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COMPARATIVE ANALYSIS OF PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF SPROUTED AND UNSPROUTED *CHENOPODIUM QUINOA* WILLD. SEEDS: INSIGHTS INTO NUTRITIONAL VALUE AND FUNCTIONAL PROPERTIES

– Research paper –

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Abstract: Our study explored the phytochemical composition and biological properties of sprouted and unsprouted quinoa seed extracts (red, black, and yellow varieties). We observed distinct differences in primary metabolites between sprouted and unsprouted seeds, particularly in carbohydrates, fats, and proteins. Sprouted seeds exhibited increased levels of simple soluble sugars, amino acids, and fatty acids. Additionally, sprouted seeds showed higher quantities of polyphenols and flavonoids, with the black variety displaying the most. However, antioxidant activity did not directly correlate with phenol and flavonoid content, with unsprouted seeds demonstrating superior performance. Conversely, unsprouted seed extracts displayed greater anti-inflammatory efficacy, attributed to saponin loss during germination. Specifically, the best carbohydrate value was found in sprouted red seeds at 21.55±1.07 mg/g of plant material, the highest protein content was in sprouted red seeds at 4.6±0.28 mg/g, and the best lipid content was in sprouted black seeds at 2.97±0.13 mg/g. The highest polyphenol content was in sprouted black seeds at 10.74±0.6 mg E GA/g Ex, while the highest flavonoid content was also in sprouted black seeds. The most effective antioxidant activity in the DPPH test was observed in non-sprouted black seeds with an IC50 of 0.06±0.01 mg/ml. The highest hemolysis inhibition was found in sprouted red seeds at 49.04% at 0.8 mg/ml, and the greatest anti-inflammatory activity was in non-sprouted yellow seeds at 1.6±0.07 mg E Dc/mg Ex.

Keywords: *Chenopodium quinoa* Willd, non-sprouted seeds, Sprouted seeds, Polyphenols, Flavonoids, Biological activity.

INTRODUCTION

Chenopodium quinoa Willd, commonly known as quinoa, hails from the Andean region of South America and is part of the Amaranthaceae family (Romero-Benavides et al., 2023). Recognized for its herbaceous nature, quinoa typically grows between 1 to 2 meters in height, featuring delicate green leaves and dense panicles of flowers (Guillaume, 2019). Its versatility extends to both its seeds and leaves, which are utilized for human nutrition (Amin et al., 2022). Rich in proteins and essential nutrients, quinoa is a valuable addition to diets, offering all essential amino acids crucial for tissue growth and development (James, 2009). Its abundance of vitamins and minerals further bolsters the immune system, reducing disease risks and enhancing productivity (James, 2009). This resilient plant exhibits remarkable resistance to environmental stressors like drought and salinity, making it suitable for cultivation in challenging conditions (Sanchez et al., 2003). Moreover, its low water requirement compared to other crops contributes to sustainability efforts (Hirich et al., 2012).

The consumption of quinoa has grown globally due to its exceptional nutritional profile and adaptability. Quinoa can be prepared in various ways, including boiling, steaming, and even baking, making it a versatile ingredient in numerous culinary applications (Mu et al., 2023). Cooking typically involves rinsing the seeds to remove saponins, which are bitter-tasting compounds, followed by boiling them in water. Quinoa can be used as a base for salads, a substitute for rice or pasta, or even as a flour for baking (Arya and Kakoli., 2019).

Quinoa varieties are diverse, with thousands of types cultivated across different regions, each adapted to specific environmental conditions. The major varieties include white, red, and black quinoa, each with unique characteristics and

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nutritional profiles (Hussain et al., 2021). White quinoa is the most common and has a mild flavor and light texture. Red quinoa holds its shape better after cooking, making it suitable for salads. Black quinoa has an earthier flavor and a crunchier texture.

Overall, *Chenopodium quinoa* Willd emerges as a nutritionally rich and adaptable species with agricultural and nutritional benefits, supporting global food security and sustainability initiatives (Adetunji et al., 2021). This study aims to compare the phytochemical composition and biological activities of sprouted and unsprouted quinoa seeds, shedding light on their nutritional value and functional properties, offering insights into the benefits of incorporating both forms into human diets.

