Effect of Incorporating Different Ratios of Quinoa (Chenopodium quinoa) on the Vitamin, Phenolic, and Antioxidant Content of Biscuit Dry Mixes

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Abstract

Quinoa (Chenopodium quinoa Willd) flour is considered one of the most promising alternatives to wheat flour due to its high content of proteins, dietary fibers, minerals, and essential amino acids, thereby enhancing the nutritional value of baked products. This study was conducted at the Food Chemistry Laboratory, Department of Food Science, College of Agriculture, Tikrit University, during the 2023–2024 season, to evaluate biscuits prepared by partially substituting wheat flour with two types of quinoa flour (white and red) at levels of 4%, 8%, 12%, and 16%. The contents of phenolics, fat-soluble vitamins, water-soluble vitamins, and antioxidant activity were assessed. The effects discovered sizeable variations ($p \leq 0.05$) in each fats-soluble and water-soluble diet contents most of the unique dry blend samples utilized in biscuit production. Vitamin concentrations regularly extended with better tiers of quinoa flour substitution. Similarly, the phenolic content of the dry blend samples exhibited sizeable variations depending on the kind and substitution degree of quinoa flour. Phenolic concentrations elevated with better substitution ratios, with red quinoa outperforming white quinoa at all substitution levels. Assuming that the optimal substitution ratio is determined by the highest percentage, Alternative A4, which has a substitution ratio of 16%, can be considered the most favorable or optimal choice. The effect of different substitution degrees on antioxidant pastime turned into evaluated by means of measuring the DPPH radical scavenging ability of the extracts at growing concentrations, alongside IC_{50} values as an indicator of common inhibitory performance. Results demonstrated a slow growth in inhibition interest with growing concentrations from 30 to 500 ppm in all samples, constant with the traditional dose–reaction curves for antioxidant pastime.

Keywords: Chenopodium quinoa, Biscuit formulation, Wheat flour substitution, Bioactive compounds ,

Introduction

Quinoa (Chenopodium quinoa Willd) has gained Growing worldwide interest over current a long time because of its excessive nutritional price and particular homes, making it an terrific meals supply [1]. belongs to the Amaranthaceae family, formerly classified under Chenopodiaceae. It is traditionally cultivated in the Andean regions of Bolivia, Peru, and Chile, but its adaptability has led to global expansion. Quinoa seed powder are rich in high-quality proteins, essential amino acids, dietary fiber, and bioactive compounds such as antioxidants. The main types include white (or ivory) quinoa (Real, Titicaca), red quinoa (Pasankalla, Kurmi), black quinoa, and tricolor or mixed quinoa, which combines white, red, and black varieties. It incorporates notable protein starting from 13–17%, encompassing all vital amino acids, together with lysine, that is frequently limited in conventional cereals which includes wheat and maize [2 .[

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Moreover, quinoa is obviously gluten-loose, making it a appropriate alternative for individuals with gluten sensitivity or celiac disease [25]. In addition, it's miles a rich source of dietary fiber, nutrients including B1, B2, B6, and E, and essential minerals including iron, magnesium, calcium, and zinc. Quinoa also contains numerous natural antioxidants, which include flavonoids, quercetin, and kaempferol [22,47]. Due to this comprehensive dietary profile, quinoa is assessed as a high-value useful meals. Its seeds offer about 11–16 g of protein in line with one hundred g of dry weight, making it one of the most distinguished plant-based resources of remarkable protein [35]. This protein includes all critical amino acids, significantly lysine, that's a proscribing amino acid in most different cereals [43.[

Such nutritional properties have directed research interest toward the utilization of quinoa in the food industry, particularly in the development of gluten-free products. The natural absence of gluten in quinoa makes it an ideal ingredient for producing healthy foods suitable for individuals with gluten intolerance or celiac disease [14]. Consequently, quinoa flour has emerged as a promising alternative that can enhance the nutritional quality of baked products and meet modern dietary demands.

This study aimed to evaluate the antioxidant activity, phenolic compounds, and flavonoid content in biscuits prepared using quinoa flour to determine its effect on the bioactive composition of the final product .

