



Gluten-Free Cupcake Enriched with Treated Black Quinoa Flour

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QUINOA is considered as a super grain due to its high nutrient content. The current study aimed to estimate the treated black quinoa flour by some treatments (sprouting, roasting and steaming) as well as the quality characteristic of gluten-free cupcakes with different substitution levels (25:100) of treated black quinoa flour. Sprouting and steaming reduced the total phenolic compounds (TPC) (78.44 and 96.06 mg GAE/100g, respectively) whereas roasting significantly increased it (102.92 mg GAE/100g) compared to the raw quinoa (98.99 mg GAE/100g). Treated quinoa flour had significantly higher antioxidant activity (AA) compared with control, and the roasting treatment showed the highest AA value. All the treatments significantly reduced the anti-nutrients content (phytates, tannins and saponins) of treated quinoa flour while enhance the functional properties (WHC and OHC) compared to the raw quinoa flour. High nutritional and functional gluten-free cupcakes for celiac patients were prepared. The crude protein, fat, ash, and fiber contents significantly increased in the produced cupcakes as the quinoa substitution levels increased (25:100). Developed quinoa cupcakes showed good characteristics due to their highest scores in numerous sensory characteristics. Concerning overall acceptability (OA) and acceptability index (AI), steamed cupcakes had the highest scores followed by roasted cupcakes. Although sprouted quinoa cupcakes had the least overall score among all processing methods, they still have a high acceptability index (more than 88%). Therefore, sprouted, roasted and steamed quinoa flour can be used as untraditional functional food ingredient for high nutritional value bakery products along with technological and health benefits.

Keywords: Gluten-free cupcake, Treated quinoa, Physicochemical properties, Functional properties, Sensory attributes, Texture profile analysis

Introduction

Recently, customers select healthier food because they are more conscious of what they eat, particularly in terms of preventing disease. As a result, cereal products play a main role in human nutrition since they are rich sources of essential nutrients and energy (Dziki et al., 2014). Pseudo cereals are plants having seeds that can be ground into cereals flour (Brady et al., 2007). They are similar to other grains such as rice, maize, millet and sorghum where they are free of gluten and can be used in therapeutic diets such as gluten free food for celiac disease (Alvarez-

Jubete et al., 2010; Saturni et al., 2010). In the recent decays, consumption of pseudo cereals, specifically quinoa has increased (FAOSTAT, 2013). This increase is mainly due to their nutritional profile, especially a high protein and mineral content (Mota et al., 2016; Nascimento et al., 2014). Quinoa (*Chenopodium quinoa* Wild) is a pseudo cereal and it is one of the natural plant-based sources of protein with high nutritional value due to higher essential amino acids content than in other cereals (Wu 2015; Haros et al., 2023). Quinoa is resistant to a variety of climatic conditions, like cold, drought, and heat stress.

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There are many quinoa varieties with different pigmentation degrees, including black, red, and rainbow (Aloisi *et al.*, 2016; Chen *et al.*, 2023). Furthermore, quinoa is considered as a super food as it contains a high content of calcium, iron, zinc and magnesium compared to the cereals. Consequently, due to the nutritional importance of these seeds, the Food and Agriculture Organization of the United Nations (FAO) declared 2013 as the International Year of Quinoa because it plays an important role in eliminating hunger, malnutrition and poverty (FAO, 2013). Quinoa contains functional ingredients which offer outstanding performance in various food applications. For example, polyphenols of quinoa flour improved the antioxidant capacity of bakery products (Alvarez-Jubete *et al.*, 2010; Chlopicka *et al.*, 2012). Omega-6 fatty acids (linoleic acid) of quinoa reduced the level of saturated fatty acids using bread enriched of γ -aminobutyric acid (Calderelli *et al.*, 2010).

Quinoa is a gluten-free, being an option for celiac disease (Morales *et al.*, 2021). Additionally, it is a good source of minerals, fiber and phytochemicals (Suarez-Estrella *et al.*, 2018). It has been reported that quinoa has phosphorus and magnesium content that may contribute with up to 55% of the daily recommended intake (Nascimento *et al.*, 2014). Interestingly, it could be used to prepare functional food products, due to its nutrient profile, functional properties and sensory acceptance (Burgos *et al.*, 2019). Quinoa has become a common raw ingredient for vegans and others who have allergies or intolerances to grains. These pseudo cereals have drawn a lot of attention from researchers as possible ingredients for gluten-free food formulations (Pasko *et al.*, 2009; Złotek *et al.*, 2019). Although the rich nutrient profile of quinoa has been extensively studied, most of the studies were realized on raw quinoa. Usually, quinoa is not consumed as a raw but it is treated in order to decrease the content of anti-nutritional component, such as saponins and phytic acid (Mhada *et al.*, 2020; Bhinder *et al.*, 2021). Phytic acid can reduce the nutritional value of quinoa because it binds divalent minerals and make them unavailable for metabolism (Demir and Bilgiçli, 2020; Miranda-Ramos and Haros, 2020). Phytic acid consumption of 5 to 10 mg/day can decrease iron solubility by 50%, potentially resulting in the iron deficiency (Siegenberg *et al.*, 1991). Saponins are the water-soluble components found in quinoa seeds as they are the bitter tasting steroid compounds. According to Thakur

et al. (2021), anti-nutritional components can be reduced or removed to safe levels for human health using appropriate processing at homes or in industries. It is reported that the aroma is a predominant factor that influences the flavor quality of quinoa. Typically, untreated quinoa has unpleasant flavors. In this regard, processing treatments could alter the sensory and nutritional aspects of the seed, serving as a potential method for reducing off-flavors (Almagueret *et al.*, 2023). Quinoa seeds like most other seeds are treated before using or consumption. They have been ground, puffed, extruded, or roasted, to make baked products, breakfast foods and snacks (Graf *et al.*, 2015; Gosine and McSweeney, 2019). Despite the nutritional benefits of quinoa, it may have unpleasant off-flavors due to the presence of bitter compounds which could limit its consumption (Suarez-Estrella *et al.*, 2018). Processing of quinoa seeds such as soaking, sprouting, fermentation, and roasting can reduce these compounds as well as increase their nutritional value. Sprouting is an important method to improve the nutritional and functional value of seeds. Numerous enzymes are activated during sprouting, which enhances the bioavailability of minerals and the protein digestibility (D'Ambrosio *et al.*, 2017). Roasting can change texture, color, flavor, along with appearance and the final product gain unique attributes like crispness and flavor that are not present in raw kernels (Chandrasekara and Shahidi, 2011). The aims of this work were to investigate the effect of pretreatment methods (sprouting, roasting, and steaming) on black colored quinoa seeds as well as the influence of gradual replacement of corn flour by different level of treated and untreated quinoa on the sensory, physicochemical, and functional properties of gluten-free cupcake.

Materials and Methods

Materials

Quinoa seeds, corn flour and other baking ingredients used to prepare cupcake (fresh egg, sugar, skimmed powdered milk, sunflower oil, baking powder, vanillin, salt and cocoa) were purchased from local markets. All chemicals and reagents used in the study were analytical grade.

Quinoa flour preparation

Quinoa seeds were cleaned from broken seeds and foreign materials and divided into four portions for raw, sprouted, roasted, and steamed quinoa seeds. The sprouting process was carried out following the method of D'Ambrosio *et al.*

(2017). To obtain sprouted quinoa, whole quinoa seeds (200 g) were soaked in water for 6 hr with successive change of soaking water. Then it was spread in a cotton cloth to germinate at 37 °C for 48 hr within consecutive change of water, and then the seeds were washed with water and consequently dried at 45-50 °C. Steaming process was performed to whole quinoa seeds (200 g) under atmospheric pressure in a food steamer (moulinex® AMA-141 Food Steamer, France) according to Motta et al. (2019) for 25 min. Whole quinoa seeds (200 g) were roasted according to Ajatta et al. (2021) with minor modifications in a hot air oven at 160 °C for 10 min, and then allowed to cool before grinding. The treated and untreated seeds were ground into flour using a grinder (Yellow line, A10; IKA-Werke, Staufen, Germany) and sieved through a 60-mesh sieve. The obtained flour samples were kept in polyethylene bags for further analysis.

