieved through methods such as breeding, cultivation, and germination. Calcium biofortification of staple food crops represents a sustainable, long-term strategy to alleviate calcium deficiency [5]. Direct addition of calcium supplements to food products predominantly involves insoluble calcium carbonate, as well as soluble calcium citrate, calcium malate, etc. Moreover, phytic acid (PA) in grains forms complexes with calcium, potentially affecting the bioavailability of calcium in grain-based foods [6]. Therefore, reducing the PA content and simultaneously promoting the production of phytochemicals has become a research focus [7,8].

During evolution, cereal grains have developed a diverse array of phytochemicals (e.g., polyphenols and flavonoids) and PA as adaptations to environmental chal-

lenges and for protective functions. Whole grains contain appreciable amounts of phytochemicals and dietary fiber, which exert beneficial effects on the human gut microbiota after consumption [9]. Biofortification is a strategy used to improve the nutritional value of crops by enriching crops with micronutrients and increasing their bioavailability during plant growth through selective breeding and cultivation practices [10]. Germinated whole grains are emerging as a promising tool in food biofortification programs, and enhancing the nutritional value and functional properties of cereals through germination has become a research hotspot [11,12,13].

To date, the interest in calcium with regard to quinoa has largely focused on agronomic biofortification to elevate the grain calcium content, and on the use of exogenous calcium to alleviate abiotic stress. Investigations into the effects of exogenous calcium chloride ($CaCl_2$) on the germination process, mineral element accumulation, and the dynamics of functional constituents such as PA and polyphenols in quinoa remain scarce. The hygienic safety of *Salmonella* during the germination process also requires further analysis.

Quinoa seeds germinated for 36–72 h show significant accumulation of free phenolic acids, bound flavonoids, and enhanced *in vitro* antioxidant activities compared with ungerminated seeds [14]. Similarly, 72 h germination significantly enhanced proteins, fiber, minerals, phenolic compounds, flavonoids, and antioxidant capacity across multiple quinoa varieties [15]. Previous studies have reported the effects of germination on minerals, PA, phenolic compounds, and antioxidant properties in quinoa and other cereals. However, the combined regulation of calcium accumulation, PA degradation, PA/Ca ratio, and antioxidant capacity by exogenous $CaCl_2$ during quinoa germination remains insufficiently understood. The aim of the present study was therefore to systematically assess the effects of a defined exogenous $CaCl_2$ concentration range (0–60 mM) during short-term quinoa germination. We simultaneously evaluated calcium enrichment, mineral balance, PA reduction, PA/Ca ratio, total phenolic content (TPC), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity, and ferric reducing antioxidant power (FRAP). By clarifying the trade-off between calcium enrichment and quality-related biochemical indicators, this study provides a scientific basis for the development of quinoa sprout food products with high calcium levels, high antioxidant activity, and a low PA/Ca ratio.

2. Materials and Methods

2.1 Materials and Reagents

White quinoa (*Chenopodium quinoa* Willd., Qinghai No. 1) was obtained commercially from Xining, Qinghai Province, China. Calcium, copper, iron, potassium, magnesium, and zinc standard solution with a purity of ≥99% was purchased from Shanghai Anpu Standard Technical Service Co., Ltd (Fengxian District, Shanghai, China). Food-grade $CaCl_2$ with a purity of ≥96% was used. ABTS (A800764, Macklin, Fengxian District, Shanghai, China) and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sinopharm Chemical Reagent Co., Ltd (Fengxian District, Shanghai, China).

2.2 Germination Treatment

To strengthen hygienic control during the germination process, the germination trays, gauze, and experimental tools were sterilized before use. Germination was conducted under controlled temperature and humidity conditions. Quinoa seeds were sterilized in 1% (v/v) sodium hypochlorite solution for 5 min, rinsed thoroughly with distilled water, and then soaked in $CaCl_2$ solutions at concentrations of 0, 10, 20, 30, 40, 50, and 60 mM at room temperature for 2 h (solid–liquid ratio 1:5, w/v) [16]. The soaked seeds were then transferred to germination trays containing the corresponding $CaCl_2$ solution. They were covered with two layers of gauze, which was kept moist with the same $CaCl_2$ solution. Germination was conducted in an incubator under controlled conditions of 25 °C and 95% relative humidity. Samples were collected every 6 h up to 24 h, washed 3–5 times with ultra-pure water, dried at 50 °C until the weight was constant, crushed, passed through a 40-mesh sieve, and stored at 4 °C for testing. Three replicates were prepared for each treatment. Sprout production involves high moisture and moderate temperature, which can favor the growth of pathogenic microorganisms. To reduce potential microbiological contamination, we used short germination times, seed disinfection, clean water, sterilized equipment, and controlled incubation. No visible spoilage or abnormal odor was observed during the 24 h germination period.

2.3 Measurement of Sprout Length

During germination, 20 seeds were randomly selected to measure sprout length. Measurement from the root tip of the seed to the apex of the tooth was made using ImageJ software (Version 1.54r, National Institutes of Health, Bethesda, Maryland, USA).

2.4 Determination of Trace Elements

Trace element levels were determined according to China's National Food Safety Standard (code GB 5009.268-2025) [17] using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) method. Quinoa powder was dried at 50 °C under vacuum to a constant weight, then ground and passed through a 40-mesh sieve. The quinoa powder samples obtained in section 2.2 (about 0.10 g/piece) were weighed into a crucible and pre-ashed on an induction cooker until no more smoke was produced. This was followed by ashing in a muffle furnace at 500–550 °C for 5–8 h until white ash was obtained. After cooling to room temperature, the residue

was dissolved in 1 mL nitric acid and diluted to 25 mL. A reagent blank was prepared simultaneously. A mixed standard calibration curve was constructed using standard solutions of calcium, magnesium, potassium, copper, iron, and zinc, enabling the subsequent determination of these elements in the samples.

2.5 Determination of Total Phenolic Content (TPC)

TPC was determined according to the method described by Wang et al. [18] with slight modifications. Briefly, quinoa powder (about 0.5 g/piece, passed through a 40-mesh sieve) was weighed, and the reaction reagents were added. The absorbance was then measured at 760 nm (optical density $(OD)_{760}$). A standard calibration curve was constructed using gallic acid as the reference standard, and the TPC was calculated according to Eqn. 1.

$$M = (C \times V \times N)/(m \times 1000) \quad (1)$$

In this equation, M is the TPC (mg/g), C is the gallic acid concentration (μg/mL), V is the volume of extract (mL), N is the sample dilution factor, and m is the sample mass (g).

2.6 Determination of Phytic Acid (PA)

The PA content was determined according to the method of Wu et al. [19] with slight modifications. The sample (about 25 mg/piece) was mixed with 2.5 mL of 3 g/L trichloroacetic acid (TCA) and 5 mL of 0.02% $NH_4Fe(SO_4)_2$ solution. The mixture was heated in a boiling water bath for 30 min, cooled under running water, and centrifuged at 4000 r/min for 10 min. An aliquot (2 mL) of the supernatant was mixed with 3 mL of 10 g/L bipyridine solution, and the absorbance was measured at 519 nm (OD_{519}). A standard curve was constructed using sodium phytate, and the PA content was calculated according to Eqn. 2. The bipyridine colorimetric method is an approach for indirectly estimating PA content based on the interaction between PA and ferric ions.

$$M = \frac{C}{m \times 1000} \quad (2)$$

In this equation, M is the PA content (mg/g dry weight (DW)), C is the concentration obtained from the standard curve (μg), and m is the sample mass (g).