Material and Methods

Source of Wheat Flour

High-quality Kuwaiti wheat flour with a 72% extraction rate was used, procured from local markets in Tikrit city. The flour was stored at room temperature in tightly sealed polyethylene bags until further analyses were conducted as part of this study.

Source of Quinoa

Quinoa seeds were obtained from local markets, imported from Bolivia, specifically from Andean Valley, one of the world's leading quinoa producers. This type of quinoa was selected due to its widespread use in international studies, recognized for its high nutritional quality and consistent properties [41]. The seeds were checked for cleanliness and absence of impurities prior to processing.

Preparation of Quinoa Flour and Substitution Ratios

Quinoa seed powder were manually cleaned to remove impurities, followed by rinsing under running water to reduce saponin content [38]. The seeds were then dried in an oven at 50°C for 12 hours. Subsequently, the dried seeds were ground using a domestic electric mill, and the resulting flour was sieved through a 60 μm mesh to obtain fine, homogeneous quinoa flour.

Four different flour blends were prepared by partially substituting wheat flour with quinoa flour at levels of 4%, 8%, 12%, and 16%, as follows:

The flour blends were prepared as follows:

.1 Sample 1 (4%): 96% wheat flour and 4% white quinoa flour.

.2 Sample 2 (8%): 92% wheat flour and 8% white quinoa flour.

.3 Sample 3 (12%): 88% wheat flour and 12% white quinoa flour.

.4 Sample 4 (16%): 84% wheat flour and 16% white quinoa flour.

.5 Sample 5 (4%): 96% wheat flour and 4% red quinoa flour.

.6 Sample 6 (8%): 92% wheat flour and 8% red quinoa flour.

.7 Sample 7 (12%): 88% wheat flour and 12% red quinoa flour.

.8 Sample 8 (16%): 84% wheat flour and 16% red quinoa flour.

The specified ratios were thoroughly mixed using a domestic electric mixer to obtain a homogeneous flour blend for each treatment. These blends were subsequently used in laboratory-scale biscuit production following standardized procedures to ensure accurate comparisons across all treatments.

Laboratory Biscuit Preparation

Biscuit production was carried out at the Food Industry Laboratory, College of Agriculture, Tikrit University, using blends of high-quality Kuwaiti wheat flour (72% extraction) partially substituted with quinoa flour at levels of 4%, 8%, 12%, and 16%, following the method described by [19]. with slight modifications. The ingredients were kneaded for 10 minutes to obtain a homogeneous dough, which was then refrigerated at 6°C for 30 minutes. The

dough was rolled to a thickness of 3 mm and cut into 45 mm diameter circles, then baked in an electric oven at 180°C for 6 minutes. After cooling for 30 minutes, the biscuit samples were stored in polyethylene bags for preservation.

Additional treatments were prepared by partially substituting wheat flour with husk powder at three levels (4%, 8%, and 12%), following the same production steps described above.

Table 1: Preparation of biscuit samples from wheat flour supplemented with varying substitution levels of red and white quinoa flour.

B2	B1	A4	A3	A2	A1	CO	Ingredients
92	96	84	88	92	96	100	**Wheat flour (g)**
45	45	45	45	45	45	45	**Butter (g)**
30	30	30	30	30	30	30	**Sugar powder (g)**
50	50	50	50	50	50	50	**Egg (g)**
2	2	2	2	2	2	2	**Vanillin (g)**
1	1	1	1	1	1	1	**Baking powder (g)**
8	4	16	12	8	4	0	**Quinoa flour (g)**

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DPPH Radical Scavenging Assay

A DPPH solution was prepared by dissolving 0.04 g of DPPH in 100 mL of methanol to obtain a concentration of 400 µg/mL[28]. The standard solution (Vitamin C) was prepared by dissolving 0.5 g of Vitamin C in a mixture of 100 mL methanol and distilled water, yielding an initial concentration of 5000 ppm. Using the dilution equation, different concentrations were prepared for both the standard and sample solutions (30, 60, 120, 250, and 500 ppm) [47.[

After thorough mixing, the solutions were left at room temperature for 30 minutes, and absorbance was measured at 517 nm using a UV–VIS spectrophotometer (Shimadzu [4]. The IC_{50} value, representing the concentration required to inhibit 50% of DPPH free radicals, was determined based on a logarithmic inhibition curve [18]. A lower absorbance indicates higher radical scavenging activity.

