

Research article

Evaluation of the nutritional and functional properties of germinated quinoa and its protein isolate

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Abstract: Germination can help improve nutrient content, bioactive compounds, and bioavailability, as well as reduce antinutritional factors. Germinated quinoa is a promising alternative to cereal starches for the production of protein isolates of high nutritional and functional value. In this study, the quinoa varieties Tunkahuan and Excelencia were germinated and analyzed. Grain germination was carried out under controlled humidity and temperature conditions, the grain and rootlets were separated and protein isolates were obtained. The nutritional content of two varieties of desaponified quinoa, the germinated grain and the rootlets was determined. Yield, digestibility and anti-inflammatory activity of protein isolates from germinated grain and rootlets were determined. Germination significantly increased the concentration of protein (39.67 g and 35.55 g/100 g dry weight [dw] in the Tunkahuan and Excelencia varieties, respectively), fat (27.10 g and 29.80 g/100 g dw in the Tunkahuan and Excelencia varieties, respectively), and minerals, especially in the rootlets, which showed higher levels of bioavailable nutrients (e.g., phosphorus, potassium, zinc, iron and manganese) in both quinoa varieties. A better protein digestibility was recorded in the germinated grain isolates (85.91–86.87 g/100 g dw) compared to the root isolates (81.5 g/100 g dw) of both varieties. Furthermore, higher antioxidant, phenol, and flavonoid content was observed in the root isolates of both varieties. These results suggest that germination improves the nutrient content and bioactive properties of quinoa, highlighting the value of rootlets that constitute a valuable source for protein extraction and the development of new food ingredients.

Keywords: germination; protein isolate; rootlets; desaponified grain; isolate protein

1. Introduction

Quinoa (*Chenopodium quinoa Willd.*) is a pseudocereal originating from the Andes of South America, whose oldest archaeological evidence dates back to 5000 BC, recognized for its remarkable nutritional value and its ability to adapt to adverse environmental conditions [1]. Among its distinctive properties are a large number of bioactive compounds with a complete protein profile and a relevant content of essential amino acids, polysaccharides, saponins, and flavonoids that make it a key resource to strengthen global food security. This pseudocereal has gained relevance as a functional ingredient in various food formulations [2].

In recent years, research interest in quinoa has experienced significant growth, mainly due to its bioactive compounds, which have antioxidant, anti-inflammatory, and antidiabetic benefits [2]. Germination is a process that increases the bioavailability of nutrients and elevates the concentration of bioactive compounds. This procedure also reduces antinutritional factors, which positions germinated quinoa as a valuable ingredient for the preparation of foods with functional properties [3]. A study in which wheat flour was partially replaced with germinated pseudocereal flour in bread concluded that partial substitution with germinated quinoa and amaranth flour provides greater nutritional benefits because during germination, and particularly with the formation of rootlets, the bioavailable of nutrients increases, phytates and saponins are reduced, and bioactive peptides are generated [4].

Protein isolates from plant sources have gained relevance in research due to their high nutritional and functional value in the food industry [3]. Germinated quinoa represents a promising alternative to cereal starches for the production of isolates, which are obtained through a process of extraction and purification from a specific source, such as grains, seeds, dairy, and legumes. During this process, most of the carbohydrates, fats, and other nonprotein components are removed, leaving a high concentration ($> 90\%$) of pure protein. This is useful for those looking to increase their protein intake without adding too many calories. Protein isolates are easy to digest and quickly absorbed into the body, making them ideal for medical nutrition, people with dairy or gluten intolerance, and postworkout consumption [3].

This study aimed to determine the nutritional content of two varieties of desaponified quinoa; to carry out the germination process of the grain and chemically characterize both the germinated grain and the roots; to isolate the protein from the germinated grain and rootlets; and to evaluate the yield, digestibility, and functional properties of the protein isolate from the grain and rootlets. This study will allow to improve the nutritional and functional value of quinoa and explore its potential for producing protein isolates with significant applications in nutrition and for individuals with specific dietary needs.

2. Materials and methods

2.1. Materials

Grains of the following quinoa varieties were used: INIAP-Tunkahuan and INIAP-Excelencia. The first is an improved variety obtained by selection of a germplasm population collected in the province of Carchi (Ecuador) and released as variety in 1992, and its average yield is 2250 kg/ha [5]. INIAP-Excelencia is an improved early variety that comes from the crossing between INIAP-Tunkahuan and INIAP-Pata de Venado, and its average yield is 1838 kg/ha. The concentration of saponins in the two varieties is low (< 0.06 g/100 dw) [6].