2.7 Determination of Antioxidant Capacity

The antioxidant capacity was determined according to the method described by Pająk et al. [20] with slight modifications. The quinoa powder (about 1.0000 g/piece) was extracted twice with 80% (v/v) ethanol by ultrasonication, and the resulting supernatants were combined and used as the sample extract.

The ABTS radical cation scavenging capacity was measured according to the method of Pająk et al. [20], with slight modification by recording the absorbance at 734 nm (OD_{734}). A standard calibration curve was prepared using different concentrations of Trolox standard solution in place of the sample extract under identical reaction conditions. The results were expressed as μmol Trolox equivalent per gram dry weight (μmol Trolox equivalent (TE)/g DW).

The ferric reducing antioxidant power (FRAP) was determined according to the method of Pająk et al. [20], with slight modifications by measuring the absorbance at 593 nm (OD_{593}). A blank control was prepared using 80% (v/v) ethanol, and a standard calibration curve was constructed using different concentrations of $FeSO_4$ standard solution in place of the sample extract under identical reaction conditions. The ferric reducing ability of the sample was expressed as μmol Fe^{2+} equivalent per gram dry weight (μmol Fe^{2+} equivalent (FE)/g DW).

2.8 Determination of Salmonella spp.

Salmonella spp. were determined according to China's National Food Safety Standard (code GB 4789.4-2024) [21]. Fresh germinated quinoa at 24 h was collected by aseptic sampling. Each sample (25 g) was placed into a sterile sampling bag with 225 mL of buffered peptone water (BPW). The sprouts were ground with a pestle for 1 min to obtain homogenates. Each homogenate was incubated at 36 °C for 18 h, after which 0.1 mL of each culture was aseptically transferred into a sterile tube with 10 mL of fresh sterile Rappaport-Vassiliadis soy (RVS) broth. At the same time, 1 mL of each culture was placed into a sterile tube with 10 mL of fresh sterile tetrathionate brilliant green broth (TTB). The tubes were incubated at 42 °C for 24 h. Subsequently, 10 μL of the RVS and TTB cultures were plated onto bismuth sulfite (BS) agar and xylose lysine tergitol (XLT) agar to identify the bacterial populations. Testing of each sample was conducted in five replicates.

2.9 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as the mean ± standard deviation (SD). Data were processed using Microsoft Excel 2019 (version 1902, Microsoft Corporation, Redmond, Washington, USA) and analyzed statistically using IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, New York, USA). The normality of data and the homogeneity of variances were evaluated using the Shapiro–Wilk test and Levene's test, respectively. When these assumptions were satisfied, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was applied to evaluate significant differences among treatments ($p < 0.05$). Graphs were generated using GraphPad Prism (version 10.3.0, Software MacKiev, Boston, MA, USA).

Table 1. Effects of different $CaCl_2$ concentrations on sprout length of quinoa (mm).

$CaCl_2$ concentration (mM)	6 (h)	12 (h)	18 (h)	24 (h)
0	3.33 ± 1.16^{a}	5.93 ± 1.69^{ab}	10.02 ± 2.67^{a}	16.41 ± 4.71^{a}
10	3.37 ± 1.37^{a}	6.67 ± 1.18^{a}	9.54 ± 2.45^{ab}	16.34 ± 4.30^{a}
20	3.20 ± 0.70^{a}	5.67 ± 1.54^{b}	9.98 ± 2.23^{a}	14.33 ± 2.28^{a}
30	2.50 ± 0.66^{b}	5.42 ± 1.11^{b}	8.80 ± 2.26^{abc}	11.96 ± 2.40^{b}
40	3.13 ± 0.85^{a}	5.25 ± 1.48^{bc}	8.51 ± 1.88^{bc}	11.53 ± 2.11^{b}
50	2.51 ± 0.67^{b}	5.04 ± 1.52^{bc}	8.10 ± 1.52^{bc}	11.12 ± 2.61^{b}
60	2.27 ± 0.74^{b}	4.41 ± 1.44^{c}	7.42 ± 1.72^{c}	10.24 ± 3.03^{b}

Note: Different lowercase letters indicate significant differences among treatments with different concentrations at the same time point ($p < 0.05$), whereas the same letter indicates no significant difference. $CaCl_2$, calcium chloride.

3. Results

3.1 Changes in Sprout Length During Germination of Quinoa

High concentrations of $CaCl_2$ were found to significantly suppress the sprout length of germinating quinoa (Table 1). This inhibitory effect intensified with longer germination time. At 24 h, sprout lengths in the 30–60 mM $CaCl_2$ groups were significantly shorter than those in the control group (0 mM) ($p < 0.05$). When the calcium concentration was increased to 50–60 mM, sprout length was significantly inhibited at 6 h ($p < 0.05$). At 24 h, the sprout length in the 60 mM group was reduced by 37.6% compared with the control group ($p < 0.05$). The relatively large SD observed in the control group at 24 h may be attributed to biological heterogeneity among individual quinoa seeds during germination, including differences in water uptake, radicle emergence time, and seed vigor. Because sprout length was measured from randomly selected seeds using calibrated images, this variation was considered to mainly reflect biological variability rather than measurement error.

High calcium concentrations have also been shown to result in shorter sprout lengths compared with the control in previous studies of alfalfa seed germination treated with $CaCl_2$ solution [22] and in common vetch germination treated with calcium acetate [23]. High calcium concentrations may provoke ionic imbalance and disrupt cell membrane permeability, induce potassium efflux, increase sodium content, and elevate the intracellular Na^+/K^+ ratio, thereby disturbing ion homeostasis and inhibiting sprout growth [3]. Excessive calcium during plant growth has been shown to inhibit development by interfering with phosphorus metabolism and blocking calcium signal transduction [24].

A possible explanation is that high calcium concentrations may disturb ion homeostasis and membrane permeability, thereby affecting the retention of potassium and ultimately inhibiting sprout elongation. However, this mechanism requires further confirmation because the Na^+/K^+ ratio was not directly measured in the present study.

3.2 Effect of Different $CaCl_2$ Concentrations on Mineral Elements in Germinated Quinoa

Calcium is an essential element for biological growth and development, acting as a versatile intracellular second messenger that participates in protein synthesis and metabolism, regulates hormone synthesis and signal transduction, and contributes to cell wall formation and stem elongation [25]. The calcium content of raw quinoa seeds (RQS) was 43.523 ± 3.265 mg/100 g DW (Table 2). During germination, the calcium content in the control group (0 mM $CaCl_2$) showed slight variations, but was not significantly different compared with RQS except at 18 h ($p > 0.05$). However, the calcium content in germinated quinoa treated with different $CaCl_2$ concentrations was significantly higher than in the control group ($p < 0.05$). Moreover, the calcium content was positively correlated with the exogenous $CaCl_2$ concentration, indicating that calcium was absorbed and accumulated during germination. The highest calcium content in germinated quinoa was observed in the 60 mM group for 24 h (304.428 ± 2.443 mg/100 g DW), which was 7-fold higher than RQS ($p < 0.05$). These results demonstrate that calcium biofortification of germinated quinoa can be effectively achieved through exogenous calcium treatment.