MATERIALS AND METHODS

Plant materials and preparation of methanolic extracts

In this study, the seeds of three varieties of quinoa (red, black, and yellow) grown in the Sidi Aoun region of the Magrane district, located northeast of the capital of El Oued Province, were selected.

We washed the unsprouted seeds to get rid of any stuck-on impurities. They were ground and stored in paper bags. As for germinated quinoa, we prepared the seedlings according to Carciochi et al. (2018). This process involves sterilizing the seeds of three quinoa varieties by soaking them in a sodium hypochlorite (NaClO) solution for 10 minutes (at a concentration of 1V/1V). Following sterilization, the seeds are rinsed with water and soaked for 24 hours. Subsequently, the seeds are filtered from the water and placed in aluminum dishes, where they are sown on leaves and irrigated. After 24 hours, germination is observed in the seeds of all three varieties, with ongoing watering for seven days. Notably, there is variation in the germination rate among the varieties: black seeds exhibit the fastest germination, followed by red seeds, while yellow seeds demonstrate slower growth.

A total of 25 grams of dehydrated botanical substance were immersed in 250 milliliters of 99% methanol at room temperature, protected from light, for a period of 24 hours. Subsequently, the solution was filtered, and the solvent was removed via evaporation using a rotary evaporator, specifically the Buchi R-200 model, maintained at a temperature of 50°C. This procedure yielded the methanolic crude extracts (Chouikh et al., 2021).

Estimation of primary metabolites (carbohydrates, proteins and lipids)

The methodology utilized in this study was adapted from Chouikh et al. (2020). One gram of plant material was mixed with 5 milliliters of trichloroacetic acid (TCA) and agitated on a magnetic shaking device for 5 minutes. Subsequently, the mixture underwent centrifugation at 3000 revolutions per minute for 10 minutes, resulting in the separation of supernatant used for carbohydrate quantification, while the first deposit (deposit 1) underwent an additional centrifugation with 2 milliliters of ether/chloroform (V/V) for 10 minutes at the same speed. The second supernatant was collected for lipid analysis, and the second deposit was treated with 2.5 milliliters of a 0.1 M solution of sodium hydroxide (NaOH) for protein determination**.**

Carbohydrate estimation followed the method described by DuBois et al. (1956), utilizing a mixture of phenol (5%) and concentrated sulfurous acid, with absorbance measured at a wavelength of λ=490 nanometers. Glucose was used as the calibration standard, and the results were expressed in milligrams of carbohydrates per gram of plant material**.**

Protein estimation was conducted following the protocol outlined by Classics-Lowry et al. (1951), involving the use of Folin-Ciocalteau reagent (V/V), 0.1 M NaOH, 0.5% $CuSO_4$, and 0.1% $KNaC_4H_4.O_64H_2O$. Bovine Serum Albumin (BSA) served as the standard, with absorbance measured at 750 nanometers using a UV spectrophotometer. The results were quantified in milligrams of proteins per gram of plant material**.**

Lipid estimation was performed following the method described in Goldsworthy et al. (1972) with certain modifications, utilizing Sulfophosphovanillinic reagent and concentrated sulfurous acid, and subjecting reaction tubes to a water bath at 100°C. Absorbance readings were taken at a wavelength of λ=530 nanometers, using Soy as the standard, and outcomes were expressed in milligrams of lipids per gram of plant material.

Quantification of phytochemical compounds

The determination of the total polyphenol content in the extract was performed following the Folin-Ciocalteu method, as described by Żurek et al. (2023), with slight modifications. In this procedure, 0.4 milliliters of the sample solution

were added to a test tube containing 2 milliliters of Folin-Ciocalteu reagent (10%) and 1.6 milliliters of Na_2CO_3 (7.5%). After incubating at room temperature for 30 minutes, absorbance was measured at a wavelength of 765 nanometers using a Shimadzu UV-Vis spectrophotometer. The total phenolic content was then calculated and expressed as milligrams of gallic acid equivalents per gram of the extract. This updated methodology ensures accurate assessment and documentation of the total phenolic content in the extract**.**

The quantification of flavonoids was carried out using the methodology established by Chaalal et al. (2023). The process involved combining 0.5 milliliters of the initial methanol plant extract with 0.5 milliliters of a 2% $AlCl_3$ solution after a 15-minute incubation period. The absorbance at 430 nanometers was measured using a spectrophotometer to determine the flavonoid content in milligrams of quercetin equivalent (QE) per gram of the extract.