2.2. *Quinoa desaponification and germination*

To remove saponin from quinoa was carried out using the technique suggested by Irigoyen and Giner [7], with certain modifications, which removes up to 80% of the saponins. The quinoa was washed with distilled water at a ratio 1:15 (grain:water) under agitation for 30 minutes to remove saponins, an antinutrient responsible for the bitter taste of the grain. Germination was carried out following the methodology described by Xing et al. [8], with some modifications. The process began by soaking the grains in distilled water for 2 h at 20 °C, after which the humidity was increased to 50%, and the seeds were placed in a germination chamber at 25 °C and 95% relative humidity. After 24 h, the germinated grains were dried in a forced-air dryer (Memmert, Büchenbach, Germany) for 5 h at 40 °C. At the end of the drying process, the rootlets were separated from the germinated grains and the rootlets and grains were ground separately using a Retsch mill (Hann, Germany) to achieve a sample size of 0.5 µm. The milled grains and roots were stored at 4 °C until analysis.

2.3. *Protein extraction*

Protein extraction was carried out using the technique suggested by Villacrés [9]. Germinated quinoa and rootlets were defatted and subsequently freeze-dried and ground to a particle size of 0.5 µm. A suspension of pulverized samples was prepared with distilled water at ratio 1:10 (w/v), the pH of the suspension was adjusted to 9.0 with 5 N NaOH solution. Each suspension was shaken for 1 h on an orbital shaker (Micromat, Lleida-Spain), then the samples were centrifuged (Wifug, Stockholm) at 3354×g for 20 min. The supernatant was recovered and acidified to pH 4.5 with 2N HCL. It was then centrifuged at 3354×g for 20 min and the precipitate containing the isolated protein was recovered, washed 3 times with distilled water, and freeze-dried in equipment (Labconco, Kansas, USA). The lyophilized samples were packed in polyethylene bags and stored at 4 °C until analysis.

2.4. *Chemical composition*

The following AOAC methodologies were used: humidity (925.09), fiber (978.10), crude protein (total N × 6.25) (955.39), ash (942.05), and the total carbohydrate content of the samples was calculated by the difference method (subtracting the percent crude protein, crude fiber, crude fat, and ash from 100%). Minerals content was determined by atomic absorption spectrophotometry in AA-700 atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan) following AOAC methods [10].

2.5. *Minerals*

The mineral content was carried out using the technique suggested by Bhinder et al. [11]. The quinoa samples were incinerated and subjected to acid digestion up to 100 ml and mineral analysis was performed. A calibration curve was made, with the values obtained from the reading of the standard (concentration vs. absorbance). The absorbance was measured in a GFA-7000 spectrophotometer (Shimadzu), and they were interpolated in the calibration curve. A value of the slope of the curve (0.0042) and the ordinate at the origin (0.0124) were developed. The values of the standard deviation of the slope and the standard deviation of the ordinate at the origin are determined to correspond to 0.01 and 0.572, respectively. Based on these data, confidence limits with a 95%

significance are established using the statistical parameter t-Student.

2.6. *In vitro digestibility of starch*

In vitro starch digestibility, including nutritionally important starch fractions (rapidly digestible [RDS], slowly digestible [SDS], and resistant starches [RS]), was determined using an enzymatic assay kit (Resistant Starch Assay Kit, Megazyme International Ireland) by the AACC method. Quinoa flour samples were incubated with a mixture of pancreatic α -amylase and amyloglucosidase in maleate buffer, pH 6.0, at 37 °C for up to 4 h with continual stirring [10]. Aliquots of the reaction solution were removed at 20 min to measure RDS, at 120 min to measure SDS, and at 240 min to measure total digestible starch (TDS) and RS. For RDS, SDS, and TDS, 1.0 mL aliquots were removed while the suspension was stirred and transferred to 20 mL of 50 mM acetic acid to terminate the reaction. These solutions were mixed thoroughly, and 0.1 mL aliquots were incubated with 0.1 mL of amyloglucosidase AMG (100 U/mL) to hydrolyze the remaining traces of maltose to glucose which was measured with glucose oxidase plus peroxidase (GOPOD) reagent.

A nonlinear model was used to describe the kinetics of starch hydrolysis and the first order equation was as following:

$$C = C\infty(1 - e^{-kt}), \quad (1)$$

where C (%) is the concentration at t (min), $C\infty$ (%) is the equilibrium concentration, k is the kinetic constant, and t is the time.

The hydrolysis index (HI) was calculated as the area under the starch hydrolysis curves, using white bread as a reference [12], and the predicted glycemic index (pGI) was estimated using the following equation:

$$pGI = 8,198 + 0,862 HI. \quad (2)$$

2.7. *In vitro digestibility of protein*

The method described by Bilgiçli et al. [13] was used with certain modifications. The multienzyme method relies on a rapid drop in pH as an indicator of proteolysis. Protein extracts were initially treated with NaOH (0.2 N) and incubated for 30 minutes. After incubation, 5 mL of HCl was added, and the pH of the solution was adjusted to 8. A multienzyme solution containing trypsin, chymotrypsin, and peptidase was subsequently added to initiate the digestion process. The pH was measured at 10 min, and the percentage of digestibility was determined with the following equation:

$$Y = 210,46 - 18,10x. \quad (3)$$

2.8. *In vitro anti-inflammatory activity*

The anti-inflammatory activity was carried out with the method described by Chandra et al. [14]. The quinoa extracts at different concentrations were incubated with egg albumin under controlled experimental conditions, with diclofenac sodium as the reference drug.