A previous study found that pea sprouts treated with calcium formate, calcium citrate and calcium chloride showed significant increases in calcium content of 242%, 155% and 134%, respectively, compared with the control [23]. This suggests that different types of calcium salts can markedly enhance the calcium content in pea sprouts, with exogenous Ca^{2+} increasing the influx and intracellular accumulation of Ca^{2+}. Zhou et al. [26] reported that treatment of mung bean sprouts with 6 mM $CaCl_2$ during germination significantly increased both the total calcium and free calcium content compared with the control, again indicating that treatment with appropriate concentrations of exogenous calcium chloride can increase the calcium content of sprouts.

In accordance with the "National Food Safety Standard for Nutrition Labelling of Prepackaged Foods" (code GB 28050-2025) [27] and the European Reference La-

Table 2. Calcium content and nutrient reference values in germinated quinoa treated with different concentrations of $CaCl_2$.

$CaCl_2$ concentration (mM)	6 (h)		12 (h)		18 (h)		24 (h)	
	Calcium (mg/100 g DW)	NRV (%)	Calcium (mg/100 g DW)	NRV (%)	Calcium (mg/100 g DW)	NRV (%)	Calcium (mg/100 g DW)	NRV (%)
D	43.523 ± 3.265^{g}	5	43.523 ± 3.265^{f}	5	43.523 ± 3.265^{g}	5	43.523 ± 3.265^{g}	5
0	42.265 ± 0.123^{g}	5	45.076 ± 1.261^{f}	6	54.723 ± 3.148^{f}	7	45.571 ± 2.248^{g}	6
10	136.914 ± 2.030^{f}	17	145.558 ± 1.479^{e}	18	141.667 ± 1.231^{e}	18	146.416 ± 0.238^{f}	18
20	170.996 ± 0.770^{e}	21	174.877 ± 1.428^{d}	22	180.625 ± 0.175^{d}	23	179.905 ± 1.088^{e}	22
30	192.372 ± 1.107^{d}	24	194.154 ± 1.190^{c}	24	214.603 ± 0.658^{c}	27	217.557 ± 3.167^{d}	27
40	214.438 ± 3.001^{c}	27	205.443 ± 4.222^{b}	26	222.963 ± 2.939^{b}	28	260.850 ± 1.445^{c}	33
50	241.960 ± 1.127^{b}	30	245.146 ± 5.023^{a}	31	242.752 ± 1.209^{a}	30	285.801 ± 0.916^{b}	36
60	245.877 ± 1.478^{a}	31	244.005 ± 0.785^{a}	31	244.994 ± 2.181^{a}	31	304.428 ± 2.443^{a}	38

Note: Different lowercase letters within the same time point indicate significant differences among concentrations ($p < 0.05$), whereas the same letter indicates no significant difference. NRV contribution (%) = X/800 mg × 100%, where X represents the calcium content per 100 g of quinoa, and NRV denotes the Nutrient Reference Value of calcium (800 mg). "D" indicates raw quinoa seeds (RQS). NRV, nutrient reference value; DW, dry weight.

belling Values (RLV) for food nutrition content, a food product can be claimed as "rich in calcium" when the percentage of nutrient reference value (NRV) or RLV exceeds 30%. In germinated quinoa at 24 h, the NRV of calcium in the 50–60 mM groups and in the 40 mM group were all more than 30% higher than in the control group, indicating these germinated quinoa of 50–60 mM groups and the 40 mM group at 24 h can may be classified as calcium-rich quinoa.

Mineral elements are essential constituents for maintaining normal physiological activities and nutritional quality in plants. Potassium plays an important role in the activation of numerous growth-related enzymes, and generally exhibits high mobility in plants. Compared to RQS, the potassium content in germinated quinoa was lower, and the iron content was higher, while the remaining elements showed no significant changes (Table 3). Under exogenous calcium treatment, the potassium content in the 60 mM $CaCl_2$ group was lowest at 18 h of germination, with a 16.15% decrease compared to the control group. The potassium content was also lower in other calcium concentration treatment groups compared to the control. This may be attributed to the leaching of potassium during soaking, and its subsequent consumption as a cofactor in the ensuing growth processes [28]. High concentrations of exogenous calcium could exert a stress effect on germinating cereals, further aggravating potassium efflux and inhibiting sprout elongation through perturbations in ion homeostasis and membrane permeability [3].

Zinc also exhibits high mobility during seed germination and plays an important role in various aspects such as protein synthesis, membrane structure, gene expression, and tolerance to oxidative stress in newly formed tissues [29]. During plant germination, iron is indispensable for key enzymes involved in plant metabolism [30]. Although exogenous calcium treatment had relatively minor effects on the accumulation of Cu, Fe, Mg, and Zn in germinating quinoa, statistically significant differences ($p < 0.05$) between certain treatment groups and the control were still observed at specific time points.

3.3 Effect of Different Concentrations of $CaCl_2$ on TPC in Germinated Quinoa

The TPC in RQS was 1.10 ± 0.03 mg gallic acid equivalent (GAE)/g DW. The TPC in the 10 mM $CaCl_2$ group was higher than that of the control after 12 h of germination (Fig. 1), but this did not reach statistical significance ($p > 0.05$). The highest TPC of quinoa germinated for 24 h was achieved in the 40 mM $CaCl_2$ group (2.15 mg GAE/g DW), which was 9.69% higher than the control group (1.96 mg GAE/g DW) and significantly different ($p < 0.05$). A possible explanation is that calcium ions exert a concentration-dependent biphasic effect on cell wall integrity and the retention of phenolic compounds. An appropriate concentration of Ca^{2+} stabilizes the cell wall and reduces the leakage of phenolic compounds, whereas a high concentration of Ca^{2+} damages the cell wall and increases permeability, leading to the loss of phenolic compounds. In the 10–40 mM $CaCl_2$ treatment groups, pectin in the cell wall contains abundant negatively charged carboxyl groups that can bind positively charged Ca^{2+}. This forms cross-linking bridges between the binding sites of pectic polysaccharide chains, thereby stabilizing cell wall integrity. Consequently, phenolic compounds are less prone to leak out of the cells and can maintain their physiological activity, leading to increased polyphenol content and antioxidant capacity in the plant [31]. Another potential reason is that exogenous calcium activates the plants' stress defense system, causing the concentration of free calcium in the extracellular space to increase. Ca^{2+} then acts as a second messenger to increase the activity of enzymes associated with the expression of phenolic substances, thereby elevating the TPC. In germinated brown rice treated by $CaCl_2$, Choe et al. [32] reported a significant increase of 128% in TPC compared

Table 3. Changes in mineral element contents in germinated quinoa treated with different concentrations of $CaCl_2$ solutions (mg/kg DW).