Estimation of antioxidant activity

The extracts were assessed for their DPPH· scavenging activity following the methodology outlined by Chouikh (2020). Briefly, 1 mL of each extract at various concentrations was mixed with 1 mL of DPPH solution (10^{-4} mol) in methanol. After incubation at room temperature for 15 minutes, the absorbance was measured at 517 nm. The inhibition activity was determined using the formula [1] (Kehal et al., 2023):

$$\boldsymbol{Inhibition}(\%) = \left[\left(\boldsymbol{Absorbance_{Control}} - \boldsymbol{Absorbance_{Sample}}\right)/\boldsymbol{Absorbance_{Sample}}\right] \times \mathbf{100} \quad [1]$$

The IC_{50} value, representing the concentration at which 50% of free radicals were scavenged by the extract, was calculated by conducting linear regression analysis on the concentration versus percentage inhibition relationship. A lower IC_{50} value indicates higher antioxidant capacity. This method provides a reliable assessment of the extracts' ability to neutralize DPPH radicals, offering valuable insights into their antioxidant potential.

Additionally, a hemolysis test was performed to evaluate the extracts' ability to protect erythrocyte blood cells from membrane damage caused by oxidative stress and free radicals, as described by Ben Ali et al. (2023). In this assay, 40 µL of human erythrocytes were mixed with 2 mL of the plant extract and incubated for 5 minutes at 37 °C. Subsequently, 40 µL each of hydrogen peroxide (30×10^{-3} M), ferric chloride (80×10^{-3} M), and ascorbic acid solution (50×10^{-3} M) were sequentially added. After incubation for 1 hour at 37 °C, the mixture underwent centrifugation at 700 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm, and the hemolysis percentage was calculated using the formula [2]:

$$\boldsymbol{Hemolysis}\% = \left(\boldsymbol{Absorbance_{Control}}/\boldsymbol{Absorbance_{Sample}}\right) \times \mathbf{100} \quad [2]$$

Anti-inflammatory activity (Albumin denaturation test)

The anti-inflammatory properties of crude extracts from Quinoa were evaluated using the albumin denaturation model, following a modified protocol based on Elias & Rao and Padmanabhan & Jangle, as described by Ben Ali et al. (2023). In this assay, 1 ml of the extract at various concentrations was mixed with 1 ml of bovine serum albumin (5%). After a 15-minute incubation period at 27°C, the samples were further incubated in a water bath at 70°C for 10 minutes. Subsequently, the absorbance of the samples was measured at 660 nm after they had cooled to room temperature. Diclofenac sodium was employed as a reference standard. The results were expressed as milligrams of diclofenac sodium equivalent per milligram of extract.

Statistical study

The results of the study were processed by applying statistical tests: ANOVA, Student T test, and Correlation Pearson Test Coefficient (R) using EXCEL and SPSS (Version 2010).

RESULTS AND DISCUSSIONS

Estimation of primary metabolites

The quantitative content values of carbohydrates, protein and lipids were estimated in milligrams per gram of plant material. As listed in Table 1.From the aforementioned observations, it is evident that the sprouted samples exhibited higher carbohydrate content compared to the non-sprouted samples. Moreover, the sprouted samples generally displayed higher protein content compared to the non-sprouted samples, except for yellow quinoa. Similarly, the lipid content was higher in germinated samples compared to non-sprouted samples, with the exception of yellow quinoa. The results of the ANOVA analysis for the quantitative content of carbohydrates, proteins, and lipids in the plant samples of germinated and non-

sprouted quinoa seeds indicated significant differences at α=0.001, signifying distinctions among the studied samples. Additionally, the results of the T-test comparing the germinated and non-sprouted seeds for each variety revealed highly significant differences at α=0.001 in the carbohydrate content across all types, in the protein content for yellow quinoa, and in the lipid content for red quinoa. This suggests that the germination process and spiking of samples have notable impacts on the nutritional composition of quinoa seeds, with variations observed among different quinoa varieties.