Absorbance and viscosity were determined to evaluate the anti-inflammatory property.

The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{Inhibición} = \left(\frac{V_t}{V_c} - 1 \right) * 100, \quad (4)$$

where, V_t is the absorbance of test sample and V_c is the absorbance of control. The extract/drug concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

2.9. *Hydrophilic antioxidant activity*

Trolox equivalent antioxidant capacity (TEAC) was determined by the 2'2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS) method. Extraction of the compounds was performed with 50% methanol and quantified with the ABTS solution. Simultaneously, a Trolox standard curve (2000 μM) was generated and the absorbance at 734 nm was measured using an Evolution 201 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). The results were expressed in μg Trolox Eq/g of dry sample [15].

2.10. *Total carotenoids*

The method described by Lachman et al. [16] was applied for the extraction of carotenoids. Approximately 0.125 g of quinoa flour samples were weighed and placed in 50 mL beakers and extracted for 2 days in the dark with 15 ml of 100% acetone. After extraction, the samples were treated with ultrasound for 15 minutes and filtered. The filtrate was made up to 25 mL with acetone. A UV-VIS spectrophotometer (Spectronic Helios γ , THERMO, GB) was used and the absorbance was measured at 662 nm, 645 nm, and 470 nm. The total carotenoid content was calculated from the following:

$$C_a = 11,75A_{662} - 2,35A_{645}, \quad (5)$$

$$C_b = 18,61A_{645} - 3,96A_{662}, \quad (6)$$

$$C_{x-c} = \frac{(1000A_{470} - 2,27C_a - 81,4C_b)}{227}, \quad (7)$$

where C_a is the content of chlorophyll a, C_b is the content of chlorophyll b, and C_{x+c} is the carotenoid content.

2.11. *Total phenolic content*

To determine the phenol content, the method described by Waterhouse [17] was used along with Folin Ciocalteu 2N reagent, which reacted with the added sodium carbonate and generated a blue color. The reagent was measured at 754 nm using an Evolution 201 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA), and the results are expressed in mg of chlorogenic acid/100 g of dry sample.

2.12. *Flavonoids determination*

Total flavonoid content (TFC) was determined according to the method described by Quettier-Deleu et al. [18]. In 1 mL of extract was added 1 mL of aluminum chloride solution. The reaction mixture was incubated at 20 °C in the dark for 30 minutes to allow the formation of the flavonoid-aluminum complex. Absorbance was measured at 510 nm against a methanol blank. The flavonoid content was expressed in mg quercetin/100 g dw.

2.13. *Statistical analysis*

All analysis were performed in triplicate. Results are expressed as mean \pm standard deviation. Data were analyzed using multifactorial ANOVA, with the INFOSTAT statistical package. Tukey's multiple range test was applied to determine significant differences at the 5% level.

3. Results and discussion

3.1. *Chemical composition*

Germination significantly affected the chemical composition of quinoa grain and rootlets ($p \leq 0.05$) as shown in Table 1. Significant decrease was observed in moisture of germinated quinoa compared to desaponified quinoa, from 11.14 to 7.06 g/100 g in Tunkahuan variety, a similar trend showed Excelencia. This behavior is consistent with results presented by Thakur et al. [19], when the humidity of germinated quinoa after 24 hours reached 9.71 g/ 100 g, and this may be related with the activation of enzymes that result in a conversion of starch and therefore reduces the water retention capacity causing the germinated quinoa to have a lower moisture content [20].

The ash content of the two germinated quinoa varieties was between 1.88 and 1.95 g/100 g dw, which is similar to the average (1.99 g/100 g dw) obtained by Thakur et al. [19]. The experimental values were lower than those shown for the desaponified quinoa. This may be due to the leaching of some minerals during the soaking of the grain prior to germination. Another factor may be the mobilization of some minerals towards the development of other structures of the grain (rootlets and cotyledons), as shown by the higher ash content of the rootlets (5.05 g/100 g dw) in the Tunkahuan variety. These results are consistent with those reported by Pathan and Siddiqui [21], who indicate that germinated quinoa has between 0.9 and 3.4 g/100 g dw.

Significant increase in fat content was observed in the germinated quinoa, particularly in Excelencia rootlets, reaching a value of 27.10, which could be due to the conversion of carbohydrates or proteins into lipids to serve as an energy reserve during rootlets and cotyledons development. Another reason could be the activation of lipases and acyltransferases promoting lipid resynthesis in different cellular structures [22].