The percentage of DPPH scavenging activity was calculated using the following equation:

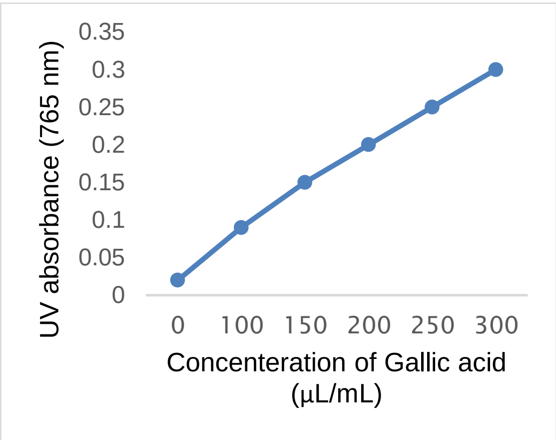

Figure 1: Standard calibration curve of gallic acid .

DPPH Scavenging Effect (%)=A0−A1A0×100\text{DPPH Scavenging Effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100DPPH Scavenging Effect (%)== (A0 - A1) / A0 × 100

Where:

A0= Absorbance of the blank

A1 = Absorbance in the presence of the tested sample . .

Total Phenolic Content Determination

Five grams of the dry mix sample used in the experiment were placed in test tubes, and 5.0 mL of 10% (v/v) diluted Folin–Ciocalteu reagent was added. The mixture was shaken and left for 6 minutes, then kept in the dark at room temperature for 2 hours. Absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated based on a standard calibration curve of gallic acid

Determination of Vitamin Content

The chemical composition of the dry mix samples, containing varying substitution levels of red and white quinoa flour used in biscuit production, was analyzed to determine their water-soluble vitamin content. The analyses were performed using High-Performance

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Liquid Chromatography (HPLC) in accordance with the standardized procedures outlined by the Official Methods of Analysis [27.[

Statistical Analysis

The experimental data were statistically analyzed using the Statistical Analysis System [42]. A Completely Randomized Design (CRD) was employed, and mean values were compared using Duncan's Multiple Range Test [12]. to determine the significance of differences among the means of the studied traits .

Results and Discussion

Fat-Soluble Vitamin Content (A, D, E, K) in the Dry Mixes for Biscuit Production at Different Quinoa Substitution Levels

The results presented in Table 2 showed significant differences ($p \leq 0.05$) in fat-soluble vitamin content among the dry mix samples used for biscuit production, reflecting the direct impact of partially substituting wheat flour with quinoa flour on vitamin levels. Generally, the concentrations of these vitamins increased progressively with higher quinoa flour substitution.

Significant differences ($p \leq 0.05$) were observed in vitamin A content with increasing levels of quinoa flour. Sample A4 recorded the highest vitamin A content (1.81 mg/100 g), followed by B4 (1.66 mg/100 g), while the lowest values were observed in samples A1 and B1 (0.89 and 0.77 mg/100 g, respectively). These findings indicate a positive effect of quinoa flour on vitamin A content, which can be attributed to the carotenoid compounds in Quinoa seed powder that are converted into vitamin A in the body [46]. These results are consistent with previous studies that reported increased vitamin A content when quinoa was incorporated into biscuit formulations, particularly at higher substitution levels [15,48.[

Vitamin E content also increased significantly ($p \leq 0.05$), especially in samples with higher quinoa flour levels. Samples A4 and B4 showed the highest values (9.03 and 8.45 mg/100 g, respectively), compared to A1 and B1, which had the lowest values (5.90 and 5.23 mg/100 g, respectively). This increase is attributed to the high content of unsaturated fats and natural antioxidants in quinoa flour, particularly tocopherols, which are the main components of vitamin E. Increasing the proportion of quinoa flour in the blends enhances vitamin E content in the final product, improving its antioxidant properties [3]. Vitamin E plays an essential role as an antioxidant, protecting cells from oxidative damage caused by free radicals [52.[