$CaCl_2$ concentration (mM)	Time (h)	Ca	Cu	Fe	K	Mg	Zn
D		43.523 ± 3.265	0.731 ± 0.061	4.825 ± 1.643	506.774 ± 9.475	148.346 ± 2.268	2.265 ± 0.074
0	6	42.265 ± 0.123^{g}	0.688 ± 0.061^{c}	8.467 ± 1.643^{a}	395.545 ± 9.475^{b}	161.819 ± 2.268^{a}	2.523 ± 0.074^{cd}
	12	45.076 ± 1.261^{f}	0.817 ± 0.012^{c}	6.599 ± 0.692^{cd}	412.188 ± 4.381^{b}	168.115 ± 1.060^{a}	2.632 ± 0.025^{bc}
	18	54.723 ± 3.148^{f}	0.686 ± 0.025^{b}	5.577 ± 0.441^{e}	378.278 ± 1.647^{b}	157.395 ± 0.806^{a}	2.434 ± 0.002^{ab}
	24	45.571 ± 2.248^{g}	0.794 ± 0.040^{c}	8.109 ± 0.344^{bc}	391.554 ± 8.701^{c}	148.213 ± 5.143^{b}	2.459 ± 0.138^{b}
10	6	136.914 ± 2.030^{f}	0.818 ± 0.031^{b}	5.850 ± 0.645^{b}	332.791 ± 2.526^{e}	151.055 ± 0.364^{b}	2.343 ± 0.012^{de}
	12	145.558 ± 1.479^{e}	0.877 ± 0.025^{bc}	6.600 ± 0.007^{cd}	360.228 ± 2.723^{e}	158.069 ± 2.723^{b}	2.819 ± 0.028^{ab}
	18	141.667 ± 1.231^{e}	0.905 ± 0.032^{a}	6.577 ± 0.384^{cde}	360.690 ± 7.058^{c}	157.483 ± 1.082^{a}	2.754 ± 0.242^{a}
	24	146.416 ± 0.238^{f}	0.991 ± 0.083^{b}	6.731 ± 0.122^{cd}	408.680 ± 3.235^{b}	152.972 ± 2.340^{a}	2.780 ± 0.159^{a}
20	6	170.996 ± 0.770^{e}	0.817 ± 0.007^{b}	5.967 ± 0.052^{b}	347.832 ± 1.965^{cde}	152.318 ± 0.314^{b}	2.487 ± 0.017^{cd}
	12	174.877 ± 1.428^{d}	0.808 ± 0.056^{c}	8.907 ± 0.015^{ab}	388.057 ± 3.328^{c}	159.934 ± 1.515^{b}	2.570 ± 0.008^{c}
	18	180.625 ± 0.175^{d}	0.832 ± 0.010^{ab}	9.188 ± 0.282^{b}	362.488 ± 0.145^{c}	155.988 ± 0.834^{a}	2.488 ± 0.019^{ab}
	24	179.905 ± 1.088^{e}	0.930 ± 0.071^{b}	7.080 ± 0.050^{cd}	357.557 ± 8.970^{d}	141.849 ± 4.313^{d}	2.765 ± 0.007^{a}
30	6	192.372 ± 1.107^{d}	0.837 ± 0.002^{b}	5.534 ± 0.118^{b}	336.484 ± 2.763^{de}	143.797 ± 1.152^{c}	2.771 ± 0.431^{a}
	12	194.154 ± 1.190^{c}	0.977 ± 0.031^{a}	7.726 ± 0.336^{bc}	373.400 ± 9.083^{cd}	152.450 ± 7.846^{c}	2.554 ± 0.133^{c}
	18	214.603 ± 0.658^{c}	0.683 ± 0.037^{b}	7.073 ± 0.658^{cd}	361.760 ± 2.500^{c}	154.229 ± 1.656^{b}	2.118 ± 0.012^{b}
	24	217.557 ± 3.167^{d}	0.930 ± 0.210^{b}	6.375 ± 0.697^{d}	344.493 ± 8.405^{d}	139.426 ± 1.754^{e}	2.351 ± 0.760^{b}
40	6	214.438 ± 3.001^{c}	0.826 ± 0.019^{b}	9.000 ± 0.168^{a}	354.493 ± 1.425^{cd}	148.402 ± 0.448^{bc}	2.556 ± 0.072^{bc}
	12	205.443 ± 4.222^{b}	0.922 ± 0.044^{ab}	8.194 ± 0.038^{ab}	356.266 ± 0.380^{d}	149.983 ± 0.368^{cd}	2.542 ± 0.057^{c}
	18	222.963 ± 2.939^{b}	0.800 ± 0.010^{ab}	6.363 ± 0.346^{cde}	368.333 ± 9.768^{c}	146.417 ± 2.465^{ab}	2.572 ± 0.120^{ab}
	24	260.850 ± 1.445^{c}	1.007 ± 0.040^{b}	9.868 ± 0.149^{a}	350.729 ± 7.121^{d}	145.138 ± 7.945^{c}	2.312 ± 0.071^{b}
50	6	241.960 ± 1.127^{b}	0.877 ± 0.142^{b}	8.877 ± 1.056^{a}	342.135 ± 5.357^{cde}	143.614 ± 1.023^{c}	2.465 ± 0.009^{cd}
	12	245.146 ± 5.023^{a}	0.934 ± 0.049^{ab}	6.630 ± 0.249^{cd}	375.744 ± 3.075^{cd}	152.699 ± 1.207^{c}	2.505 ± 0.065^{cd}
	18	242.752 ± 1.209^{a}	0.885 ± 0.030^{a}	7.344 ± 0.070^{c}	341.624 ± 1.523^{cd}	151.854 ± 0.544^{a}	2.488 ± 0.041^{ab}
	24	285.801 ± 0.915^{b}	1.130 ± 0.003^{a}	7.164 ± 0.910^{cd}	356.169 ± 5.230^{d}	149.252 ± 1.792^{b}	2.520 ± 0.023^{b}
60	6	245.877 ± 1.478^{a}	0.958 ± 0.086^{a}	5.735 ± 0.749^{b}	358.689 ± 3.465^{c}	151.372 ± 1.142^{b}	2.651 ± 0.078^{b}
	12	244.005 ± 0.785^{a}	0.935 ± 0.052^{ab}	9.184 ± 0.092^{a}	371.520 ± 5.287^{cd}	149.141 ± 0.669^{d}	2.849 ± 0.004^{a}
	18	244.994 ± 2.181^{a}	0.796 ± 0.118^{ab}	10.778 ± 0.514^{a}	317.174 ± 7.367^{d}	146.663 ± 1.955^{ab}	2.492 ± 0.115^{ab}
	24	304.428 ± 2.443^{a}	0.947 ± 0.018^{b}	9.012 ± 0.201^{ab}	326.100 ± 1.828^{e}	136.220 ± 0.211^{f}	2.347 ± 0.013^{b}

Note: Different lowercase letters within the same time point indicate significant differences among concentrations ($p < 0.05$), whereas the same letter indicates no significant difference.

with the control. Yang et al. [33] found that the addition of Ca^{2+} significantly influenced polyphenol metabolites in the AMP-activated protein kinase (AMPK) signaling pathway.