Fiber content showed an increase in germinated grain and its roots compared to desaponified grain. These results agreed with those reported by Thakur et al. [19], when in grain germination trials they found an increase in crude fiber from 5.56 to 6.66 g/100 g dw. Some authors explain that this increase is due to the structural modification of polysaccharides and cell wall biosynthesis, which generates the production of new dietary fiber, since as the seed germinates, the cell wall structure is modified and part of this insoluble fiber is converted into soluble forms [20].

One of the most relevant findings was the significant increase of protein content in germinated and quinoa roots. The results obtained are consistent with those mentioned by Thakur et al. [19], when they initially reported a content of 14.94 g/100 g dw and after 24 h of germination this value increased to 16.14g/100 g dw. According to Suárez-Estrella et al. [23], this increase could be due to the release of proteins from the seed during germination when there is an increase in α -amylase activity causing the breakdown of the starch granule, resulting in a higher protein content in germinated quinoa.

On the other hand, carbohydrates in germinated quinoa decreased considerably. Thakur et al. [19] reported a decrease in quinoa carbohydrate content from 60.12 to 59.87 g/100 g dw after 24 h of germination. Lan et al. [24] indicated that carbohydrate content after germination is low. This is due to the conversion of starches into simple sugars during the germination process. This change has implications for the digestibility and bioavailability of nutrients [3].

Table 1. Effect of germination on the proximal composition of the grain and rootlets of two varieties of quinoa*.

	Tunkahuan			Excelencia		
	Desaponified grain	Germinated grain	Rootlets	Desaponified grain	Germinated grain	Rootlets
Moisture	11.14 \pm 0.05 ^a	7.06 \pm 0.07 ^d	9.08 \pm 0.56 ^b	10.61 \pm 0.05 ^a	6.09 \pm 0.11 ^e	8.15 \pm 0.23 ^c
Ash	2.89 \pm 0.05 ^b	1.88 \pm 0.03 ^d	5.05 \pm 0.13 ^a	2.37 \pm 0.05 ^c	1.95 \pm 0.05 ^d	5.05 \pm 0.13 ^a
Fat	6.59 \pm 0.05 ^f	9.20 \pm 0.05 ^d	27.10 \pm 0.05 ^b	7.63 \pm 0.05 ^e	9.40 \pm 0.05 ^c	29.80 \pm 0.05 ^a
Fiber	4.10 \pm 0.05 ^e	4.41 \pm 0.05 ^{bc}	4.28 \pm 0.05 ^{cd}	4.20 \pm 0.05 ^{de}	5.09 \pm 0.05 ^a	4.50 \pm 0.05 ^b
Protein	13.17 \pm 0.05 ^e	19.23 \pm 0.09 ^c	39.67 \pm 0.26 ^a	13.08 \pm 0.05 ^e	15.81 \pm 0.06 ^d	35.55 \pm 0.41 ^b
Carbohydrates	61.80 \pm 0.25 ^{ab}	58.52 \pm 0.14 ^c	14.82 \pm 0.41 ^e	62.55 \pm 0.08 ^a	61.22 \pm 0.25 ^b	16.95 \pm 0.40 ^d

Note: *g/100 g dw. Different letters in the same row indicate significant differences ($p \leq 0.05$). Mean value \pm SD (n = 3).

3.2. Minerals

Germination caused an increase in the concentration of most minerals in the rootlets, while in the germinated grain decreased (Table 2). This is because during germination the grain metabolizes its nutrient reserves to activate metabolic pathways, such as enzyme synthesis and the formation of new cells [23]. In the germination process, the radicle develops the ability to absorb minerals from the grain, causing a higher concentration of these nutrients in the rootlets [25]. This result suggests that these components of the germinated grain could be a rich source of bioassimilable nutrients collected in the early phase of its development [26].

The P values increased significantly ($p \leq 0.05$) in the rootlets of Tunkahuan to an average of 0.83 g/100 g dw, while in the germinated grain 0.32 g/100 g dw and in the desaponified grain 0.37 g/100 g dw were recorded. The same trend was observed for the Excelencia variety. The potassium content increased in the roots of the two quinoa varieties, up to 2.38 g/100 g dw in Tunkahuan and 2.48 g/100 g dw in Excelencia. Calcium concentration did not vary significantly ($p \leq 0.05$) with the germination process. These values are consistent with those mentioned by Bhinder et al. [11] who reported slight variations of this mineral in germinated grains, radicles and rootlets.

In the two quinoa varieties, the magnesium content of the rootlets increased at the expense of the germinated grains, which showed a lower content compared to the desaponified grains. The sulfur

values increased from 0.12 g/100 g dw in desaponified grain to 0.16 g/ 100 g dw in germinated grain and 0.255 g/ 100 g dw in the rootlets of Tunkahuan variety. While in the Excelencia variety, an increase from 0.09 to 0.15 and 0.20 g/100 g dw was recorded.