Color characteristics

Color attributes (L^* , a^* , and b^* values) are obtained from the Minolta lab scale measurement according to McGurie (1992). The L^* value indicates lightness to darkness, a^* scale indicates red to green (a positive value indicate red and a negative value indicate green), and b^* scale represents yellow to blue (a positive values indicate yellow and negative values indicate blue).

Water activity (a_w)

The LabStart-aw (Novasina, Switzerland) apparatus was used to measure the water activity (a_w). For completely dehydrated food and pure water, a_w values varied from 0 to 1, respectively.

Proximate chemical composition

Chemical characteristics (moisture, protein, ash, fat, crude fiber and carbohydrates) of flour samples (corn, untreated quinoa, and treated quinoa) and the produced cupcake samples were determined according to the methods of AOAC (2019). The fat, protein, and total carbohydrate contents were used to calculate the energy value of the cupcake samples according to the AOAC methods (2019). It was expressed as the following equation:

$$\text{Energy (kcal/100 g)} = (\text{Fat} \times 9) + (\text{Protein} \times 4) + (\text{Carbohydrates} \times 4)$$

Phytochemical components of flour samples

Total phenolic content (TPC) of extracts were determined using the Folin-Ciocalteu reagent method according to Singleton and Rossi (1965).

A 0.25 mL of methanol extract was added to 0.25 mL of Folin-Ciocalteu's reagent (diluted 10 times) and 0.5 mL of Na_2CO_3 (10% w/v). The mixtures were kept in dark for 30 min at room temperature before measuring absorbance at 725 nm using a spectrophotometer (Jenway UV-Vis, Cole-Parmer Ltd., Staffordshire, UK). TPC was expressed as Gallic acid equivalent per gram dry weight of the flour. The free radical scavenging activity was measured using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method according to Fischer et al. (2013). Using the following formula, the scavenging activity was determined:

$$\text{DPPH radical-scavenging activity (\%)} =$$

$$[(A_c - A_f) / A_c] \times 100$$

where A_c is the absorbance of the control sample and A_f is the absorbance of the flour samples extract.

Anti-nutrients of flour samples

The contents of phytic acid, tannins, and saponins in corn flour, raw and treated quinoa flour were determined as follows:

Phytic acid content

Phytic acid content was determined based on the method of Mohamed et al. (1986). Trichloroacetic acid (TCA) extracts of each flour sample (1 mL) were introduced to columns packed using anion exchange column (Dowex AG1-X8) and washed with 6 mL of NaCl solution (0.2M). The phytate was then eluted from the column with NaCl solution (1.0M) and collected for phytate determination. The extracted phytate (0.2 mL) was mixed with distilled water (4.6 mL) and chromogenic solution (0.2 mL), then heated at 95°C for 30 min in a water bath and finally allowed to cool. The developed blue color was measured at 830 nm using a spectrophotometer (Jenway UV-Vis, Cole-Parmer Ltd., Staffordshire, UK). Phytate content was expressed as g/100g flour sample.

Tannins content

Tannins content was determined following the method described by Osman (2004). Methanol extract (1 mL) of flour sample was mixed with vanillin/HCl mixture (5 mL) in a test tube, kept for 20 min at room temperature, and then absorbance was measured at 500 nm by using spectrophotometer (Jenway UV-Vis, Cole-Parmer Ltd., Staffordshire, UK). Tannins were calculated as mg catechin equivalent (CE /100g) on dry weight basis.

Saponin content

For extraction of the saponin, one gram of each flour samples was mixed with 30 mL ethanol and the suspension was left at ambient temperature for 30 min. Subsequently, the suspension was cooled to ambient temperature and filtered. The clear extracts (0.25 mL) were placed in a water bath at 65 °C to dryness for about 5 min (until the methanol was evaporated). Then, 0.5 mL of vanillin in ethanol (4%) was added to each tube followed by 2.5 mL of H₂SO₄ (72%), vortexed, incubated in a water bath at 60 °C for 15 min and then left to cool at room temperature. Finally, the absorbance of the solutions was measured using spectrophotometer (Biosystem 310) at 560 nm (Le et al., 2018). The absorbance values obtained were plotted against the concentrations to construct a standard curve. Total saponin content was expressed as mg aescin equivalents per gram dry weight of the flour (mg/100gm).

Functional properties of flour samples

The water and oil holding capacity (WHC and OHC) of raw and treated quinoa flour, as well as corn flour, were measured using the Traynham et al. (2007) method. Briefly, in centrifuge tubes, 10 milliliters of either sunflower oil (OHC) or distilled water (WHC) were mixed with one gram of flour for 30 minutes and then centrifuged (at 4000 rpm for 20 minutes at room temperature). The weight difference between the sample before and after centrifugation was used to calculate

the amount of water or oil that was absorbed indicating the ability of flour to retain water and oil. Water solubility index (WSI) determines the amount of polysaccharide release from the granule upon the addition of excess of water. WSI is the weight of dry solids in the supernatant from the water holding capacity test, represented as percentage of the initial weight of the sample (Yousf et al., 2017).

Cupcake preparation

The formula and procedure for baking the gluten-free cupcakes were carried out based on the method of Hoover (2009) with some modifications (Table 1). The control cupcake sample contained 100% corn flour was substituted with different amount of raw and treated quinoa flour (25, 50, 75, and 100%). A Kitchen Aid Mixer (Model K45SS, St. Joseph, MI, USA) was used for batter mixing. Batters were baked in at 180 °C for about 30 minutes. Cupcakes were allowed to cool to room temperature before any testing.

Baking quality of cupcakes

After the cupcakes were allowed to cool at room temperature, they were weighed (g) and the volume (mL) was measured using the rapeseed displacement method (AACC, 2000). Specific volume was calculated as the ratio of volume to weight (mL/g). Baking loss (%) was calculated as the weight difference between the batter and the produced cupcake.

TABLE 1. Formulas of gluten-free cupcake with different substitution levels of quinoa flour

Ingredients (g)	Q0 (Control)	Q25	Q50	Q75	Q100
Corn flour	100	75	50	25	0
Quinoa flour	0	25	50	75	100
Sugar	85	85	85	85	85
Fresh egg	50	50	50	50	50
Skimmed powdered milk	10	10	10	10	10
Vanillin	1	1	1	1	1
Salt	1	1	1	1	1
Baking powder	2	2	2	2	2
Sunflower oil	30	30	30	30	30
Cocoa	6	6	6	6	6
Water	Variable	Variable	Variable	Variable	Variable

Q0, Q25, Q50, Q75, and Q100: formulas with 0, 25, 50, 75, and 100% quinoa flour (raw or treated quinoa) substitution levels, respectively.

Texture Profile Analysis (TPA)

Texture profile of cupcakes in terms of hardness, springiness, and cohesiveness were measured using a texture analyzer (Brookfield Texture Pro CT V1.8 Build 31, Stable Micro Systems, USA). TPA measurements were conducted under ambient conditions at 0, 4 and 8 days storage periods.

Sensory evaluation of cupcake

The cupcake samples were sliced after cooling and evaluated by ten panelists (staff in Food Technology Research Institute, Agricultural Research Center, Egypt) using a nine-point hedonic scale. Appearance, crust and crumb color, odor, taste, and overall acceptability were assessed according to the method of Stone and Sidel (1993). The average score for overall acceptability was used to calculate the acceptability index (%).

Alkaline Water Retention Capacity (AWRC)

Cupcake freshness was evaluated after 0, 4, and 8 days of storage at room temperature (25°C) using the AWRC method following Yamazaki (1953) method which was modified by Kitterman and Rubenthaler (1971).

Statistical analysis

In order to investigate statistically significant differences between the analysis means of the experimental data. Data was subjected to one-way analysis of variance (ANOVA) using Costat statistical software according to Steel and Torrie (1980). The mean values were compared at the ($P \leq 0.05$) level using Duncan's multiple range tests.