The TPC of quinoa germinated for 24 h in the 50 and 60 mM $CaCl_2$ groups was 1.90 and 1.81 mg GAE/g DW, respectively (Fig. 1). These values were 3.06% and 7.65% lower compared with the control group (each $p < 0.05$), indicating that the accumulation of phenolic compounds in germinated quinoa was inhibited by excessively high exogenous calcium concentrations. A possible reason is that high Ca^{2+} concentrations impose stress on quinoa seeds and damage the cell walls. This would lead to increased cell wall permeability, facilitating the leaching and oxidation of TPC substances, and consequently reducing the TPC [33]. The TPC was increased in the 10–40 mM calcium groups, but inhibited in the 50–60 mM groups. Germinated quinoa with high TPC could thus be obtained under appropriate calcium concentrations. Although 50–60 mM $CaCl_2$ treatments were favorable for calcium accumulation, they did not maximize TPC. In particular, the 60 mM group showed a lower TPC than the control at 24 h, suggesting a trade-off between calcium biofortification and phenolic accumulation at high $CaCl_2$ concentrations. Therefore, higher $CaCl_2$ concentrations may be suitable for calcium enrichment, whereas moderate concentrations such as 40 mM may be more appropriate with regard to phenolic accumulation and mineral bioavailability.

3.4 Effects of Different Concentrations of $CaCl_2$ on PA Content in Germinated Quinoa

PA, or myo-inositol hexakisphosphate, is the primary storage form for phosphorus in plants and is a typical antinutritional factor. It can form insoluble complexes with mineral elements such as Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}, thereby

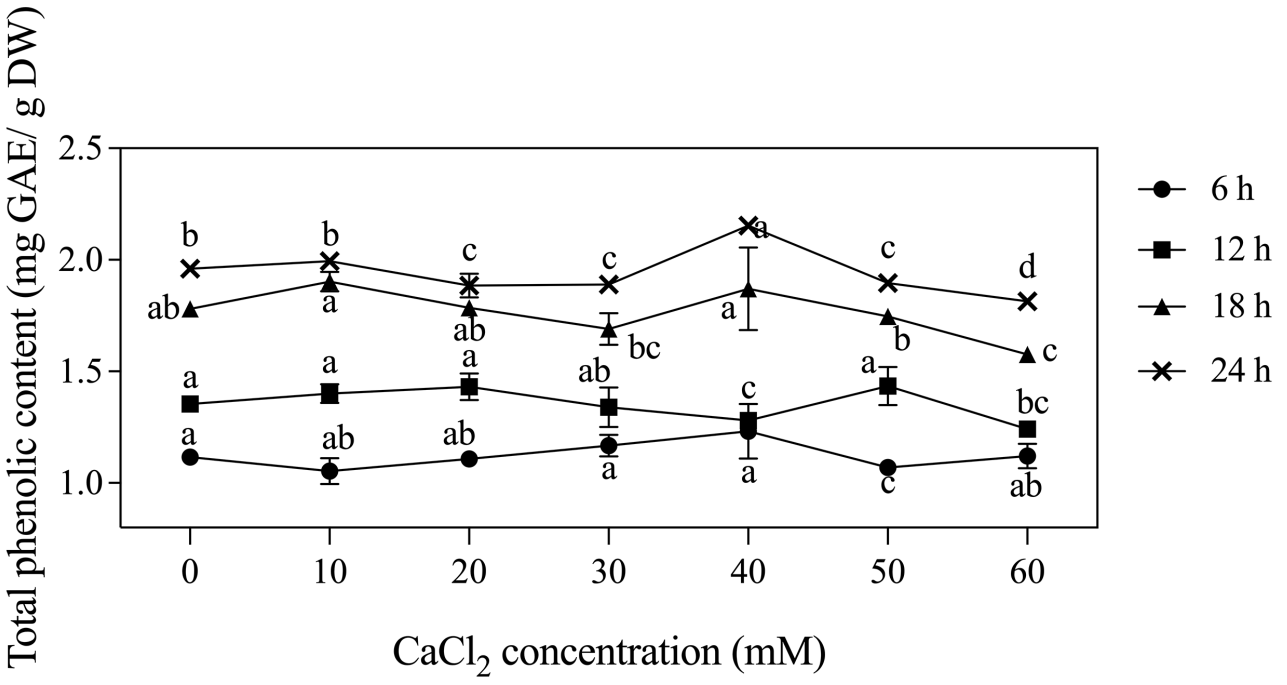

Fig. 1. Changes in total phenolic content (TPC) of germinated quinoa over 24 h treated with different concentrations of $CaCl_2$ solution. Note: Different lowercase letters indicate significant differences among concentrations at the same germination time; for each concentration, values at different germination times are all significantly different ($p < 0.05$), whereas the same letter indicates no significant difference.

reducing their bioavailability [34]. Considering the low calcium intake in the populations of low- to middle-income countries and their predominant reliance on plant-based dietary sources, the external addition of calcium to processed cereal grains is a potentially feasible strategy to address this issue. Initiatives aimed at increasing the plant calcium content through strategies such as crop biofortification, innovation in processing technology, and policy interventions have become critically important [35].

The PA content in RQS was 5.29 ± 0.01 mg/g DW. As shown in Fig. 2, the PA content in the control group decreased continuously with longer germination time, falling to 2.45 ± 0.15 mg/g DW at 24 h. This corresponds to a 53.69% decrease compared with RQS ($p < 0.05$). The PA content in germinated quinoa was further reduced by the addition of exogenous calcium. At 24 h of germination, the PA content declined progressively from the 10 mM group to the 40 mM group, reaching a minimum of 0.39 ± 0.01 mg/g DW in the 40 mM group. This represents a decrease of 84.08% compared to the control group (0 mM groups) ($p < 0.05$). The PA content was higher in the 50–60 mM groups compared with the 40 mM group. A possible explanation is that exogenous calcium increases the intracellular free calcium level, thereby enhancing phytase activity in germinated quinoa and promoting PA degradation. Degradation of PA is beneficial for phosphorus metabolism [26]. Zhou et al. [26] demonstrated that $CaCl_2$ (6 mM) significantly improved extracellular and intracellular calcium precipitates and calcium content, elevated phytase and acid phosphatase activity, and further enhanced phytic acid degradation.

The PA/Ca ratio is an important indicator reflecting the bioavailability of calcium in the diet. A lower PA/Ca ratio indicates reduced interference of PA on calcium ions, and therefore higher availability for calcium absorption. The PA/Ca ratio in RQS (12.93 ± 1.12) was significantly higher than in all germinated groups ($p < 0.001$), suggesting that calcium ions in RQS are strongly inhibited by PA. As shown in Fig. 3, the PA/Ca ratio was significantly reduced in germinating quinoa. In the control group, the PA/Ca ratio (5.05 ± 0.20) at 18 h was 60.94% lower compared with RQS ($p < 0.001$). These results indicate that the potential bioavailability of calcium could be improved by germination.