For vitamin D, significant differences ($p \leq 0.05$) were also observed. Sample A4 recorded the highest content (1.30 mg/100 g), while A1 had the lowest (0.50 mg/100 g). For samples B4 and B1, the values were 1.12 and 0.35 mg/100 g, respectively. The addition of Quinoa seed powder to biscuits contributed to the increase in vitamin D content, likely due to the presence of notable amounts of vitamin D or its precursors. This enhances the nutritional value of the biscuits and supports preventive nutrition [6]. The increase may also be related to the accumulation of vitamin D in the added fats during preparation or the total content present in Quinoa seed powder, as lipids in quinoa can enhance the absorption of this vitamin [37].Significant differences ($p \leq 0.05$) were observed in vitamin K content among the samples. Samples A4 and B4 had the highest values (2.50 and 2.30 mg/100 g, respectively), while A1 and B1 recorded the lowest values (1.33 and 1.01 mg/100 g, respectively). These results indicate that increasing quinoa flour substitution significantly affects vitamin K content.

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Vitamin K, a fat-soluble vitamin abundant in plant oils and whole grains such as quinoa, is essential for bone health and blood coagulation. The increase in vitamin K content in the biscuits is attributed to the high levels of this vitamin, particularly phylloquinone (K1), present in Quinoa seed powder Quinoa seed powder, thereby enhancing the nutritional value of the final product [36]. These findings are consistent with those reported [7.[

Table 2: Effect of substituting red and white quinoa flour at levels of 4%, 8%, 12%, and 16% on fat-soluble vitamins (A, E, D, K) in the dry mix used for biscuit production.

Vitamin K (mg/100g)	Vitamin D (mg/100g)	Vitamin E (mg/100g)	Vitamin A (mg/100g)	Treatments
1.33 ± 0.01 g	0.50 ± 0.01 g	5.90 ± 0.01 g	0.89 ± 0.01 g	A1
1.78 ± 0.01 e	0.75 ± 0.01 e	6.80 ± 0.01 e	1.10 ± 0.01 e	A2
2.10 ± 0.01 c	0.97 ± 0.01 c	7.98 ± 0.01 c	1.50 ± 0.01 c	A3
2.50 ± 0.01 a	1.30 ± 0.01 a	9.03 ± 0.04 a	1.81 ± 0.01 a	A4
1.01 ± 0.01 h	0.35 ± 0.01 h	5.23 ± 0.01 h	0.77 ± 0.01 h	B1
1.56 ± 0.01 f	0.66 ± 0.01 f	6.33 ± 0.01 f	0.98 ± 0.01 f	B2
1.99 ± 0.01 d	0.89 ± 0.01 d	7.20 ± 0.01 d	1.23 ± 0.01 d	B3
2.30 ± 0.01 b	1.12 ± 0.01 b	8.45 ± 0.01 b	1.66 ± 0.01 b	B4

*Different lowercase letters inside the identical column indicate big variations ($p \leq 0.05$) among remedies in line with Duncan's more than one range test.

A1: Red quinoa flour at 4% substitution; A2: Red quinoa flour at 8% substitution; A3: Red quinoa flour at 12% substitution; A4: Red quinoa flour at 16% substitution.

B1: White quinoa flour at 4% substitution; B2: White quinoa flour at 8% substitution; B3: White quinoa flour at 12% substitution; B4: White quinoa flour at 16% substitution

In the dry mixture the water soluble vitamin content is used for the production of biscuits with a variety of substitute levels

The effects presented in Table three discovered considerable variations ($p \leq 0.05$) within the concentrations of water-soluble vitamins, which includes thiamine (B1), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and vitamin C, the various studied samples with varying substitution tiers of quinoa flour. Overall, nutrition concentrations improved proportionally with better substitution degrees, specially in samples A4 and B4, compared to lower substitution levels including B1.