Results and discussion

Color characteristics of flour samples

Color is a crucial attribute that determines the acceptance of food products. The color attributes (L^* , a^* , and b^*) of flour samples are listed in Table 2. Since black quinoa samples were milled as whole meal, the quinoa flour included bran layer and husk, resulted in a lower L^* value than in the corn flour. It may be due to the presence of pigments in the black quinoa outer layers. Sprouted quinoa was soaked prior to germination, which results in a decrease in a soluble saponin content, it showed a significantly ($P \leq 0.05$) lower L^* value than raw quinoa (Vega-Gálvez et al., 2010). Regarding a^* and b^* values of all quinoa flour, they are a positive values indicating redness and yellowness, respectively. Treated quinoa flour had higher a^* values than raw quinoa flour. Quinoa seed redness is attributed to the betacyanin pigment, which is mainly found in the outer coat

of the seeds (Dularia et al., 2024). These findings were in agreement with the reported results that sprouting process decreased the L^* value of the sprouted quinoa, while increased a^* and b^* values which could be attributed to the hydrolysis of starch and proteins during germination (Rico et al., 2020). Thermal treatment of seeds at high temperature may cause protein denaturation as well as reaction of released amino acids with sugars to form melanoidins (Maillard reaction) giving a darker color (Dularia et al., 2024).

Water activity (a_w) of flour samples

Water activity is an important indicator for predicting the safety and quality of food since it represents the amount of water available to microorganisms. It was reported that, the activity and growth of all microorganisms can be prevented at a water activity less than 0.6 (Abbas et al., 2009). Moreover, a lower water activity to be less than 0.25, it is linked to a faster rate of lipid oxidation which may shorten the product's shelf life (Jensen and Risbo, 2007). The availability of water determined as the water activity in the raw and treated quinoa flour is shown in Table 2. It ranged from 0.27 to 0.43 in roasted quinoa flour and raw quinoa flour, respectively indicating high storage and microbiological stability of all quinoa samples and they could be considered as safe and stable ingredient for food applications. As seen from the results, all treated quinoa had significantly lower a_w than raw quinoa and corn flour.

Proximate chemical composition of flour samples

The changes in chemical composition of treated quinoa flour by sprouting, steaming, and roasting compared to the native/raw quinoa are presented in Table 2. The moisture content in treated quinoa samples was less than that of raw quinoa (9.02%). The highest value (7.90%) was found in sprouted quinoa while the lowest value (4.97%) was in roasted quinoa. Raw and different treated quinoa had significantly higher protein content than corn flour. The protein content of quinoa samples ranged between 12.16% and 14.37% in treated quinoa, compared to 7.38% in corn flour. The total protein content of the sprouted quinoa sample significantly decreased due to the soaking process of seeds before germination while it increased in steamed and roasted samples compared to the untreated one. The protein content in roasted seeds was the highest which is in agreement with the reported results of Mariod et al. (2012) and Upadhyaya et al. (2023).

The ash content in quinoa flour samples ranged from 1.95% to 2.99%. The sprouting process resulted in a significantly ($p \leq 0.05$) lower ash content compared to raw quinoa which could be attributed to minerals leaching during the soaking process prior to germination as well as the utilization of minerals as coenzymes during the bioconversion of carbohydrates (Quesada et al., 2020; Maldonado-Alvarado et al., 2023). This result obtained is consistent with other reported findings that roasting increases the ash content of food samples (Lawal, 2019).

Regarding to the crude fiber content, it ranged from 1.70% to 5.20%. In comparison to corn flour, quinoa flour has been found to have a higher fiber content, which may enhance the food product's nutritional value. Increased fiber content could reduce the risk of high cholesterol and chronic heart disease via improving food digestion, regulating blood lipids and the glycemic index (Chen et al., 2020). According to Giami (1993), sprouting of seeds results in a significant rise in crude fiber content because more cell wall material is synthesized to protect the shoots and rootlets during sprouting. In term of crude fat content, it was significantly higher in quinoa samples (ranged from 6.31 to 7.41%) compared to corn flour (3.56%). The high fat content of quinoa seeds may improve taste and acceptability in addition to serving as a good source of energy. Among quinoa samples, fat content was significantly the highest in raw quinoa (7.41%) and was the lowest in roasted quinoa (6.31%). During germination and sprouting, the lower fat content as compared to raw is due to hydrolysis of lipid and oxidation of fatty acids, since lipids are most likely used as an energy source (Kumari and Srivastava, 2000). The decrease in fat content

in roasted and steamed quinoa may contribute to either enzymatic hydrolysis or lipid oxidation as a result of thermal treatment (Perera, 2005; Stenberg et al., 2005). Concerning carbohydrates content, they were significantly lower in treated quinoa samples as compared to corn flour (86.12%). The carbohydrate content was found to be the highest in sprouted quinoa (73.52%) followed by steamed, roasted, and then raw quinoa (71.63, 71.51, and 70.85, respectively). According to Wright et al. (2002), quinoa seeds had 73.60% of carbohydrate content. Bhathal et al. (2017) reported that the sprouted and roasted quinoa had carbohydrate content higher than raw quinoa, on dry weight basis.

Phytochemicals content of flour samples

The phenolic compounds in quinoa seeds mainly exist in the outer seed coat and might distribute also within the seeds (Gómez-Caravaca et al., 2014). TPC and AA of flour samples are shown in Fig. 1. Raw and treated quinoa flour had significantly higher TPC and AA values than corn flour. Sprouting and steaming treatments lowered the TPC (78.44 and 96.06 mg GAE/100g, respectively) while roasting process significantly increased it (102.92 mg GAE/100g) compared to the raw quinoa (98.99 mg GAE/100g). According to the obtained results, dry thermal treatment (roasting) increased TPC and AA value of quinoa seeds. These findings align with earlier research demonstrated that roasting process led to TPC value increment compared to the raw material (Gallegos-Infante et al., 2010). Concerning the antioxidant activity, sprouting significantly lowered AA (19.29%) than raw quinoa (34.49%) while the roasting significantly increased it (37.48%). Furthermore, steaming process had no significant effect on the AA relative to raw quinoa

TABLE 2. Physicochemical characteristics of flour samples.

Parameter	Corn (Control)	Raw quinoa	Sprouted quinoa	Roasted quinoa	Steamed quinoa
L*	96.22±0.39 ^a	82.26±0.14 ^b	81.68±0.03 ^c	81.86±0.18 ^{bc}	78.11±0.31 ^d
a*	-0.40±0.06 ^c	1.32±0.05 ^b	1.62±0.03 ^a	1.37±0.03 ^b	1.58±0.02 ^a
b*	11.58±0.10 ^a	8.71±0.08 ^d	8.80±0.06 ^d	9.79±0.05 ^b	9.07±0.04 ^c
a _w	0.52±0.02 ^a	0.43±0.02 ^b	0.39±0.01 ^c	0.27±0.01 ^d	0.35±0.02 ^c
Moisture (%)	10.72±0.12 ^a	9.02±0.24 ^b	7.90±0.04 ^c	4.97±0.11 ^c	7.38±0.05 ^d
Protein (%)	7.38±0.08 ^c	13.98±0.06 ^c	12.16±0.01 ^d	14.37±0.09 ^a	14.21±0.03 ^b
Ash (%)	1.23±0.02 ^d	2.78±0.02 ^b	1.95±0.03 ^c	2.99±0.04 ^a	2.80±0.05 ^b
Crude fiber (%)	1.70±0.02 ^c	4.97±0.18 ^{ab}	5.20±0.05 ^a	4.81±0.14 ^b	4.90±0.10 ^b
Fat (%)	3.56±0.05 ^d	7.41±0.09 ^a	7.17±0.08 ^b	6.31±0.07 ^c	6.46±0.11 ^c
TC (%)	86.12±0.16 ^a	70.85±0.18 ^d	73.52±0.07 ^b	71.51±0.11 ^c	71.63±0.09 ^c

Values are means ± SD (n=3) and different superscript letters in the same row are significantly different at $p \leq 0.05$. Protein, ash, fat, crude fiber, and TC (total carbohydrates) on dry weight basis.