The observed difference in carbohydrate content between germinated and non-germinated quinoa seeds in our study can be attributed to the physiological changes associated with germination. During germination, the storage tissue, mainly starch in the endosperm, undergoes degradation facilitated by enzymes like amylase, leading to the breakdown of starch into simple sugars (Joshi, 2018). The embryo relies on these stored reserves, including endosperm starch, to sustain its growth. As water enters the seeds, metabolic processes are activated, supporting embryo growth and the development of seedling organs through respiratory processes that provide metabolic energy (Bewley et al., 2013).

The significant increase in protein content observed in germinated quinoa seeds compared to non-sprouted seeds can be attributed to the biochemical changes occurring during seed germination. This process initiates with imbibition and seed swelling upon water uptake, activating enzymatic reactions and respiration. The increase in protein content is facilitated by the utilization of carbohydrates and fats during respiration, leading to a loss in dry weight (Ongol et al., 2013; Jan et al., 2017). Additionally, the heightened synthesis of proteins during germination, outpacing their decomposition facilitated by protease enzymes, contributes to this phenomenon (Moongngarm and Saetung, 2010). This elevated protein synthesis is crucial for nucleic acid production and enzyme synthesis necessary for seedling growth. However, it's worth noting that the obtained results may be lower than previous studies due to environmental stresses (Kaushal et al., 2013).

There is a notable difference in lipid content between sprouted and non-sprouted quinoa seeds, primarily attributed to the germination process. Morita et al. (2001) conducted a study on sprouted quinoa seeds, indicating a decrease in free lipid content and a significant increase in bound fat content after 72 hours of germination. Specifically, the free lipid content decreased from 6.0% (at 24 hours) to 4.3% (at 48 hours) and further to 4.0% (at 72 hours), while the bound fat content increased from 2.1% to 2.9% to 4.8% during the same germination periods. This suggests that lipase activity during early germination rapidly metabolizes glycerol and free fatty acids, leading to the recombination of membrane-bound lipids. Additionally, an increase in non-polar fats, including oleic acid, linoleic acid, and palmitic acid, was observed during quinoa seed germination, while polar fats decreased. This indicates the utilization of phosphate from polar fats in the synthesis of essential compounds containing phosphate during the germination process.

Yield and Quantification of phytochemical compounds

The results obtained in the process of estimating the yield of extracts from germinated and non-sprouted quinoa seeds in their three varieties, red, black and yellow, showed that there were noticeable differences between the yield percentages, which witnessed a superiority of the extracts in the germinated state (Table 2).

Table 1. Estimation of primary metabolites in milligrams per gram of plant material.

Quinoa type	Seed state	Carbohydrates	Proteins	Lipids
Red	Sprouted	21.55 ±1.07^{***+++}	4.6±0.28***	2.23 ±0.22^{***+++}
Red	non-sprouted	4.3±0.68^{***+++}	2.01 ±0.47***	0.48±0.1^{***+++}
Black	Sprouted	20.28 ±1.42^{***+++}	1.71 ±0.21***	2.97 ±0.13***
Black	non-sprouted	10.78 ±0.93^{***+++}	1.49 ±0.36***	2.28±0.1***
Yellow	Sprouted	14.89 ±0.86^{***+++}	1.35±0.27^{***+++}	1.43 ±0.21***
Yellow	non-sprouted	7.16±1.01^{***+++}	2.19±0.18^{***+++}	1.54±0.18***

***: p≤0.001 when comparing the six samples.

$^{+++}$: p≤0.001, comparison between the sprouted and non-sprouted states for each variety.

Table 2. Yield of methanolic extracts of sprouted and non-sprouted quinoa seeds and quantitative determination of chemical compounds.