The two desaponified quinoa varieties showed the lowest sodium content (0.01 mg/kg dw), which increased with the germination process to 64.00 mg/kg dw in Tunkahuan and 75.50 mg/kg dw in Excelencia. The highest content was found in the rootlets, 99.00 mg/kg dw (Tunkahuan) and 94.00 mg/ kg dw (Excelencia). In the two quinoa varieties, the content of iron and zinc increased in the rootlets at the expense of the germinated grain, which showed the lowest concentration of these microelements. In this regard, Demir and Bilgiçli [27], reported that germination for 48 h produces an increase in the content of these minerals, while Darwish et al. [3] reported an increase of these minerals after 72 h of germination.

The copper content increased in the germinated quinoa and in the roots, which showed the highest content (14.30 mg/ kg dw) in Tunkahuan and 10.60 mg/kg dw in Excelencia variety. These values are similar those reported by Thakur et al. [19], when they indicated that the copper content in quinoa increased from 6.55 to 8.25 mg/kg after 24 h of germination. The manganese content showed a significant increase ($p \leq 0.05$) in the roots with values of 56.00 mg/kg dw (Tunkahuan) and 38.30 mg/kg dw (Excelencia), in relation to desaponified and germinated quinoa.

Table 2. Effect of germination on the mineral content of the grain and rootlets of two varieties of quinoa*.

Tunkahuan			Excelencia		
	Desaponified grain	Germinated grain	Rootlets	Desaponified grain	Germinated grain
g/100 g dw	P	0.37 ± 0.03 ^c	0.32 ± 0.02 ^c	0.83 ± 0.05 ^a	0.47 ± 0.02 ^b
	K	0.67 ± 0.01 ^b	0.43 ± 0.05 ^c	2.38 ± 0.05 ^a	0.75 ± 0.03 ^b
	Ca	0.07 ± 0.01 ^a	0.06 ± 0.01 ^a	0.07 ± 0.01 ^a	0.07 ± 0.01 ^a
	Mg	0.18 ± 0.02 ^{ab}	0.13 ± 0.05 ^b	0.21 ± 0.04 ^{ab}	0.24 ± 0.03 ^a
	S	0.12 ± 0.01 ^{bc}	0.16 ± 0.04 ^{abc}	0.25 ± 0.05 ^a	0.09 ± 0.02 ^c
mg/kg	Na	0.01 ± 0.01 ^e	64.00 ± 0.05 ^d	99.00 ± 0.21 ^a	0.01 ± 0.01 ^e
	Zn	75.50 ± 0.71 ^c	66.90 ± 0.02 ^d	103.60 ± 0.05 ^a	24.03 ± 0.06 ^f
	Cu	8.19 ± 0.41 ^c	8.60 ± 0.05 ^c	14.30 ± 0.05 ^a	5.70 ± 0.01 ^d
	Fe	95.53 ± 0.28 ^c	57.50 ± 0.05 ^d	226.00 ± 0.03 ^a	47.07 ± 0.12 ^e
	Mn	22.40 ± 0.56 ^c	15.30 ± 0.01 ^d	56.00 ± 0.05 ^a	10.51 ± 0.01 ^e

Note: Mean value ± SD (n = 3). Different letters in the same row indicate significant differences ($p \leq 0.05$).

3.3. *In vitro digestibility of starch*

Starch and starchy foods can be classified according to their digestibility. TDS, RDS and SDS of

desaponified quinoa, germinated quinoa, and roots are shown in Table 3. Total digestible starch (TDS) showed higher values in the germinated grain with respect to the desaponified quinoa and the rootlets, this could be linked to the germination process that favors the enzymatic hydrolysis of starch [8]. Sai Srujana [28] reported 58.20 g/100 g dw for the TDS of germinated quinoa for 4 h and indicated that a longer germination time could increase this value. This behavior is consistent with results presented by Guardianelli et al. [26] for germinated quinoa. They reported that germination could be related to the activation of amylases and other enzymes, which reduces the structure of the starch, acting on the surface of the grain and forming pores. Rootlets showed the lowest TDS in relation to desaponified and germinated quinoa. This may be related to the lower starch content in the rootlets and its partial hydrolysis by amylases to provide energy for radicle development, which can make the starch in the root less accessible to digestive enzymes.

RDS also showed a significant increase in germinated grains, indicating a higher availability of rapidly digestible starch compared to desaponified grains. Similar results have been obtained by other authors when germination grains such as barley, amaranth and quinoa [7]. SDS decreased significantly in germinated grains and rootlets. This may be related to the biochemical changes that starch undergoes in the germination process [28].