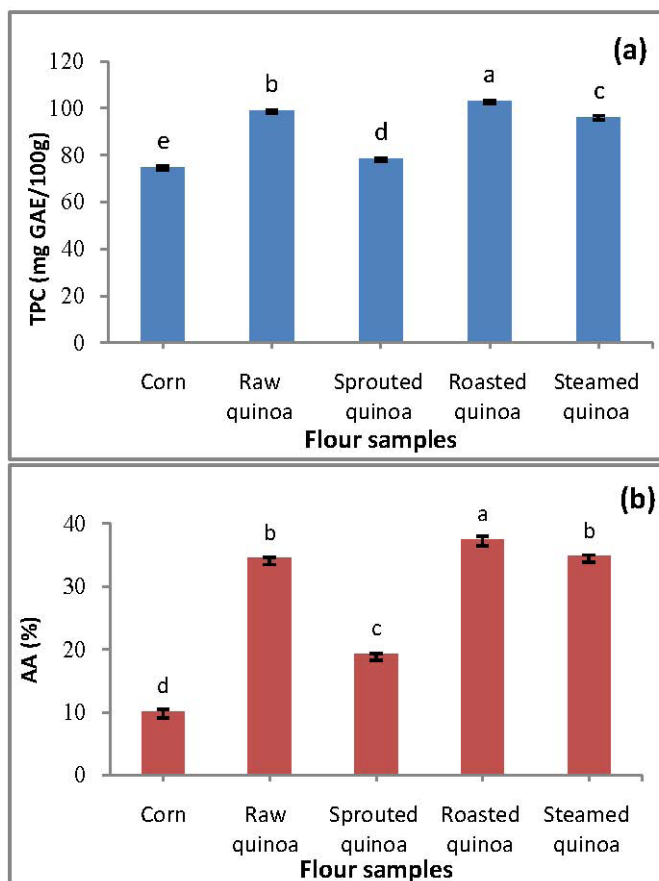
(34.83% and 34.49%, respectively). Higher TPC and AA in roasted quinoa could be attributed to Maillard reaction products which increase as processing temperature increase. Maillard products cause an overestimation of TPC content using Folin-Ciocalteu's method (Chandrasekara and Shahidi, 2011; Carciochi et al., 2016).

Anti-nutrients of flour samples

Quinoa seeds include anti-nutritional components such as phytic acid, tannins, saponins, trypsin inhibitors, and oxalates. Unlike other cereals, phytic acid is distributed in the endosperm of quinoa as well as in the outer layers (Vega-Gálvez et al., 2010). Moreover, phytates may link to cations such as iron, zinc, calcium, and magnesium, which restricts their absorption (Jukanti et al., 2012). Furthermore, tannins can join with protein through non-covalent bond, decreasing their nutritional availability (Zia-Ul-Haq et al., 2007).

Meanwhile, phytates can be beneficial because of their antioxidant activity (Miranda-Ramos et al., 2019). Phytic acid, tannins, and

saponins of raw and treated quinoa flour are presented in Table 3. Results indicated that all treated quinoa had significantly lower phytic acid content (0.95: 1.12 g/100g) compared to raw quinoa (1.30 g/100g). According to Thakur et al. (2021), the phytic acid content in raw quinoa seeds ranged from 1.05 to 1.35 g/100g. Sprouted quinoa had the lowest phytic acid content (0.95 g/100g) among all quinoa samples and corn flour. It has been reported that, the soaking and sprouting treatments significantly decreased the phytic acid content in beans (Luo et al., 2013). Moreover, endogenous phytase activity (an enzyme that degrades phytic acid) significantly increased as a result of sprouting and phytic acid content consequently decreased (Maldonado-Alvarado et al., 2023). Notably, the data obtained (Table 3) showed that the phytic acid content was not significant different among the two thermal treatments (roasting and steaming). In terms of tannins content, it was significantly ($P \leq 0.05$) high in quinoa flour samples compared to the corn flour. All processing methods significantly reduce tannin content when compared to raw



Data are means \pm SD and the different small letter indicates significant difference ($p \leq 0.05$).

Fig. 1. TPC (mg GAE/100g) and AA (%) of flour samples.

quinoa (Table 3). It ranged from 26.14 mg/100g in steamed quinoa to 36.80 mg/100g in untreated quinoa. It was shown that the tannins content was decreased approximately 26, 14.5, and 29% after sprouting, roasting, and steaming treatments, respectively. From the results obtained, the most effective process for lowering tannin content was found to be hydrothermal treatment (steaming) followed by sprouting. This result was in consistence with the finding of Le *et al.* (2021). During sprouting, many enzymes are activated leading to the hydrolysis of some components such as proteins and high molecular weight phenolic compounds (tannins) (Kumari *et al.*, 2015).

Regarding total saponin content, both raw and treated quinoa flour contained significantly higher content than corn flour (Table 3). Among quinoa flour samples, raw quinoa had significantly ($P \leq 0.05$) the highest saponin content (5.63 mg AE/100gm) and it decreased to 5.10, 4.78, and 4.10 mg AE/100gm after roasting, sprouting, and steaming, respectively. It was reported that thermal treatment degraded saponins, which may directly affect sensory perception and reduce the bitter flavor that saponins provide (Brady *et al.*, 2007). The minimum content of saponin after steaming process (Table 3) could be attributed to the high moisture content during steam treatment, since the starch particles are completely expanded by thermal suction water which releases the embedded saponin starch molecules (Yang *et al.*, 2020). Washing, soaking, sprouting and steaming of quinoa has been reported to reduce its high saponin content due to the leaching out of saponin from the seed (Bhathal *et al.*, 2015; Padmashree *et al.*, 2019).

Functional properties of flour samples

Functionality should be investigated because the water holding capacity (WHC) and oil holding capacity (OHC) influence the utilization of samples in food (Joshi *et al.*, 2015). WHC and OHC are quality control indices in the food

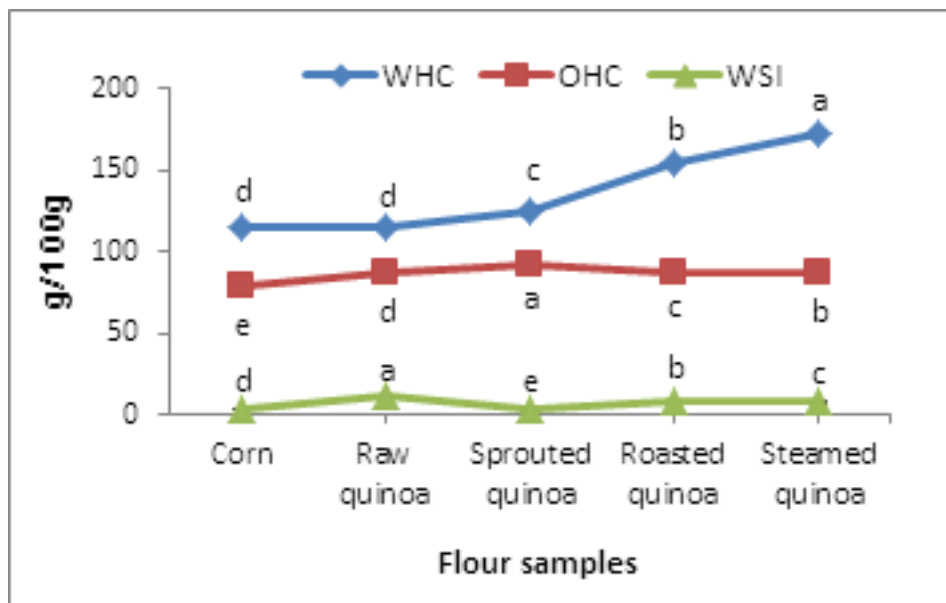
industry given that they may affect the food texture and taste. The effect of different treatments on WHC and OHC of raw and treated quinoa flour were assessed and shown in Fig. 2. WHC is used to measure the amount of water absorbed by starch as a starch gelatinization index since native starch does not absorb water at room temperature (Seth and Rajamanickam, 2012). Interestingly, all the treatments of quinoa significantly increased the water and oil holding capacity ($p \leq 0.05$) which ranged from 125.37 to 172.81% and from 87.13 to 91.35%, respectively. Noticeably, WHC of thermal treatments (roasting and steaming) were the highest values which may be attributed to the starch damage caused by thermal gelatinization, as well as the denaturation and dissociation of the protein (Jogihalli *et al.*, 2017). Compared to the untreated and other treated quinoa samples, WHC of steamed quinoa was significantly ($p \leq 0.05$) the highest. It was found that moist-heat treatment might significantly increase WHC of seeds, which could be due to structural changes of components in kernel during heating, that would lead to absorbing more water and improving the hydration property (Choe *et al.*, 2022).