Quinoa type	Seed state	Yield%	Quantification of phytochemical compounds	
			Polyphenols (mg E GA/g Ex)	**Flavonoids (mg E Qu/g Ex)**
Red	Sprouted	4.73***	8.56±0.35	6.29±0.68***
Red	non-sprouted	1.46***	8.21 ±0.52	4.08±0.44***
Black	Sprouted	5.71***	10.74 ±0.6	6.85 ±0.73***
Black	non-sprouted	1.9***	7.34 ±0.47	4.1±0.51***
Yellow	Sprouted	3.87***	10.24±0.95	5.56 ±0.34***+++
Yellow	non-sprouted	1.56***	7.49±0.74	2.93±0.28***+++

***: *p≤0.001 when comparing the six samples.*
+: *p≤0.05, comparison between the sprouted and non-sprouted states for each variety.*
+++: *p≤0.001, comparison between the sprouted and non-sprouted states for each variety.*

Additionally, there is consistency in the polyphenol content values across all examined plant extracts (Table 2). The ANOVA statistical analysis revealed no significant differences ($p \leq 0.05$) in the total polyphenol content between germinated and non-germinated quinoa seed samples, indicating no discernible distinction among the studied samples. Similarly, the T-test affirmed the absence of a difference between germinated and non-sprouted extracts for each variety. It was observed that the quantitative content of flavonoids in germinated quinoa exceeded that of non-sprouted quinoa (Table 2). The ANOVA results for the total flavonoid content in plant extracts demonstrated significant differences between germinated and non-sprouted quinoa seeds, with ($p \leq 0.001$), indicating variations among the analyzed samples. This distinction was further corroborated by the T-test conducted for the yellow seed variety, revealing significant differences with ($p \leq 0.001$)between sprouted and non-sprouted seeds. Conversely, such differences were not observed for both red and black seed varieties.

Upon comparison with previous studies such as those conducted by Zouaoui et al. (2018) on the same plant species, a slight variance in yield percentage was noted, with the highest yield reaching 9.65%, despite similar experimental conditions. This difference can be attributed to several factors: Plant physiological activity during growth stages (Tlili et al., 2014), influencing the production or conversion of chemical compounds, thus impacting yield. Varietal differences within the species (Chouikh et al., 2015) due to genetic factors. Harvest timing, affecting the plant's exposure to various stresses, thereby altering the nature and quality of produced compounds, both quantitatively and qualitatively (Ebrahimi et al., 2008). Variations in extraction methods, influenced by factors such as temperature, duration, solvent quality, molecular weight, structure, and polarity of compounds, as well as their complexity and carbon chain length (Chouikh et al., 2018).

The significant differences observed in the total content of polyphenols and flavonoids between various sprouted and non-sprouted quinoa seed extracts can be attributed to the changes occurring during the seed germination process, which typically leads to an increase in the total content of these compounds. Studies by Pellegrini et al. (2010) and Carciochi et al. (2014) have demonstrated that germination causes a doubling of phenols and flavonoids in sprouted quinoa seeds. This increase can be explained by biochemical changes during germination, such as increased activity of starch hydrolysis enzymes, leading to a surge in compound production (Carciochi et al., 2016).

Phenolic compounds play a crucial role during germination, particularly in protecting seeds from pests and insects (Tang and Tsao, 2017). The increase in germination may also result in the release of phenolic compounds from cell walls or the synthesis of new phenolic compounds via endoesterase enzymes (Díaz-Batalla et al., 2006). Regarding the varieties, it's evident that black germinated quinoa exhibited the highest quantitative content of polyphenols and flavonoids. This finding aligns with previous studies indicating that different quinoa varieties possess varying amounts of phenolic compounds, influenced by genotype (Miranda et al., 2012). Comparing with other studies, our results are somewhat consistent with Carciochi et al. (2014), which also found higher polyphenol content in germinated quinoa seed extracts. However, variations in environmental conditions, harvest time, and extraction methods may contribute to differences observed among studies (Ojeil et al., 2010).