A gradual increase in thiamine (B1) content was determined with higher substitution ratios. Sample A4 recorded the very best thiamine awareness (19.65 mg/100g), whereas pattern B1 exhibited the bottom (13.61 mg/100 g). This increase highlights quinoa's richness in thiamine, a nutrition vital for carbohydrate metabolism and energy manufacturing, thereby supporting vital body functions [5]. These findings are consistent with [48,49]. And [who stated that quinoa includes higher thiamine ranges compared to most

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conventional cereals, making it an super thing for gluten-free diets and for addressing diet B1 deficiencies.

For niacin (B3), sample A4 demonstrated the highest concentration (30.15 mg/100 g), followed by sample B4 (29.65 mg/100 g), while the lowest level was recorded in sample B1 (22.15 mg/100 g). This trend confirms that quinoa flour enhances niacin availability, which plays a crucial role in nervous system functions and circulatory health [48,3.[

Pantothenic acid (B5) levels also increased significantly, with sample A4 showing the highest content (25.99 mg/100 g) compared to the lowest in B1 (18.90 mg/100 g). This improvement is attributed to quinoa's natural richness in pantothenic acid, a coenzyme precursor that is fundamental for lipid and carbohydrate metabolism, thereby contributing to improved metabolic performance [,39,29,34.[

Similarly, pyridoxine (B6) levels increased gradually with higher substitution levels. Sample A4 recorded the highest concentration (12.66 mg/100 g), while B1 had the lowest (6.25 mg/100 g). The enrichment effect is due to quinoa's natural content of B6, which is essential for amino acid metabolism, neurotransmitter synthesis, and immune regulation [8,30,31 .[

Vitamin C content also exhibited a progressive increase with higher substitution levels. The highest value was detected in sample A4 (1.60 mg/100 g), followed by B4 (1.41 mg/100 g), whereas B1 showed the lowest (0.65 mg/100 g). This increase reflects quinoa's notable antioxidant profile, particularly its vitamin C content, which enhances immune function, supports iron absorption, and reduces oxidative stress [44,22,46 .[

These results collectively demonstrate that incorporating quinoa flour into biscuit formulations significantly enhances the content of essential water-soluble vitamins, thereby improving the nutritional and functional value of the final product.

Table (3). Effect of substituting red and white quinoa flour at levels (4, 8, 12, 16%) on the water-soluble vitamin content in the dry mix used for biscuit production (mg/100 g.(

C (Ascorbic acid)	B6 (Pyridoxine)	B5 (Pantothenic acid)	B3 (Niacin)	B1 (Thiamine)	Treatments
0.78 ± 0.01 g	7.22 ± 0.01 g	19.51 ± 0.01 g	23.65 ± 0.01 g	14.22 ± 0.01 g	A1
0.99 ± 0.01 e	9.33 ± 0.01 e	21.65 ± 0.01 e	25.45 ± 0.01 e	16.22 ± 0.01 e	A2
1.30 ± 0.01 c	11.65 ± 0.01 c	23.89 ± 0.01 c	27.88 ± 0.01 c	17.65 ± 0.01 c	A3
1.60 ± 0.01 a	12.66 ± 0.01 a	25.99 ± 0.01 a	30.15 ± 0.01 a	19.65 ± 0.01 a	A4
0.65 ± 0.01 h	6.25 ± 0.01 h	18.90 ± 0.01 h	22.15 ± 0.01 h	13.61 ± 0.01 h	B1
0.89 ± 0.01 f	8.12 ± 0.01 f	20.33 ± 0.01 f	24.56 ± 0.01 f	15.04 ± 0.01 f	B2
1.10 ± 0.01 d	10.36 ± 0.01 d	22.65 ± 0.01 d	26.98 ± 0.01 d	16.90 ± 0.01 d	B3
1.41 ± 0.01 b	12.25 ± 0.01 b	24.56 ± 0.01 b	29.65 ± 0.01 b	18.22 ± 0.01 b	B4

*Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$) among treatments according to Duncan's multiple range test (Duncan, 1955).A1: Red quinoa flour substitution at 4% A2: Red quinoa flour substitution at 8%A3: Red quinoa flour substitution at 12%A4: Red quinoa flour substitution at 16%B1: White quinoa flour substitution at 4%B2: White quinoa flour substitution at 8%B3: White quinoa flour substitution at 12%B4: White quinoa flour substitution at 16%.