Additionally, this may be contributed to the starch swelling, gelatinization, or damaging during steaming treatment, which lead to more exposed hydroxyl groups that interacting with water (Choe *et al.*, 2022). On the other hand, the sprouted quinoa showed the highest OHC value (Fig. 2). Regarding the water solubility index (WSI), all the studied treatments significantly decreased it ($p \leq 0.05$), which ranged from 3.18% in sprouted quinoa to 8.38% in roasted quinoa compared to 11.51% in raw quinoa. The WSI is an indicator to the quantity of soluble solids and is frequently used as indication to starch molecules degradation. It is also a parameter for measuring the rate of starch conversion and represents the amount of liberated polysaccharide from starch granules during processing (Jogihalli *et al.*, 2017).

TABLE 3. Anti-nutrients of raw flour samples.

Flour samples	Phytic acid (g/100g)	Tannins (mg/100g)	Saponin (mg/100g)
Corn (Control)	1.23±0.07 ^b	3.94±0.14 ^c	3.57±0.07 ^c
Raw quinoa	1.30±0.03 ^a	36.80±0.24 ^a	5.63±0.11 ^a
Sprouted quinoa	0.95±0.02 ^d	27.10±0.12 ^c	4.78±0.17 ^c
Roasted quinoa	1.08±0.03 ^c	31.47±0.10 ^b	5.10±0.12 ^b
Steamed quinoa	1.12±0.02 ^c	26.14±0.25 ^d	4.10±0.12 ^d

Values are means±SD (n=3) and different superscript letters in the same column are significantly different at $p \leq 0.05$.



Values are means \pm SD (n=3) and different small letters on the same curve are significantly different at $p\leq 0.05$. WHC, OHC and WSI are Water Holding Capacity, Oil Holding Capacity and Water Solubility Index, respectively.

Fig. 2. Functional properties (WHC, OHC and WSI) of flour samples

Color characteristics of cupcakes

Color is one of the most important quality indicators that people consider when choosing food product. The color attributes of cupcakes prepared using various substitution ratios of treated and raw quinoa flour are displayed in Table 4. The L^* , a^* , and b^* values of the crust and crumb in the control cupcake samples were higher than quinoa substituted cupcakes. These values decreased gradually by increasing the amount of quinoa flour. The lower lightness (L^*) values of crumb and crust with increasing quinoa flour may be attributed to the original color of black quinoa flour, which is darker than the corn flour. Additionally, the higher quinoa substitution, the more darkness (lower L^* value) of both cupcake crust and crumb due to the pigments in black quinoa flour. Karimi et al. (2021) reported that, the lower L^* value of cupcake crust and crumb were due to the higher protein content that interacts with reducing sugars (Maillard reaction) during baking. The redness (a^*) and yellowness (b^*) values of cupcake crust and crumb, enriched with black quinoa flour, gradually decreased as the amount of quinoa flour increased due to natural pigments in whole meal quinoa flour and higher protein content that causing the darkness of color.

Proximate chemical analysis of cupcakes

The chemical composition of corn cupcake substituted with different levels of quinoa flour (25:100%) is presented in Table 5. Protein, ash, fat, and crude fiber contents were gradually increased as the substitution level of quinoa flour

increased in the formula of cupcake. Cupcake with 100% roasted quinoa flour recorded the highest protein content (10.34%), followed by 100% steamed quinoa cupcake (9.88%). This enhancement in protein content is due to the higher protein content of quinoa flour than corn flour as shown in Table 2. Among all treatments, sprouted quinoa cupcake significantly showed the lowest protein content (8.71%). Ash and crude fiber contents of cupcake enriched with quinoa flours also were higher than the control sample due to using the whole meal quinoa flour that had higher fiber and ash content than corn flour (Table 2). Ash content in all cupcake samples ranged from 1.58% to 2.52%. Cupcake with 100% steamed quinoa flour significantly recorded the highest ash content due to the higher ash content in steamed quinoa flour than other treated quinoa flour. The lowest ash content was observed in the 100% corn flour cupcake (control). Fiber content of cupcake substituted with quinoa flour also were higher than the control sample due to using the whole grain quinoa flour that had higher fiber content compared to corn flour (Table 2). Fiber content increased from 1.18% in control up to 2.82% in sprouted quinoa cupcakes. These findings are in the same line with the previously reported data (Repo-Carrasco et al., 2003; Park et al., 2005) which showed that the bakery products supplemented with quinoa flour increased the fiber and minerals content. Fat content of cupcakes ranged from 15.12 to 17.26%. Cupcake with 100%

TABLE 4. Color characteristics of cupcakes crust and crumb.

Substitution level (%)	Crust			Crumb		
	L*	a*	b*	L*	a*	b*
Raw quinoa						
0 (Control)	49.83±0.21 ^a	7.94±0.07 ^a	6.54±0.09 ^a	51.83±0.17 ^a	7.30±0.05 ^a	10.99±0.12 ^a
25	46.15±0.13 ^b	5.94±0.06 ^b	5.41±0.10 ^b	50.04±0.12 ^b	6.05±0.07 ^b	8.89±0.05 ^b
50	45.16±0.23 ^c	5.14±0.07 ^c	4.67±0.03 ^c	48.13±0.16 ^c	5.16±0.07 ^c	6.86±0.08 ^c
75	43.04±0.30 ^d	3.41±0.08 ^d	3.45±0.09 ^d	47.36±0.07 ^d	4.22±0.04 ^d	5.94±0.12 ^d
100	42.18±0.06 ^c	2.67±0.12 ^c	3.08±0.08 ^c	45.00±0.21 ^c	3.83±0.01 ^c	5.19±0.03 ^c
Sprouted quinoa						
0 (Control)	49.83±0.21 ^a	7.94±0.07 ^a	6.54±0.09 ^a	51.83±0.17 ^a	7.30±0.05 ^a	10.99±0.12 ^a
25	48.21±0.22 ^b	7.24±0.12 ^b	5.44±0.06 ^b	49.90±0.16 ^b	6.48±0.02 ^b	9.42±0.05 ^b
50	46.63±0.17 ^c	4.76±0.12 ^c	4.88±0.11 ^c	47.01±0.14 ^c	4.62±0.08 ^c	6.94±0.10 ^c
75	44.26±0.27 ^d	3.62±0.09 ^d	3.93±0.06 ^d	46.09±0.08 ^d	4.20±0.08 ^d	6.00±0.10 ^d
100	43.40±0.14 ^c	3.33±0.10 ^c	3.33±0.09 ^c	44.87±0.07 ^c	3.76±0.05 ^c	5.25±0.09 ^c
Roasted quinoa						
0 (Control)	49.83±0.21 ^a	7.94±0.07 ^a	6.54±0.09 ^a	51.83±0.17 ^a	7.30±0.05 ^a	10.99±0.12 ^a
25	48.28±0.23 ^b	5.23±0.06 ^b	5.93±0.06 ^b	49.95±0.18 ^b	6.41±0.05 ^b	10.28±0.03 ^b
50	46.42±0.15 ^c	4.87±0.07 ^c	5.24±0.09 ^c	47.12±0.20 ^c	5.06±0.07 ^c	8.63±0.06 ^c
75	44.44±0.17 ^d	3.74±0.06 ^d	4.28±0.16 ^d	46.05±0.11 ^d	4.82±0.06 ^d	6.98±0.05 ^d
100	49.83±0.21 ^c	2.97±0.07 ^c	3.57±0.11 ^c	42.76±0.14 ^c	3.73±0.07 ^c	5.69±0.07 ^c
Steamed quinoa						
0 (Control)	49.83±0.21 ^a	7.94±0.07 ^a	6.54±0.09 ^a	51.83±0.17 ^a	7.30±0.05 ^a	10.99±0.12 ^a
25	49.09±0.22 ^b	5.46±0.09 ^b	5.60±0.09 ^b	50.95±0.16 ^b	6.47±0.03 ^b	10.01±0.05 ^b
50	48.27±0.17 ^c	4.43±0.08 ^c	4.96±0.07 ^c	48.76±0.07 ^c	4.92±0.02 ^c	7.17±0.05 ^c
75	46.12±0.17 ^d	3.55±0.07 ^d	4.14±0.13 ^d	46.96±0.25 ^d	4.42±0.06 ^d	6.09±0.07 ^d
100	44.05±0.10 ^c	3.09±0.05 ^c	3.47±0.08 ^c	44.76±0.28 ^c	4.13±0.04 ^c	5.53±0.03 ^c

Values are means±SD (n=3) and different superscript letters in the same column, for each treatment separately, are significantly different at $p \leq 0.05$. Substitution level: 0, 25, 50, 75 and 100% are (100% corn and 0% quinoa), (75% corn and 25% quinoa), (50% corn and 50% quinoa), (25% corn and 75% quinoa), and (0% corn and 100% quinoa), respectively.

sprouted and steamed quinoa had the highest fat content while control cupcake showed the lowest content. Regarding total carbohydrates content of cupcakes enriched with quinoa flour up to 100%, has significantly lower content than control due to the higher protein, ash, fat, and fiber contents in quinoa flour samples compared to corn flour (Table 2). Total energy of the prepared cupcakes ranged from 464.58 to 470.04 kcal/100g. Results showed that among each treatment method, 25% substitution level had the highest energy value. The obtained results of the current study are in line the reported findings by Stikic *et al.* (2012) and El-Sohaimy *et al.* (2020), who found that bread fortified with quinoa flour had a higher nutritive value contained more protein, fiber, and fat.