Estimation of antioxidant and anti-inflammatory activity

In the DPPH test and through the IC_{50} values, we noticed that the non-sprouted samples achieved better antioxidant activity than the germinated quinoa samples. The results of the ANOVA analysis to test the radical displacement of the free radical DPPH• for the studied plant extracts also showed a significant difference ($p \geq 0.001$), meaning that there is a difference in the difference between the studied samples (Table 3).

As for anti-inflammatory activity, we noticed that the germinated quinoa samples achieved better activity than the non-sprouted quinoa samples. The results of the ANOVA analysis in this test for plant extracts of germinated and non-germinated quinoa seeds show significant differences at ($\alpha = 0.001$), which proves that there are differences between the studied samples. The results of the T-test also confirm the presence of significant differences in the black variety at ($\alpha = 0.05$) compared to the rest of the germinated and non-sprouted quinoa varieties (Table 3).

From the results obtained, it's evident that unsprouted black quinoa seed extract exhibited the most potent DPPH free radical scavenging activity compared to other extracts. According to the rule stating that lower IC_{50} values indicate greater antioxidant activity (Elisha et al., 2016), it can be inferred that both germinated and unsprouted quinoa extracts exhibit weak DPPH inhibition compared to the standard reference, ascorbic acid.

Interestingly, in this study, the concentration of polyphenols didn't correlate with antioxidant activity, contrary to findings in most studies on germinated quinoa seeds. This discrepancy might be attributed to the varying behavior of phenolic compounds and flavonoids. Previous research by Yordil et al. (2012) highlighted that polyphenols exhibit diverse mechanisms in inhibiting free radicals. Some form stable complexes with reactive oxygen species (ROS), while others break valent bonds, leading to the restoration of oxidizing elements. Additionally, some polyphenols act as chelating agents and donors of protons and electrons.

The results demonstrated significant effectiveness in preventing hemolysis of red blood cells, with comparable efficacy to ascorbic acid, a standard reference. Notably, germinated quinoa seed extracts showed inferior performance to ascorbic acid, while non-sprouted extracts exhibited similar effectiveness. Red quinoa outperformed ascorbic acid, consistent with Yael et al's findings (2012), indicating its superior protective effect against hemolysis. Chaudhuri et al. (2007) suggested that this protective ability stems from flavonoids' presence in plant extracts, which integrate into red blood cell membranes, shielding them from oxidation.

In this study, we utilized protein denaturation as a method to assess inflammatory responses. Protein denaturation involves the loss of a protein's functional structure due to external factors such as pressure, heat, or certain compounds, leading to the disruption of its receptors. This process contributes to tissue inflammation and damage. Additionally, research by El Hazzam et al. (2020) demonstrated that saponins isolated from quinoa seeds enhance antibody response (IgG and IgA) against inflammatory agents.

Studies by Yao et al. (2014) and support the anti-inflammatory properties of quinoa saponins, particularly 3-O-β-D-Glucopyranosyloleanolic acid and triterpenoid compounds, respectively. However, germinated extracts showed diminished anti-inflammatory activity, likely due to reduced saponin content caused by soaking and washing during the germination process.

Table 3. Antioxidant and anti-inflammatory activity of methanolic extracts from sprouted and non-sprouted quinoa seeds.

Quinoa type	Seed state	Antioxidant activity		Anti-inflammatory activity (mg E Dc/mg Ex)
		DPPH• test IC_{50} (mg/mL)	% Hemolysis (C= 0.8mg/mL)	
Red	Sprouted	$0.56\pm0.01^{***}$	49.04^{***}	$1.13\pm0.08^{***}$
Red	non-sprouted	$0.21\pm0.01^{***}$	11.14^{***}	$1.45\pm0.04^{***}$
Black	Sprouted	$0.11\pm0.01^{***}$	37.09^{***}	$1.31\pm0.07^{***+}$
Black	non-sprouted	$0.06\pm0.01^{***}$	16.62^{***}	$0.89\pm0.04^{***+}$
Yellow	Sprouted	$0.54\pm0.01^{***}$	38.17^{***}	$1.3\pm0.06^{***}$
Yellow	non-sprouted	$0.53\pm0.01^{***}$	30.2^{***}	$1.6\pm0.07^{***}$
Ascorbic acid		0.02±0.01	16.32	

***: p≤0.001 when comparing the six samples.

$^{+}$: p≤0.05, comparison between the sprouted and non-sprouted states for each variety.