Under exogenous calcium treatment, the PA/Ca ratio in germinated quinoa showed further highly significant ($p < 0.001$) and progressive declines compared with the control (Fig. 3). At 24 h, the PA/Ca ratio of 0.15 ± 0.01 in the 40 mM group was reduced by 97.33% compared to the control ($p < 0.001$). This indicates that calcium bioavailability in germinated quinoa is highest in the 40 mM $CaCl_2$ group. According to Nkhata et al. [36], phytase activity was greatly increased during grain germination, thereby contributing to the reduction in PA and improvement in mineral bioavailability. Germination has been shown to enhance the utilization of minerals in cereals. The increase in calcium content during germination may be related to the PA content and phytase activity, leading to a progressive weak-

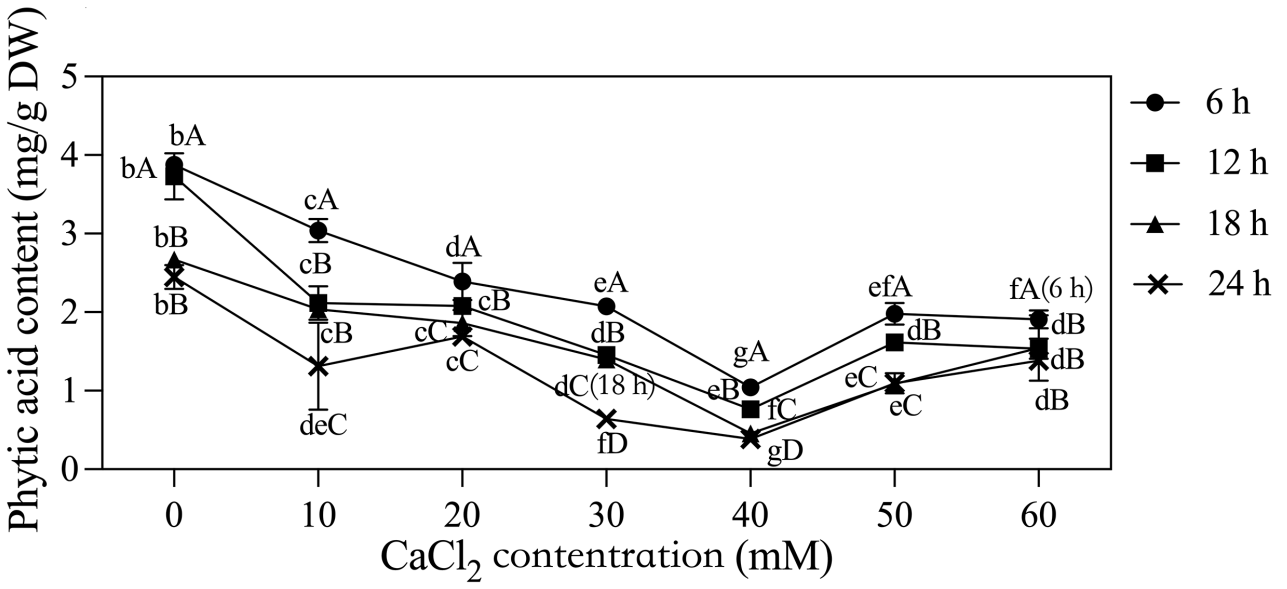

Fig. 2. Changes in phytic acid (PA) content in germinated quinoa over 24 h under different $CaCl_2$ treatments. Note: Different lowercase letters indicate significant differences among treatments with different concentrations at the same time point, whereas different uppercase letters indicate significant differences among different time points at the same concentration ($p < 0.05$), whereas the same letter indicates no significant difference.

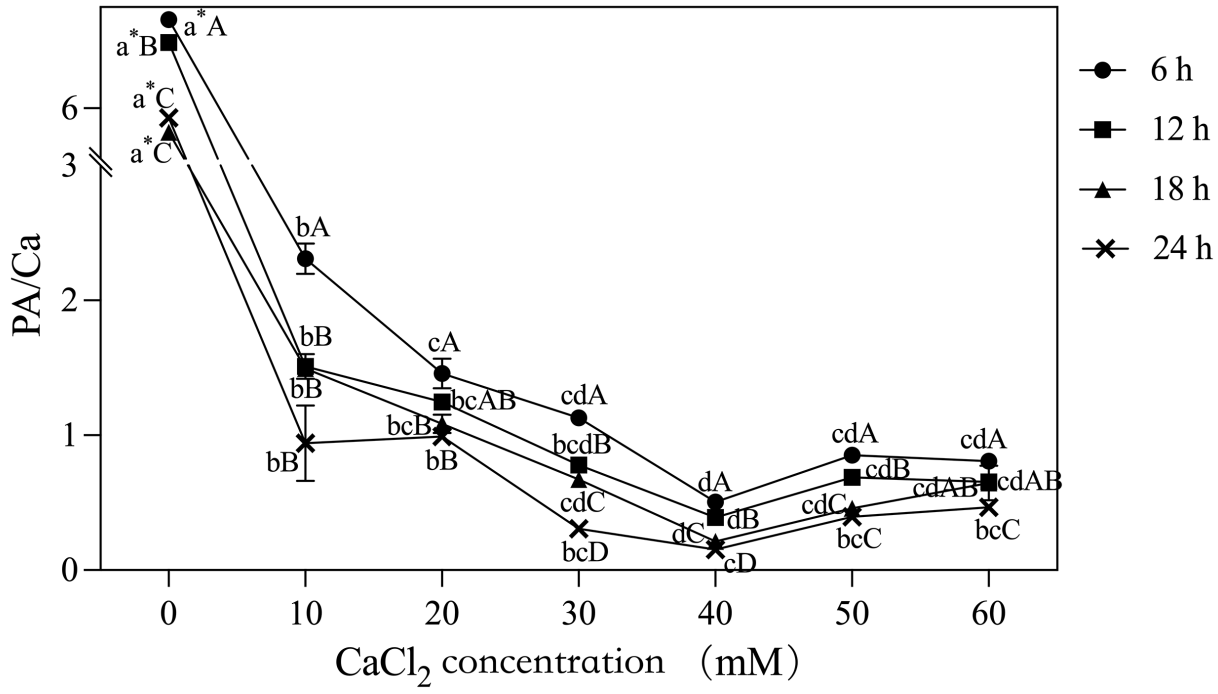

Fig. 3. Changes in the PA/Ca ratio in germinated quinoa over 24 h under different $CaCl_2$ treatments. Note: Different lowercase letters indicate significant differences among treatments with different concentrations at the same time point, whereas different uppercase letters indicate highly significant differences among different time points at the same concentration ($p < 0.05$). Lowercase letters marked with an asterisk (*) indicate highly significant differences ($p < 0.001$) between different concentration treatments at the same time point, whereas the same letter indicates no significant difference.

ening of metal chelation [7]. Exogenous calcium treatment could not only increase the calcium content of germinated quinoa, but also improve the potential utilization of calcium by reducing the PA content and PA/Ca ratio.