Moisture content (%) of the cupcakes during storage periods

The moisture content (%) of the cupcakes during storage periods (up to 8 days) is shown in Fig. 3. Within each treatment process, the control sample (0% substitution level) had the lowest moisture content. As the substitution level of both raw and treated quinoa flour increase, the moisture content significantly increased. This trend could be contributed to the higher protein and fiber content where the water retention capacity of the flour is related to protein and fiber content (Torbica *et al.*, 2010; Shobeiri *et al.*, 2023). Flour with high soluble dietary fiber content shows higher water absorption of the dough in baking products which allows more interaction with water. It was reported that quinoa has high level of dietary fiber that can improve moisture maintenance (Turkut *et al.*, 2016; Shobeiri *et al.*, 2023).

TABLE 5. Proximate chemical composition of quinoa cupcakes with different substitution levels.

Substitution levels (%)	Crude Protein (%)	Ash (%)	Fat (%)	Crude Fiber (%)	TC (%)	Energy (kcal/100 g)
Raw quinoa						
0 (Control)	7.06±0.02 ^c	1.58±0.02 ^c	15.12±0.04 ^d	1.18±0.01 ^c	75.29±0.10 ^a	465.51±0.07 ^d
25	8.03±0.02 ^d	1.93±0.02 ^d	16.70±0.05 ^c	1.43±0.04 ^d	71.91±0.11 ^b	470.04±0.01 ^a
50	9.03±0.23 ^c	2.21±0.01 ^c	16.85±0.02 ^b	1.72±0.05 ^c	70.19±0.28 ^c	468.51±0.34 ^b
75	9.38±0.06 ^b	2.28±0.02 ^b	16.87±0.02 ^b	1.85±0.03 ^b	69.62±0.05 ^d	467.86±0.22 ^{bc}
100	9.83±0.06 ^a	2.41±0.05 ^a	16.97±0.03 ^a	2.01±0.05 ^a	68.78±0.01 ^c	467.17±0.56 ^c
Sprouted quinoa						
0 (Control)	7.06±0.02 ^c	1.58±0.02 ^d	15.12±0.04 ^d	0.95±0.01 ^d	75.30±0.10 ^a	465.51±0.07 ^c
25	7.65±0.04 ^d	1.68±0.01 ^c	16.66±0.07 ^c	1.85±0.15 ^c	72.16±0.14 ^b	469.14±0.98 ^a
50	7.81±0.07 ^c	1.71±0.01 ^c	16.92±0.08 ^b	2.39±0.09 ^b	71.17±0.11 ^c	468.22±0.02 ^{ab}
75	8.27±0.17 ^b	1.78±0.01 ^b	17.18±0.09 ^a	2.65±0.01 ^a	70.12±0.25 ^d	468.19±0.48 ^{ab}
100	8.71±0.06 ^a	1.99±0.03 ^a	17.26±0.10 ^a	2.82±0.04 ^a	69.22±0.15 ^c	467.05±0.51 ^b
Roasted quinoa						
0 (Control)	7.06±0.02 ^c	1.58±0.02 ^c	15.12±0.04 ^d	0.95±0.01 ^d	75.29±0.10 ^a	465.51±0.07 ^d
25	7.94±0.11 ^d	1.95±0.04 ^d	16.43±0.13 ^c	1.18±0.01 ^c	72.50±0.30 ^b	469.63±0.39 ^a
50	8.41±0.07 ^c	2.22±0.02 ^c	16.64±0.07 ^{bc}	1.34±0.05 ^{bc}	71.39±0.18 ^c	468.97±0.23 ^b
75	9.72±0.15 ^b	2.31±0.02 ^b	16.72±0.10 ^{ab}	1.53±0.11 ^{ab}	69.72±0.03 ^d	468.26±0.13 ^c
100	10.34±0.31 ^a	2.42±0.05 ^a	16.92±0.08 ^a	1.63±0.15 ^a	68.69±0.28 ^c	468.35±0.02 ^c
Steamed quinoa						
0 (Control)	7.06±0.02 ^c	1.58±0.02 ^c	15.12±0.04 ^c	0.95±0.01 ^c	75.30±0.10 ^a	465.51±0.07 ^{bc}
25	7.64±0.01 ^d	1.91±0.01 ^d	16.34±0.02 ^b	1.59±0.09 ^d	72.52±0.11 ^b	467.68±0.21 ^a
50	8.38±0.17 ^c	2.10±0.02 ^c	16.39±0.07 ^b	1.80±0.01 ^c	71.33±0.22 ^c	466.31±0.39 ^b
75	9.29±0.04 ^b	2.39±0.01 ^b	16.88±0.02 ^a	2.41±0.07 ^b	69.03±0.01 ^d	465.24±0.32 ^{cd}
100	9.88±0.08 ^a	2.52±0.03 ^a	17.03±0.11 ^a	2.63±0.06 ^a	67.94±0.23 ^c	464.58±0.47 ^d

Values are means±SD (n=3) on the dry weight basis and different superscript letters in the same column, for each treatment separately, are significantly different at $p \leq 0.05$. TC is total carbohydrates calculated by difference. Substitution level: 0, 25, 50, 75 and 100% are (100% corn and 0% quinoa), (75% corn and 25% quinoa), (50% corn and 50% quinoa), (25% corn and 75% quinoa), and (0% corn and 100% quinoa), respectively.

Water activity and baking quality of gluten-free cupcakes

Water activity (a_w) of cupcakes is shown in Fig. 4a. Water activity is an important indicator to determine the shelf life of food product. The 0.9 water activity significantly shortened the shelf life of cake and facilitated the growth of bacteria, mold, and yeast. The value of a_w of cake samples increased as the quinoa substitution level increased and it ranged from 0.79 to 0.82, 0.80 to 0.81, 0.79 to 0.83, and 0.78 to 0.80 in the raw, sprouted, roasted, and steamed cupcakes, respectively. Among all cupcakes, the lowest a_w value was found in the control sample. It was previously mentioned that a_w was associated with

the variation in moisture content (Lazaridou et al., 2007).

Effect of using raw and different treated quinoa flour on specific volume (mL/g) and baking loss (%) of cupcakes are presented in Fig. 4b and 4c. Compared to 100% corn flour cupcake, there was no significant difference in the specific volume when using raw quinoa flour. Meanwhile, the specific volume was significantly higher using sprouted and roasted quinoa flour up to 75% replacement compared to corn flour sample (control). Furthermore, cupcakes prepared with steamed quinoa flour had significantly

higher specific volume than control up to 100% replacement. During the mixing stage of ingredients, as the level of quinoa flour increased, the batter becoming harder which could explain why the 100% quinoa flour cupcake had the lowest specific volume. This result is attributed to the higher water holding capacity of quinoa flour than corn flour as shown in Fig. 2. Gluten-free bread formulated with 50% quinoa flour was prepared with significantly increased specific volume (Aprodu and Banu, 2021).