RS content was higher in desaponified quinoa compared to germinated quinoa and roots, suggesting that in desaponified quinoa the starch is in its compact form, a fraction of which (5.14 g/100 g dw) is able to resist digestion and remains intact throughout the gastrointestinal tract. However, due to the effect of germination, the RS content decreased in the grain and in the rootlets (Table 3). This could be linked to the greater accessibility of starch to enzymatic action during grain hydration and germination. In this process, enzymes such as α -amylase and β -amylase are activated, which break down starch into simpler and fermentable molecules, such as maltose and glucose [28].

Table 3. In vitro digestibility of starch and Glycemic Index of two quinoa varieties.

	TDS*	RDS*	SDS*	RS*	Glycemic index
Tunkahuan	Desaponified grain	48.25 \pm 0.05 ^c	9.93 \pm 0.07 ^c	23.04 \pm 0.04 ^b	5.14 \pm 0.01 ^a
	Germinated grain	69.38 \pm 0.07 ^a	46.95 \pm 0.05 ^a	18.29 \pm 0.07 ^c	0.64 \pm 0.07 ^b
	Rootlets	28.07 \pm 0.04 ^d	17.56 \pm 0.04 ^c	7.76 \pm 0.03 ^e	0.42 \pm 0.02 ^{cd}
Excelencia	Desaponified grain	48.33 \pm 0.01 ^c	10.07 \pm 0.04 ^e	22.16 \pm 0.03 ^a	5.15 \pm 0.04 ^a
	Germinated grain	67.25 \pm 0.02 ^b	46.10 \pm 0.07 ^b	12.90 \pm 0.07 ^d	0.57 \pm 0.03 ^{bc}
	Rootlets	20.62 \pm 0.04 ^e	14.75 \pm 0.06 ^d	4.32 \pm 0.02 ^f	0.36 \pm 0.03 ^d
Note: *g/100 g dw. Different letters in the same column indicate significant differences ($p \leq 0.05$). Mean value \pm SD ($n = 3$). TDS = Total Digestible Starch, RDS = Rapidly Digestible Starch, SDS = Slow Digestible Starch, RS = Resistant starch.					

The predicted glycemic index (pGI) for desaponified quinoa, germinated quinoa, and its roots are shown in Table 3. pGI results differed significantly varying between 43.74 of Excelencia rootlets to 62.111 of desaponified Excelencia variety. The pGI was affected by the amount of RDS present. In particular, the RDS content had an inverse relationship with pGI. Higher percentage of RDS in starch generally relate to a higher degree of starch digestion and consequently with a lower degree of pGI [29]. pGI is related to nutritional quality of food and a product with a low GI is preferable not only in individuals with diabetes, but also in healthy individual [29]. Considering the in vitro digestibility results of germinated quinoa and its rootlets, these might be a potential alternative to cereal

starches in the formulation of products for diabetics and weight management and could lead to the formulation of novel foods characterized by the slow release of glucose (i.e., low glycemic index and prevention of fasting hypoglycemia). Moreover, the bland taste of germinated quinoa could represent an advantage over the cereal starches as uncooked ingredient because to its high TDS content and the absence of the specific cereal flavor [29].

3.4. Protein extraction yield

The high protein content of the germinated grain and roots led to the isolation of this nutrient in order to determine its digestibility, functional properties and enhance the properties of quinoa. The results of this trial are shown in Table 4.

The protein extraction yield was significantly higher from the rootlets (15.36%) compared to the protein extracted from germinated grains (7.80%) of Tunkahuan variety, a similar trend showed the Excelencia variety. These results relate to the protein content of the germinated grain and the rootlets and are consistent with those reported by Mir et al. [30], when they obtained quinoa protein isolate yields of 8.12%–12.22%. Interestingly, the germination process helped to concentrate the protein in the grain and rootlets, however, in the extraction process a better yield is obtained from the latter, this could be linked to the lower carbohydrate content in the rootlets, which facilitates the protein extraction process [30].

3.5. In vitro digestibility of protein

Digestibility of germinated quinoa protein isolates was between 81.5% and 86.8% (Table 4) calculated using Eq 3. Vilcacundo et al., [32] mentions that the digestibility of quinoa protein isolate is 82.10%. The increase in digestibility in germinated quinoa isolates may be related to the enzymatic activation of proteases, the reduction of grain antinutrients and the increase of free amino acids due to germination [32]. However, the digestibility of the protein from the roots was lower than that of raw quinoa grain, which could be linked to the higher concentration of structural fiber (cellulose and lignin) that hinders the accessibility of digestive enzymes to the proteins. Other factors may be the presence of insoluble complexes formed from tannins, phytates, and protein and the lower enzyme activity, which hinders the hydrolysis of proteins into peptides and amino acids and interferes with their digestion and absorption [33].