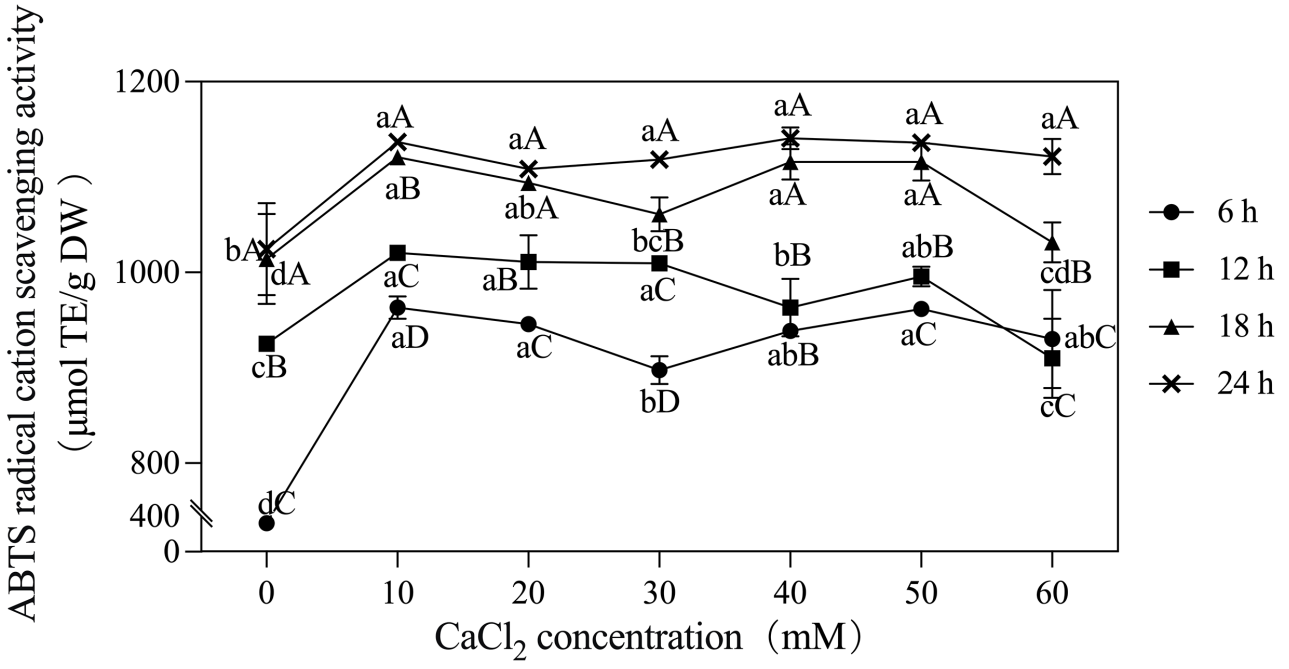

Fig. 4. Changes in ABTS radical scavenging activity of germinated quinoa under different $CaCl_2$ treatments. Note: Different lowercase letters indicate significant differences among treatments with different concentrations at the same time point, whereas different uppercase letters indicate significant differences among different time points at the same concentration ($p < 0.05$), whereas the same letter indicates no significant difference. ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TE, Trolox equivalent.

3.5 Changes in the Antioxidant Capacity of Germinated Quinoa Treated With Different Concentrations of $CaCl_2$

The ABTS radical scavenging capacity of RQS was 709.688 ± 12.014 μmol TE/g DW, while that of the control group reached a maximum value of 1024.372 ± 48.288 μmol TE/g DW at 24 h (Fig. 4), representing a 44.34% increase compared to RQS ($p < 0.05$). The ABTS radical scavenging capacity of the various calcium concentration groups was significantly higher than that of the control group ($p < 0.05$). The 10 mM group (1136.742 ± 6.060 μmol TE/g DW) at 24 h showed a 10.97% increase compared with the control group at 24 h ($p < 0.05$). Previous studies have demonstrated that the antioxidant capacity of lentils [37] and maize [38] is significantly increased after germination processing ($p < 0.05$). A possible mechanism is that the intracellular Ca^{2+} concentration is elevated by exogenous calcium, and the negatively charged carboxyl groups in the cell wall are subsequently bound by these Ca^{2+} ions. Cross-linking bridges are then formed between pectin polysaccharide chains to stabilize the cell wall, thereby increasing the polyphenol content and antioxidant capacity of plants [31,39].

The ferric reducing power of RQS was 46.234 ± 3.481 μmol FE/g. As shown in Fig. 5, the ferric reducing power of germinating quinoa increased significantly over time ($p < 0.05$). This increased even further under exogenous calcium treatment, showing a non-linear relationship with the calcium concentration. The ferric reducing power reached a peak in the 40 mM group (62.221 ± 0.551 μmol FE/g) at 24 h, which was 13.1% higher than in the control group ($p < 0.05$). Li et al. [40] reported that an iron biofortification process strongly enhanced the antioxidant capacity of brown rice. Overall, the effect of exogenous $CaCl_2$ on germinated quinoa was concentration-dependent. The release of free polyphenols and flavonoids in cells is promoted by exogenous salt ions, thereby enhancing antioxidant activity [41]. In this work, $CaCl_2$ treatment increased calcium accumulation in a concentration-dependent manner, with the 60 mM treatment achieving the highest calcium level after 24 h. However, functional indicators did not increase linearly with the $CaCl_2$ concentration. The 40 mM treatment produced the lowest PA content and PA/Ca ratio and also showed favorable TPC and FRAP values. The 50–60 mM treatments favored calcium enrichment but partly reduced phenolic accumulation, making them more suitable for product developments in which calcium fortification is the primary goal. These results indicate that the response of germinating quinoa to exogenous $CaCl_2$ is concentration-dependent and involves a trade-off between mineral biofortification and functional quality.

Different calcium concentrations affect a hormone-related signal transduction pathway in cells that serves as an essential component for the synthesis of organic compounds such as proteins, nucleic acids, and chlorophyll. Calcium ions are involved in the regulation of various metabolic pathways, and an appropriate calcium concen-

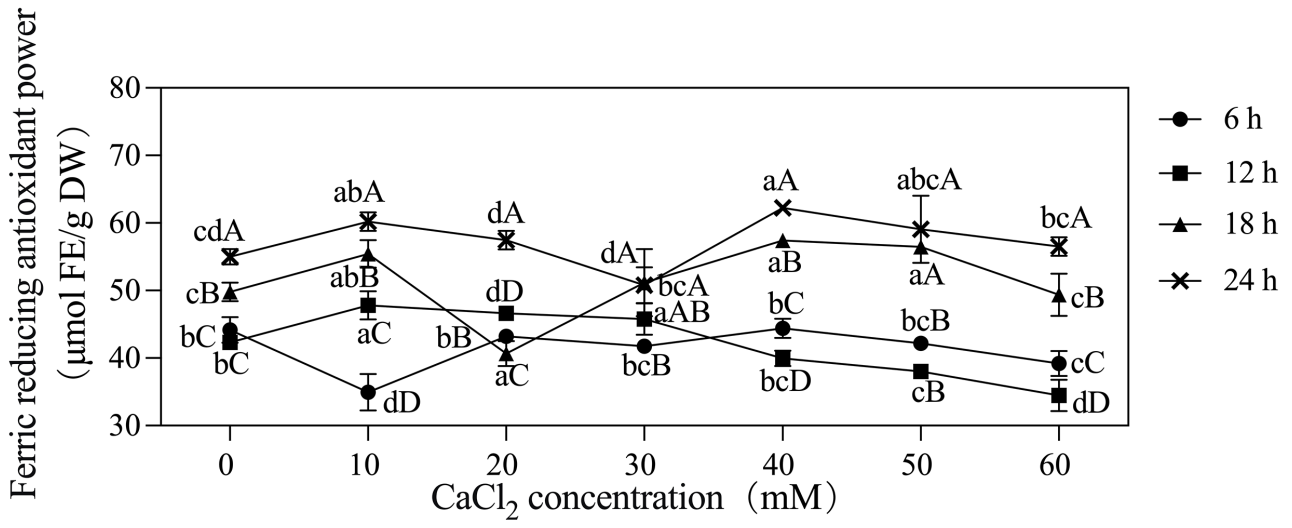

Fig. 5. Changes in ferric reducing antioxidant power (FRAP) of germinated quinoa under different $CaCl_2$ treatments. Note: Different lowercase letters indicate significant differences among treatments with different concentrations at the same time point, whereas different uppercase letters indicate significant differences among different time points at the same concentration ($p < 0.05$), whereas the same letter indicates no significant difference. FE, Fe^{2+} equivalent.

tration can promote the synthesis and accumulation of organic compounds in plants [42]. Excessively high exogenous calcium concentrations can induce osmotic stress and ion toxicity, thereby disrupting normal physiological functions of plant cells, inhibiting seed germination, and impairing overall plant growth [22]. Gao et al. [41] reported that exogenous calcium could enhance the salt tolerance of peanut seedlings by increasing the contents of soluble sugar and antioxidants such as flavonoids, as well as the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD).