Regarding baking loss, it ranged from 11.68% to 17.06% in raw quinoa cupcake (in 25% substitution level) and roasted quinoa cupcake (in 100% substitution level), respectively (Fig. 4c). The baking loss (%) of both raw and steamed quinoa cupcakes (up to 100% quinoa flour substitution) was less than the control. Baking loss of sprouted quinoa cupcakes (up to 75% of

substitution level) was lower than control, but it was higher in the cupcake with 100% substitution level. Among all prepared cupcakes, the baking loss values of roasted quinoa cupcakes (up to 100% substitution level) were higher than other treated quinoa due to the lower specific volume. The lowest specific volume (100% roasted cupcake) could be attributed to the denser and tightly packed crumb structure which leads to a higher crumb hardness and lower specific volume. Furthermore, enhanced starch gelatinization in thermal treated quinoa (roasted and steamed) may support the internal structure formation and volume retention upon baking. Additionally, lipid loss followed heat treatment of quinoa seeds (Table 2) is another factor, since lipids could reduce the interfacial stability of the protein layer and hence reducing its ability to trap gas (Li *et al.*, 2023).

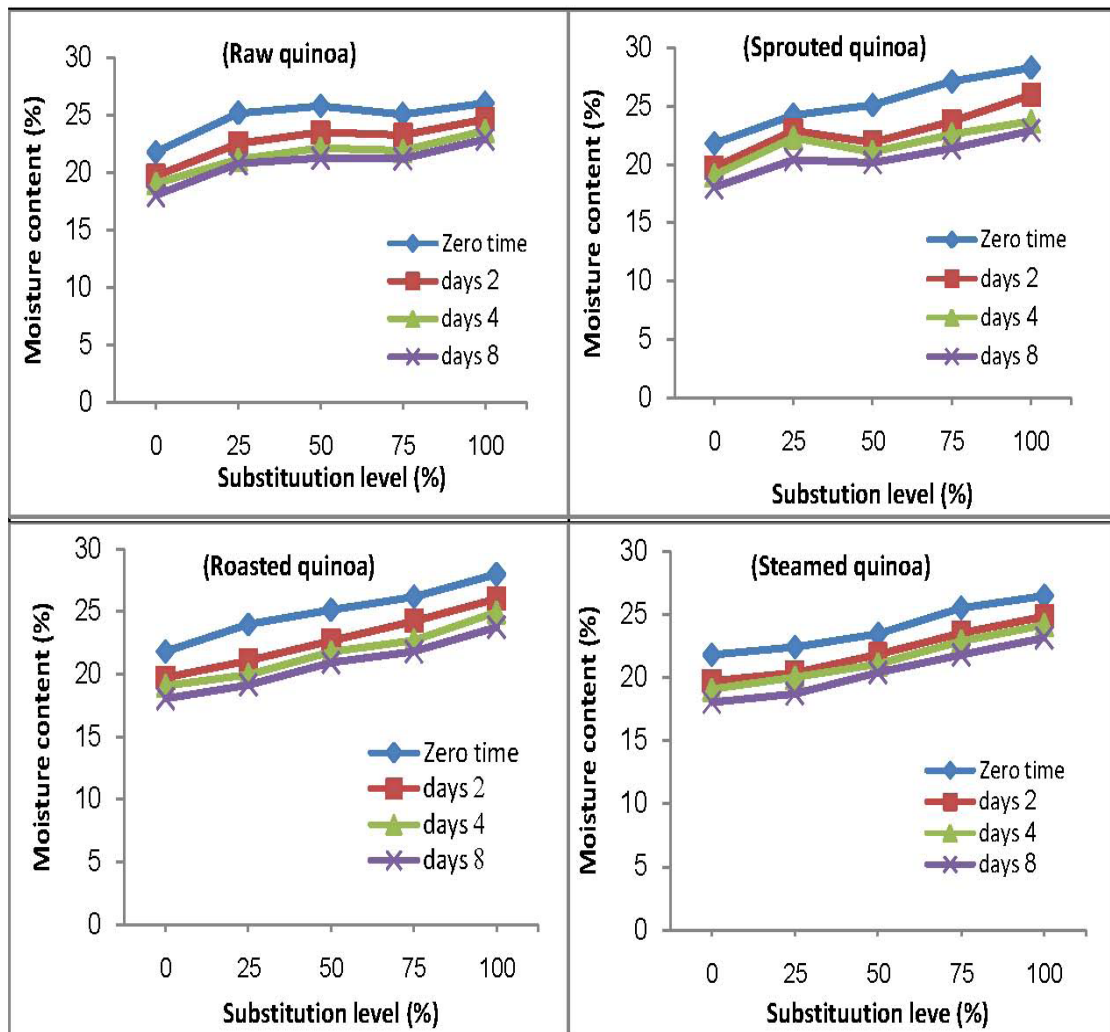
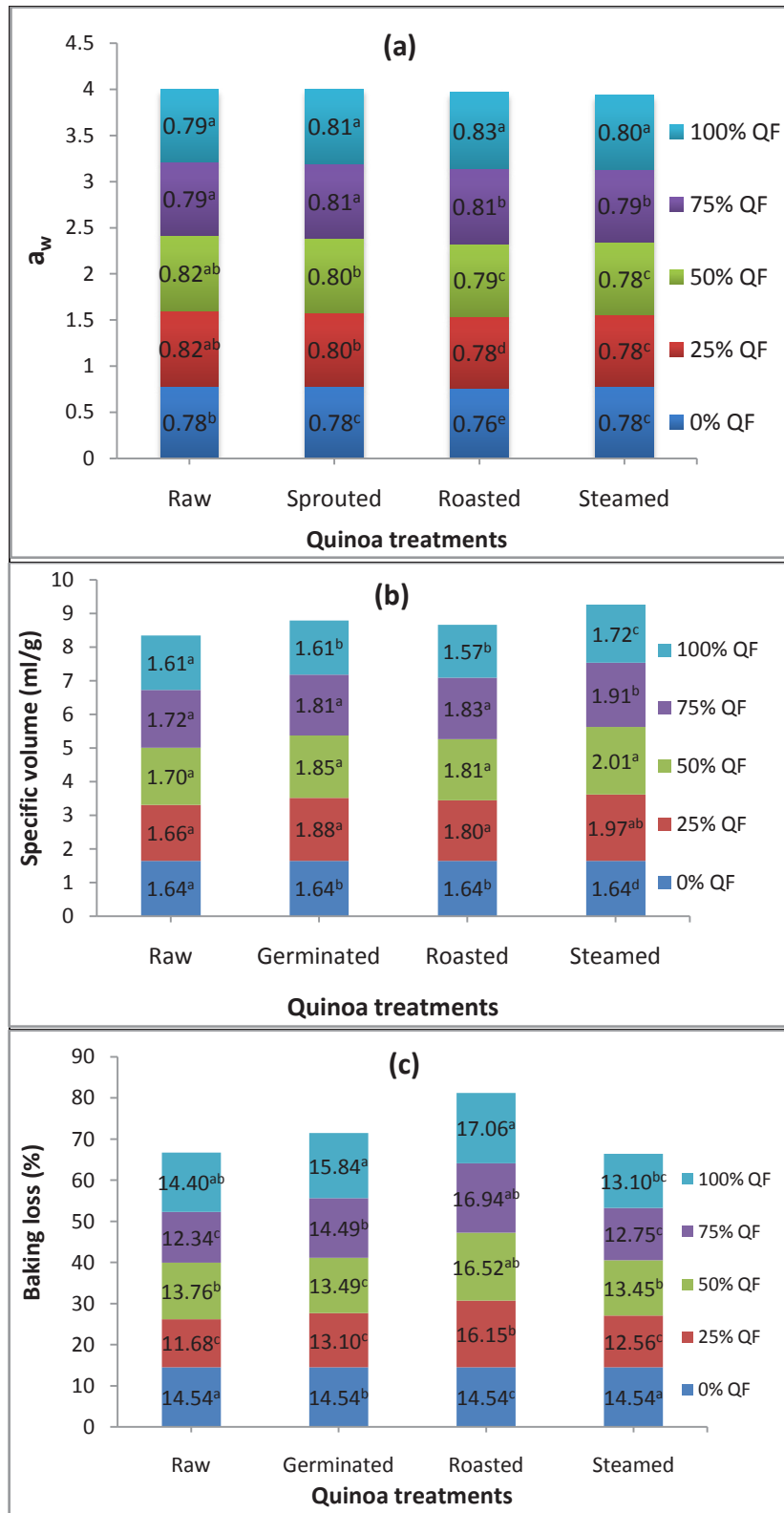


Fig. 3. Moisture content (%) of gluten-free cupcakes during storage periods.