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Antioxidant Activity (DPPH) and IC_{50} Values in Biscuit Dry Mixes with Different Substitution Levels

Table (4) illustrates the impact of substituting wheat flour with varying stages of crimson and white quinoa flour at the antioxidant pastime of the dry biscuit mixes. The assessment became finished by means of measuring the potential of the extracts to scavenge DPPH unfastened radicals at increasing concentrations, in conjunction with IC_{50} values as an indicator of overall inhibitory performance.

The results demonstrated a progressive increase in radical scavenging activity with rising concentrations (30–500 ppm) across all studied samples, consistent with conventional dose–response curves for antioxidant activity. The highest inhibition at 500 ppm was recorded in sample A4 (78.22%), followed by A3 (77.60%) and B4 (77.89%), whereas the lowest inhibition was observed for the standard compound, vitamin C (69.80%). Although vitamin C is recognized as a potent antioxidant, the superior performance of certain quinoa-enriched samples may be attributed to the diverse array of bioactive compounds in Quinoa seed powder, including polyphenols (quercetin and kaempferol derivatives), flavonoids, and tocopherols. These compounds act synergistically to enhance the overall antioxidant potential of biscuits and other baked products, thereby improving their nutritional and functional value [33 .[

Notably, samples A4, B4, and A3 exhibited significantly higher scavenging activity across most concentrations compared to B1, highlighting the contribution of quinoa flour substitution to improved antioxidant capacity. This effect is closely linked to the phenolic compound content of quinoa, which is strongly associated with antioxidant activity. These findings are consistent with Kaur et al. (2021), who reported higher antioxidant activity in quinoa-enriched bakery products compared to wheat-based controls, and with [23]. who observed DPPH inhibition rates approaching 80% in quinoa-based formulations.

IC_{50} values, representing the concentration required to scavenge 50% of free radicals, served as a key parameter for evaluating antioxidant efficacy. Lower IC_{50} values indicate stronger free radical scavenging activity. The lowest IC_{50} was recorded in sample A4 (112.70 ppm), followed by A3 (113.80 ppm) and B4 (113.40 ppm), whereas higher values were observed in other treatments. This demonstrates that higher substitution levels of quinoa flour effectively enhance the biological capacity of the mix to neutralize free radicals. The observed improvement is attributed to the high content of active phenolic compounds in quinoa, particularly quercetin, kaempferol, flavonoids, and tocopherols [33]. These compounds act synergistically to maximize antioxidant efficiency.

Similar observations were reported by [45]. who found that Quinoa seed powder of Puno and Titicaca varieties were rich in polyphenols and exhibited strong in vitro free radical scavenging activity, explaining the reduction in IC_{50} values. [33]. also demonstrated that incorporating quinoa flour into baked products significantly lowered IC_{50} values compared to conventional products, reflecting improved total antioxidant capacity. Furthermore, [22]. emphasized that the lower IC_{50} values in quinoa-containing products are directly associated with the concentrations of quercetin and kaempferol, compounds known for their strong free radical scavenging activity. Thus, the reduction in IC_{50} observed in high-substitution samples underscores quinoa's role as a potent source of bioactive compounds, making it a promising ingredient for enhancing the functional and health-promoting properties of baked goods

Table (4). Effect of substituting red and white quinoa flour at levels of 4, 8, 12, and 16% on antioxidant activity (DPPH, % inhibition) at different concentrations and IC_{50} values (ppm) in dry mix samples used for biscuit production.