3.6. In vitro anti-inflammatory activity

Protein isolated from the germinated grains showed higher in vitro anti-inflammatory activity compared to protein isolated from the rootlets (Table 4). This may be related to the higher antioxidant capacity and presence of bioactive compounds with anti-inflammatory activity in the protein isolated from the germinated grain [34]. The lower in vitro anti-inflammatory activity of protein isolated from roots correlated ($r^2 = 0.91$) with its lower flavonoid content and antioxidant capacity (Table 5), a value obtained using equation 4. Other compounds that could increase the anti-inflammatory activity of the protein isolated from germinated quinoa are peptides from the partial hydrolysis of proteins during the germination process. Bioactive peptides may influence the regulation of inflammation through molecular mechanisms and may act by modulating inflammatory cytokines and reducing the release

of proinflammatory mediators [35]. The results of this study highlight the importance of protein isolated from germinated quinoa as a functional food with potential to alleviate inflammatory processes.

Table 4. Yield, digestibility and anti-inflammatory activity of protein isolate of two quinoa varieties.

	Source of protein isolate	Yield (g/100 g dw)	Protein digestibility (g/100 g dw)	Anti-inflammatory activity (IC50 μ g/mL)
Tunkahuan	Germinated grain	7.80 \pm 0.09 ^c	86.87 \pm 0.11 ^a	82.48 \pm 0.04 ^b
	Rootlets	15.36 \pm 0.21 ^a	81.51 \pm 0.25 ^c	80.89 \pm 0.03 ^d
Excelencia	Germinated grain	7.14 \pm 0.06 ^c	85.91 \pm 0.06 ^b	84.30 \pm 0.08 ^a
	Rootlets	12.04 \pm 0.23 ^b	81.54 \pm 0.11 ^c	81.88 \pm 0.04 ^c

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$). Mean value \pm SD ($n = 3$).

3.7. *Hydrophilic antioxidant activity*

Significant differences ($p \leq 0.05$) were observed in antioxidant activity, carotenoids concentration, phenols, and flavonoids of Tunkahuan germinated quinoa (GQT), Tunkahuan protein isolated from GQT (PIGQT), Tunkahuan rootlets (RT), Tunkahuan protein isolated from RT (PIRT), Excelencia germinated quinoa (GQE), Excelencia protein isolated from GQE (PIGQE), Excelencia rootlets (RE), and Excelencia protein isolated from RE (PIRE) (Table 5).

The antioxidant activity of PIGQT, PIRT, PIGQE, and PIRE, determined by the DPPH method was higher than that determined by the ABTS method, suggesting that the protein isolates have a higher efficiency in neutralizing DPPH free radicals than the free radicals generated in the ABTS assay due to differences in the reaction mechanisms and chemical characteristics of both methods [12,35]. On the other hand, protein isolated from germinated grains and roots showed higher antioxidant capacity than germinated grains and their rootlets. These results were directly related to the higher content of phenols and flavonoids in the protein isolated and agreed with Piñuel et al.'s [36] results of higher biological activity of protein isolates from germinated grains.

Ramos-Pacheco et al. [37] also reported that phenolic compounds (simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, and lignans) increased upon germination, which influenced the increase in antioxidant capacity. They reported 21.92 μ mol TE/g for the antioxidant activity of white quinoa after 24 hours of germination and 25.28 μ mol TE/g for black quinoa after 72 hours of germination. Our results confirm the relationship between quinoa variety, germination time, protein isolated origin and antioxidant capacity.

3.8. *Total carotenoids*

Significant differences ($p < 0.05$) were observed in the total carotenoids of germinated quinoa and protein isolated (Table 5), determined using equation 7. Germinated grains and roots of the Excelencia variety showed higher carotenoid levels (22.93 and 24.13 μ g/g), while the lowest values corresponded to the protein isolates of the two quinoa varieties. Similar results have been obtained by other authors when quinoa was germinated for 24 h [3]. The lower carotenoid content in the protein isolated may be related to the degradation of carotenoids in the protein isolation process, the solubility

differential of these two nutrients, the elimination of lipid fractions, oxidation or heat sensitivity, and the interaction of carotenoids with other cellular fractions that are not part of the protein isolate [38]. During protein precipitation, different components of the food are separated. Carotenoids tend to be associated with lipid fractions of the food, such as cell membranes, which are generally not included in the protein fraction. As a result, carotenoids are not concentrated in the protein fraction, which can lead to a decrease in their concentration in the final product [3].

3.9. Total phenolic and flavonoids

The concentration of phenols and flavonoids increased considerably in protein isolated from germinated grains and rootlets (Table 5); the latter presented the highest levels of these bioactive compounds in the two quinoa varieties, suggesting that protein extraction improves the content of these metabolites. Similar values of total phenols (671 mg/100 g dw) in germinated quinoa were reported by Sai Srujana [28]. Regarding flavonoids, Ramos-Pacheco et al. [37] reported values between 58.02–120.51 mg quercetin/100 g dw after 24 h of germination, these values are similar to those obtained in this study.