According to the Pharmacopoeia of the People's Republic of China (2020 and 2025 editions), sprouts of limited lengths (3–12 mm) of millet, barley, rice, and other cereals have the functions of promoting digestion, harmonizing the stomach, strengthening the spleen, and stimulating appetite. Quinoa with appropriately restricted germination can moderately reduce the starch content, promote protein synthesis, and lower the glycemic index (GI) and glycemic load (GL), all of which are beneficial for reducing and stabilizing blood glucose levels. However, excessive germination of quinoa could result in a considerable loss of dry matter. This study showed that the sprout length of germinating quinoa was inhibited by exogenous calcium treatment, thereby promoting the synthesis of polyphenolic substances and enhancing the antioxidant capacity of germinated quinoa.

During plant growth, environmental factors such as drought, temperature and ion concentration can act as stimuli or stressors, while plants synthesize phytochemicals for seed protection or to facilitate seed dispersal [43]. Based on this seed evolutionary trait, exogenous calcium stress was applied here for quinoa germination. This led to the accumulation of calcium content and an increase in polyphenol content.

3.6 Recovered Salmonella spp. on Germinated Quinoa

From the perspective of food safety and industrial application, germinated quinoa should be considered not only as a calcium-biofortified product, but also as a sprout-type food requiring strict hygienic control. The warm and humid conditions used for germination can increase microbiological risks if there is inadequate seed disinfection, water quality, equipment sanitation, and incubation control. Salmonella can contaminate seeds and germinated seeds [44], thereby posing a health threat. In the current study, no suspected colonies of Salmonella were observed after culturing all fresh quinoa sprouts from up to 24 h of germination on BS and XLT agar plates, indicating negative test results for Salmonella. The pH range of the 10–60 mM calcium chloride solution was 5.75–6.55, and the solution contained no nutrients. A germination time of only 24 h means that such conditions are not conducive to the growth of Salmonella. Compared with previous reports of quinoa germination for 3 days, the sprouts in this study were shorter (<10 mm) and had a shorter germination time, which is more favorable for ensuring their microbiological safety [45]. For industrial-scale production, these results suggest that $CaCl_2$-assisted germination could be integrated with standardized hygiene management, routine microbial monitoring, process validation, and cold-chain control after germination. Therefore, the proposed approach has implications not only for improving calcium enrichment and functional quality, but also for developing safe and quality-controlled quinoa sprout products.

4. Discussion

In this work, $CaCl_2$ treatment increased calcium accumulation in a concentration-dependent manner, with the 60 mM treatment achieving the highest calcium level after 24 h. However, the functional indicators did not increase linearly with increasing $CaCl_2$ concentration. The 40 mM treatment produced the lowest PA content and PA/Ca ratio and also showed favorable TPC and FRAP values, whereas the 50–60 mM treatments favored calcium enrichment but partly reduced phenolic accumulation. These results indicate the response of germinating quinoa to exogenous $CaCl_2$ is concentration-dependent and involves a trade-off between mineral biofortification and functional quality.

Based on calcium content and nutrition-labelling considerations, the 50–60 mM $CaCl_2$ treatments met the criterion for calcium-rich germinated quinoa. However, when functional quality indicators were considered together, including PA content, PA/Ca ratio, TPC, ABTS and FRAP, the 40 mM $CaCl_2$ treatment showed a more balanced performance. Therefore, 50–60 mM $CaCl_2$ may be suitable when the main purpose is to obtain a calcium-rich product, whereas 40 mM $CaCl_2$ is recommended when the goal is to balance calcium enrichment with lower antinutritional factors and better antioxidant-related quality.

From a food safety perspective, germinated seeds are typically prepared under conditions of high humidity and suitable temperature. These are favorable for seed germination, but may also increase the risk of microbial contamination. Therefore, when developing germinated quinoa food products, attention should be paid to raw material cleaning, seed disinfection, hygienic control during germination, and drying and storage conditions. In the current study, quinoa seeds were surface-disinfected with sodium hypochlorite before germination, the germination process was carried out under controlled temperature and humidity conditions, and the germinated quinoa was tested for Salmonella. However, for practical food development, further evaluation of the microbiological safety, residual $CaCl_2$ levels, sensory quality, and storage stability of germinated quinoa products is needed. In particular, for calcium-fortified germinated quinoa, it is essential to ensure that the product meets food safety requirements and consumer acceptance while improving its mineral nutritional value.

The reduction in PA content and PA/Ca ratio should be interpreted as indirect evidence of improved potential calcium bioavailability rather than direct evidence of calcium absorption. This is because *in vitro* digestion, Caco-2 cell uptake, animal experiments, or human absorption trials were not performed in the current study. Further *in vitro* and *in vivo* studies are required to confirm the actual bioavailability of calcium from $CaCl_2$-treated germinated quinoa.

5. Conclusion

This study systematically investigated calcium accumulation, phenolic accumulation, PA reduction, and antioxidant capacity in germinated quinoa treated with different exogenous $CaCl_2$ concentrations. Exogenous $CaCl_2$ at 30–60 mM significantly inhibited sprout elongation while markedly promoting calcium accumulation. Based on calcium content and nutrition-labelling considerations, the 40 mM group after 24 h of germination and the 50–60 mM $CaCl_2$ treatment groups met the criterion for calcium-rich germinated quinoa. $CaCl_2$ treatment also reduced the PA content and PA/Ca ratio, with the 40 mM group showing the lowest PA/Ca ratio, suggesting improved potential calcium bioavailability. However, TPC exhibited a concentration-dependent biphasic response: moderate $CaCl_2$ concentrations promoted phenolic accumulation, whereas high $CaCl_2$ concentrations (especially 60 mM) decreased the TPC compared with the control group. $CaCl_2$ treatment also enhanced antioxidant capacity, including ABTS radical scavenging activity and ferric reducing power. Overall, 40 mM $CaCl_2$ may be more suitable for improving phenolic accumulation, antioxidant capacity, and the PA/Ca ratio, whereas 50–60 mM $CaCl_2$ may be better suited for calcium fortification when lower TPC and inhibition of sprout elongation are acceptable. Therefore, the selection of $CaCl_2$ concentration is dependent on the intended product target. These findings provide a practical basis for developing calcium-biofortified germinated quinoa products with improved nutritional and functional quality.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

DW: Writing—original draft, Methodology, Formal analysis, Data curation. MY: Writing—review & editing, Methodology, Conceptualization. HZ: Performed the experiments and collected the data, Writing—review & editing, Methodology. YL: Contributed to conceptualization and study design, reviewed and edited the manuscript. LL: Writing—review & editing, Methodology, Formal analysis, Conceptualization. XX: reviewed and edited the manuscript, Substantial contributions to the conception and design of the work. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

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