IC_{50} (ppm)	500 ppm	250 ppm	120 ppm	60 ppm	30 ppm	Sample
131.50 ± 0.01 a	69.80 ± 0.01 I	60.25 ± 0.01 i	45.69 ± 0.01 i	20.11 ± 0.01 i	12.05 ± 0.01 i	Vit C
116.50 ± 0.01 c	76.60 ± 0.01 G	68.30 ± 0.01 g	51.50 ± 0.01 g	28.33 ± 0.01 g	19.45 ± 0.01 g	A1
115.30 ± 0.01 e	77.11 ± 0.01 e	68.89 ± 0.01 e	52.04 ± 0.01 e	28.70 ± 0.01 e	20.00 ± 0.01 e	A2
113.80 ± 0.01 g	77.60 ± 0.01 c	69.57 ± 0.01 c	52.74 ± 0.01 c	29.33 ± 0.01 c	20.45 ± 0.01 c	A3
112.70 ± 0.01 i	78.22 ± 0.01 a	70.00 ± 0.01 a	53.20 ± 0.01 a	29.70 ± 0.01 a	20.88 ± 0.01 a	A4
117.00 ± 0.01 b	76.25 ± 0.01 h	68.08 ± 0.01 h	51.23 ± 0.01 h	27.08 ± 0.01 h	19.08 ± 0.01 h	B1
115.80 ± 0.01 d	76.85 ± 0.01 f	68.55 ± 0.01 f	51.80 ± 0.01 f	28.50 ± 0.01 f	19.80 ± 0.01 f	B2
114.70 ± 0.01 f	77.45 ± 0.01 d	69.25 ± 0.01 d	52.36 ± 0.01 d	28.98 ± 0.01 d	20.23 ± 0.01 d	B3
113.40 ± 0.01 h	77.89 ± 0.01 b	69.88 ± 0.01 b	52.98 ± 0.01 b	29.50 ± 0.01 b	20.69 ± 0.01 b	B4

Different lowercase letters within the same column indicate the presence of significant differences ($p \leq 0.05$) between the effects of the treatments.A1: Red quinoa at 4% A2: Red quinoa at 8% A3: Red quinoa at 12%A4: Red quinoa at 16% B1: White quinoa at 4% B2: White quinoa at 8% B3: White quinoa at 12% B4: White quinoa at 16.

Total phenolic content in the dry mix used for biscuit production.

The results presented in Figure (2) show a significant difference in the phenolic content of the dry mix samples with different substitution levels of wheat flour with red and white quinoa flours used for biscuit production. The phenolic content increased with increasing the substitution ratio of wheat flour with quinoa flour, and red quinoa outperformed white quinoa at all substitution levels.

In the red quinoa samples (A1, A2, A3, A4), a gradual increase in phenolic content was observed with increasing substitution from 4% to 16%, reaching 1.60, 1.90, 2.35, and 3.00 mg gallic acid equivalent/g, respectively, compared to the control sample, which recorded the lowest phenolic content of 0.5 mg gallic acid equivalent/g.

On the other hand, the increase in phenolic content in white quinoa (B1, B2, B3, B4) was less pronounced. The highest value was recorded at the 12% substitution level (B3) at 1.95 mg gallic acid equivalent/g, which is higher than the control but lower than that of red quinoa.These results are consistent with the findings of [21,40]. who indicated that substituting wheat flour with quinoa flour, especially red quinoa, contributes to increasing the phenolic content of biscuits, thus providing enhanced functional properties such as higher antioxidant activity. This effect depends on both the substitution ratio and the type of quinoa.