Interestingly, black quinoa extracts displayed superior anti-inflammatory effects over non-germinated seeds. This can be attributed to their high polyphenol content, which has been shown to exhibit potent anti-inflammatory properties by reducing inflammatory signals targeting tissues, as suggested by Santangelo et al. (2007) and Gonzalez-Gallego et al. (2010). Specifically, flavonoids found in black quinoa inhibit the NOS enzyme responsible for nitric oxide production, thus preventing inflammation at the molecular level.

Correlation coefficient test results

As shown in Table 4,a direct correlation between phenolic compounds and flavonoids in extracts is noted, given that flavonoids are categorized within phenolic compounds (Sulaiman and Balachandran, 2007). This relationship also extends to anti-inflammatory activity, as phenolics, including flavonoids, play a significant role in inflammation suppression (Gonzalez-Gallego et al., 2010).

The results also reveal a strong linear correlation between flavonoids, carbohydrates, and yield. This trend is consistent with the observed increase in these compounds during the germination stage, indicating a correlation between primary and secondary metabolic pathways. Additionally, a significant positive linear correlation is observed between flavonoids and yield, particularly evident in germinated seed extracts, where changes in seed physiological state contribute to enhanced phenolic compound synthesis.

Furthermore, a notable correlation exists between yield and the hemolysis test, as well as between DPPH radical scavenging tests and the hemolysis test, indicating increased antioxidant activity in non-sprouted samples for both assays. Weak correlations are observed between the DPPH radical scavenging test and the amount of polyphenols and yield, attributed to the test's emphasis on compound structure and quality rather than quantitative content.

These findings highlight intricate relationships between phenolic compounds, flavonoids, antioxidants, and biochemical processes, underscoring the importance of both quantitative and qualitative assessments in understanding their biological activities (Jimenez Martiınez et al., 2012).

Table 4. Correlation coefficient (R^2) between the different variables studied.

Variable	Yields	polyphenols	Flavonoids	DPPH	Hemolysis	Anti-inflammatory	Protein	Lipid	Carbohy-drates
Yields	1	0.82	0.91	0.08	0.79	-0.2	0.29	0.59	0.95
polyphenols		1	0.84	0.01	0.51	0.98	-0.19	0.69	0.62
Flavonoids			1	-0.27	0.48	-0.15	0.07	0.75	0.77
DPPH				1	0.63	0.39	0.49	-0.17	0.15
Hemolysis					1	-0.02	0.56	0.05	0.85
Anti-inflammatory						1	-0.1	-0.52	-0.41
Protein							1	-0.41	0.44
Lipid								1	0.45
Carbohydrates									1

CONCLUSIONS

In conclusion, this study investigated the impact of germination on the nutritional composition, phytochemical content, antioxidant, and anti-inflammatory activities of quinoa seeds. The findings revealed significant variations in the levels of carbohydrates, proteins, lipids, polyphenols, and flavonoids between germinated and non-sprouted quinoa seeds across different varieties. Germination was associated with higher carbohydrate, protein, and lipid contents, indicating metabolic changes and utilization of stored reserves during seedling growth. Moreover, germination led to a significant increase in the total content of polyphenols and flavonoids, highlighting the potential health benefits of sprouted quinoa seeds due to their enhanced antioxidant and anti-inflammatory properties. The results also demonstrated a strong correlation between phenolic compounds, flavonoids, and antioxidant activities, emphasizing the importance of these phytochemicals in promoting health. Furthermore, the study identified varietal differences in phytochemical content, with black quinoa exhibiting the highest levels of polyphenols

and flavonoids. However, it's worth noting that variations in environmental conditions, extraction methods, and genetic factors may influence these differences. Overall, the findings underscore the importance of germination as a bio-processing technique to enhance the nutritional and functional properties of quinoa seeds, making them a valuable addition to a healthy diet.

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