The higher concentration of phenols and flavonoids in the rootlets may be due to the higher metabolic activity of the grain during germination, a process that activates the synthesis of secondary metabolites as part of the plant's natural defenses. In addition, in the process of protein isolation, solvents are generally used to separate proteins from other fractions, such as carbohydrates and fats [3]. In the process of protein isolation, a higher concentration of flavonoids and polyphenols can be extracted, these compounds are soluble in water or organic solvents [37]. Other authors indicate that proteins can act as a protective matrix for flavonoids and polyphenols, which facilitates the preservation of these compounds [28].

Table 5. Functional compounds and antioxidant capacity of germinated quinoa and protein isolated of two varieties of quinoa.

		ABTS	DPPH	Carotenoids	Phenols	Flavonoids
		uM Trolox Eq./g		ug/g	mg chlorogenic/100 g dw	mg quercetin/100 g dw
Tunkahuan	GQT	45.77 ± 0.06 ^f	48.23 ± 0.07 ^f	21.18 ± 0.02 ^d	654.63 ± 0.07 ^g	68.97 ± 0.01 ^h
	PIGQT	56.21 ± 0.04 ^d	75.31 ± 0.05 ^d	8.54 ± 0.06 ^f	1182.07 ± 0.06 ^c	167.97 ± 0.02 ^d
	RT	32.14 ± 0.06 ^h	34.18 ± 0.02 ^h	23.64 ± 0.05 ^b	871.80 ± 0.04 ^e	79.51 ± 0.03 ^f
	PIRT	64.44 ± 0.05 ^b	76.47 ± 0.02 ^b	3.31 ± 0.02 ^g	1531.01 ± 0.04 ^a	177.41 ± 0.04 ^b
Excelencia	GQE	47.17 ± 0.06 ^e	49.85 ± 0.03 ^e	22.93 ± 0.03 ^c	606.20 ± 0.07 ^h	71.08 ± 0.07 ^g
	PIGQE	56.41 ± 0.02 ^c	75.84 ± 0.05 ^c	9.71 ± 0.07 ^e	1068.68 ± 0.10 ^d	169.63 ± 0.08 ^c
	RE	32.76 ± 0.02 ^g	35.17 ± 0.02 ^g	24.13 ± 0.03 ^a	836.37 ± 0.02 ^f	86.50 ± 0.01 ^e
	PIRE	66.24 ± 0.04 ^a	77.22 ± 0.02 ^a	2.20 ± 0.02 ^h	1464.54 ± 0.07 ^b	186.68 ± 0.04 ^a

Note: Different letters in the same column indicate significant differences ($p \leq 0.05$). Mean value $\pm SD$ ($n = 3$). GQT = Tunkahuan's germinated quinoa, PIGQT = Tunkahuan's protein isolated from GQT, RT = Tunkahuan's rootlets, PIRT = Excelencia's protein isolate from RT. GQE = Excelencia's germinated quinoa, PIGQE = Excelencia's protein isolated from GQE, RE = Excelencia's rootlets, PIRE = Excelencia's protein isolate from RE.

4. Conclusions

The germination had a positive impact on the chemical composition of quinoa Tunkahuan and Excelencia, showed an increase in fat and protein content in the grain and rootlets. These showed a higher content of ash and minerals, such as phosphorus, potassium, magnesium, iron, zinc, and copper. The study on in vitro starch digestibility, including nutritionally important starch fractions, showed an increase in TDS and RDS and a decrease in SDS and RS of the germinated grain, with respect to the desaponified grain. This behavior influenced the decrease of glycemic index in the germinated grain, which might be a potential alternative to cereal starches in the formulation of products for diabetics and weight management and could lead to the formulation of novel foods characterized by the slow release of glucose and low glycemic index. The yield of protein isolate was higher in the Tunkahuan rootlets. However, the in vitro digestibility of the protein isolated from the germinated grain was higher than that obtained from the rootlets, which could be related to the higher concentration of structural fiber that hinders the accessibility of the digestive enzymes to the proteins. Protein isolates from germinated grain and rootlets showed higher phenol and flavonoid content, which influenced their higher antioxidant and anti-inflammatory activity. The results of this study highlight the importance of protein isolate from germinated quinoa as a functional food with potential to alleviate oxidative and inflammatory processes. These products can also help the food industry to have suitable proteins for the development of new foods with high digestibility and adapted functional properties.

Author contributions

Pamela Venegas: Conceptualization, Methodology, Investigation, Writing – Original Draft; Elena Villacrés: Supervision, Validation, Writing – Review & Editing; María Quelal: Supervision, Validation, Writing – Review & Editing; María Morales: Supervision, Writing – Review & Editing. All authors have read and agreed to the published version of the manuscript.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

All authors declare that they have no conflict of interest.

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