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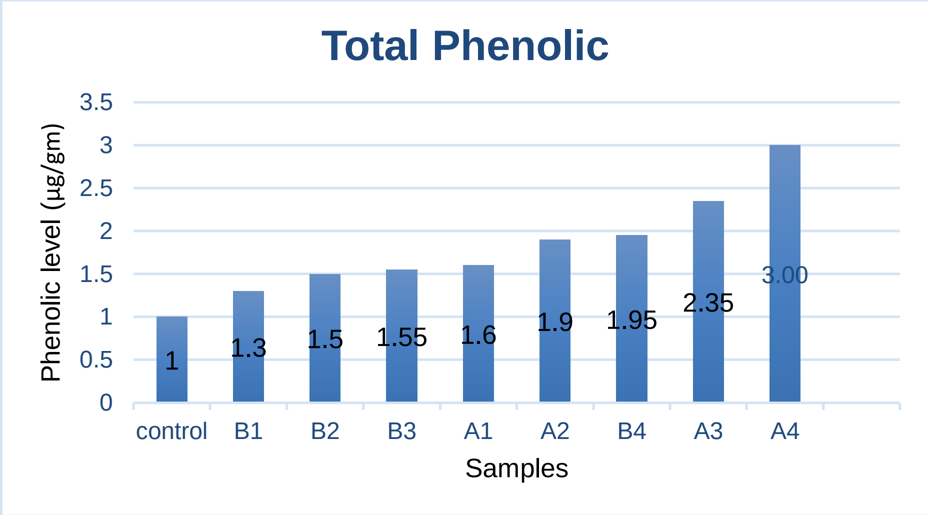

Figure (2): Total phenolic content in the dry mix of the prepared biscuits.

Control = Control sample mix A1 = Addition of 4% red quinoa flour A2 = Addition of 8% red quinoa flour

A3 = Addition of 12% red quinoa flour A4 = Addition of 16% red quinoa flour B1 = Addition of 4% white quinoa flour B2 = Addition of 8% white quinoa flour B3 = Addition of 12% white quinoa flour B4 = Addition of 16% white quinoa flour.

The current results are consistent with those reported by [20]. who demonstrated that whole Quinoa seed powder contain high levels of phenolic compounds, which play an important role in enhancing antioxidant activity. The observed increase in phenolic content in biscuit models fortified with quinoa flour, particularly red quinoa, has been attributed to the higher phenolic content of this type, especially in the outer layers of the seeds [13]. also confirmed that the addition of quinoa flour to bakery products resulted in a significant increase in total phenolics and improved antioxidant activity compared to samples without it. Similarly, [11]. reported that incorporating quinoa flour at traditional ratios into bread dough significantly increased phenolic content compared to wheat-only bread, in addition to noticeably enhancing antioxidant activity.

On the other hand,[10]. indicated in a recent study that using red quinoa flour in the development of innovative food products contributed to elevated phenolic levels and enhanced antioxidant activity [50]. confirmed that moderate thermal processing of Quinoa seed powder helps release free phenolic compounds, thereby increasing their nutritional value.

Moreover [46]. reported that red and black Quinoa seed powder contain higher levels of phenolic compounds compared to white quinoa, which aligns with the current study's findings showing the clear superiority of red quinoa.) [25]. also demonstrated that colored quinoa, particularly red and black, is characterized by high concentrations of flavonoids and phenolic compounds compared to white quinoa, making it a valuable dietary choice for improving the health benefits of food products.

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The observed superiority of red quinoa over white quinoa is attributed to its higher concentrations of natural phenolic compounds and flavonoids associated with darker grain colors. [51]. noted that red or black colors indicate higher levels of bioactive compounds compared to lighter-colored grains. [46]. supported this explanation by showing that red and black quinoa contain double the phenolic content compared to white quinoa, which explains the higher antioxidant activity in red quinoa.

Based on these findings, this study confirms that partially replacing wheat flour with quinoa flour, especially red quinoa, is an effective strategy to increase phenolic content and enhance the functional and health-promoting properties of biscuits compared to the control sample without quinoa.

Conclusion

The study showed that replacing wheat flour with quinoa flour at different levels improved the nutritional and functional quality of the laboratory-produced biscuits. The content of fat- and water-soluble vitamins increased, along with a notable rise in total phenolics and flavonoids as the substitution levels increased, with red quinoa flour having a greater effect compared to white quinoa. This was also reflected in enhanced antioxidant activity, as the extracts demonstrated a higher capacity to scavenge DPPH free radicals, accompanied by a gradual improvement in IC_{50} values. These results suggest that quinoa flour can serve as a promising alternative in the production of gluten-free bakery products, while providing added nutritional and health benefits that align with current trends toward functional foods.

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