Values are means±SD (n=3) and different superscript letters are significantly different at $p \leq 0.05$. QF, is quinoa flour.

Fig. 4. Water activity (a_w) and baking quality of gluten-free cupcakes.

Texture profile analysis (TPA) of gluten-free cupcakes

Effect of quinoa flour substitution on the textural characteristics (hardness, springiness, and cohesiveness) of gluten-free cupcakes is displayed in Table 6. Hardness describes the force needed to deform food when bitten (Esteller *et al.*, 2004). Cupcakes without quinoa flour (100% corn flour) showed the highest hardness values which could be attributed to lower moisture content and specific volume (Fig. 3 and 4). Due to the lower specific volume, the cupcakes were denser, had a packed crumb structure and had higher hardness. Firmness is inversely correlated with the specific volume (Hera *et al.*, 2014). Also, the fiber-protein complex at high baking temperature of cakes can cause the hardening of the final product (Jan *et al.*, 2018). As shown in Table 6, the hardness (firmness) of cupcakes increased as the substitution level of quinoa flour increased during all storage periods of cupcakes (zero, 4 and 8 days) at room temperature ($25\pm 2^{\circ}\text{C}$). These results are consistent with the previous finding that hardness of pan bread was gradually increased by increasing the amount of quinoa flour (Park *et al.*, 2005; Cotovanu *et al.*, 2021).

Springiness of the cupcake indicates a fresh and elastic product and has been associated with protein aggregation (Shevkani and Singh, 2014). The springiness of the cupcakes enriched with various quinoa flour levels (raw and treated) was higher than control due to the higher protein content compared to corn flour. In the term of cohesiveness, it describes the ability of food structure to resist compression and it is an important factor to produce a high quality product to meet consumer and packaging standards (Bozdogan *et al.*, 2019). The cohesiveness values of all quinoa cupcakes were found to be higher than control which could be attributed to the higher WHC of quinoa than corn flour (Fig. 2). This result supports the finding of Madadi *et al.* (2024), who reported that quinoa flour at medium and high substitution levels increased the cohesiveness value.

Sensory evaluation of gluten-free cupcake

Sensory attributes for the freshly prepared cupcake samples with different quinoa flour substitution assessed on a 9-point hedonic scale are summarized in Table 7. The more corn flour substituted with black quinoa flour; the browner (darker) cake color (Fig. 5) due to the colored pigments. Regarding appearance parameter, no significant differences among all quinoa substitution levels and they were all higher than control (100% corn flour). Cupcakes prepared using thermally treated quinoa (roasted and steamed) had higher scores compared to raw and sprouted quinoa.

The higher quinoa substitution, the more darkness (lower L^* value, Table 4) and the browner color of both cake crust and crumb, due to the higher protein content (Table 5) that interacts with reducing sugars during baking in the Maillard reaction. The final products of Maillard reaction (melanoidins) as well as the resultant brown pigments are responsible for the brown color development in roasted quinoa seeds (Dularia *et al.*, 2024). Compared to control (100% corn flour), no significant difference in the odor scores up to 25% and 50% of substitution levels for raw and sprouted quinoa, respectively and they tended to decrease as substitution level more increased than these levels. While all roasted and steamed quinoa cupcakes (up to 100% substitution level), showed no significant difference in the odor scores and they were higher than control. In terms of taste characteristic, roasted and steamed quinoa cupcakes recorded higher taste scores compared to raw and sprouted cupcakes due to their nutty flavor and enhanced cocoa color. Whole grains have cell wall structures, biopolymers, and flavor-active components that can alter their flavor characteristics during different treatments (Heinio *et al.*, 2016). Roasting has been reported to enhance the flavor of foods like peanuts and coffee and decrease anti-nutrients (Moon and Shibamoto, 2009). The taste score increased gradually as the roasted and steamed quinoa substitution level increased up to 100%. Interestingly, roasted and steamed quinoa highly produced cocoa, caramel and roasted nut flavor (taste and aroma) to cupcakes. The obtained results are consistent with other reported work (Peng *et al.*, 2024). Concerning overall acceptability (OA) and acceptability index (AI, %), steamed cupcakes had the highest scores (ranged from 8.56 to 8.74 and 95.08 to 97.06%, for OA and AI, respectively) followed by roasted (ranged from 8.49 to 8.63 and 94.37 to 95.87%, for OA and AI, respectively). From the obtained results, it could be concluded that treated quinoa cupcakes are good functional gluten-free alternatives with good characteristics due to their highest scores in numerous sensory characteristics. Although sprouted quinoa cupcakes had the least overall score among all processing methods, they still have a high acceptability index (more than 88%) as shown in Table 7. These findings indicated that the substitution of corn flour with thermally treated quinoa flour had a positive impact on the sensory attributes of the cupcakes. Furthermore, the results revealed that the panelists showed a preference for the dark brown color observed in thermally treated black quinoa. On the other hand, the raw quinoa sample received lower scores for most sensory attributes.

TABLE 6. Texture profile analysis (TPA) of gluten-free cupcakes.

Substitution levels (%)	Hardness (N)			Springiness			Cohesiveness		
	Zero time	4 days	8 days	Zero time	4 days	8 days	Zero time	4 days	8 days
Raw quinoa									
0 (Control)	34.21±0.42 ^a	36.54±0.41 ^a	39.14±0.59 ^a	11.46±0.04 ^d	10.51±0.05 ^d	10.06±0.09 ^d	0.31±0.01 ^d	0.23±0.01 ^e	0.19±0.01 ^d
25	24.30±0.49 ^e	25.73±0.62 ^e	28.95±0.16 ^c	15.76±0.11 ^c	13.94±0.06 ^b	12.48±0.04 ^b	0.33±0.01 ^d	0.26±0.01 ^d	0.21±0.01 ^d
50	27.19±0.31 ^d	28.01±0.61 ^d	30.22±0.98 ^c	16.24±0.07 ^b	13.60±0.05 ^d	12.10±0.07 ^c	0.35±0.01 ^c	0.32±0.01 ^c	0.24±0.01 ^c
75	29.18±0.42 ^c	30.72±0.90 ^c	33.26±0.69 ^b	18.76±0.23 ^a	13.75±0.04 ^c	12.18±0.06 ^c	0.41±0.01 ^b	0.37±0.01 ^b	0.30±0.01 ^b
100	32.08±0.07 ^b	33.30±0.35 ^b	35.24±0.52 ^b	18.84±0.14 ^a	14.31±0.09 ^a	13.27±0.10 ^a	0.44±0.01 ^a	0.41±0.01 ^a	0.33±0.01 ^a
Sprouted quinoa									
0 (Control)	34.21±0.42 ^a	36.54±0.41 ^a	39.14±0.59 ^a	11.46±0.04 ^d	10.51±0.05 ^d	10.06±0.09 ^d	0.31±0.01 ^d	0.23±0.01 ^e	0.19±0.01 ^d
25	14.00±0.32 ^e	17.66±0.39 ^e	20.99±0.13 ^d	19.56±0.21 ^c	14.31±0.14 ^c	12.41±0.19 ^c	0.33±0.02 ^c	0.25±0.01 ^d	0.21±0.01 ^c
50	17.30±0.19 ^d	19.67±0.25 ^d	23.84±0.37 ^d	21.42±0.26 ^b	14.95±0.06 ^b	12.65±0.13 ^b	0.35±0.01 ^b	0.27±0.01 ^c	0.22±0.01 ^c
75	21.14±0.22 ^c	23.08±0.47 ^c	28.16±0.42 ^c	22.38±0.19 ^a	15.19±0.14 ^b	12.56±0.08 ^b	0.36±0.01 ^b	0.30±0.01 ^b	0.26±0.01 ^b
100	24.61±0.17 ^b	29.23±0.43 ^b	31.66±0.31 ^b	21.46±0.18 ^b	15.86±0.12 ^a	13.66±0.12 ^a	0.39±0.01 ^a	0.34±0.01 ^a	0.29±0.01 ^a
Roasted quinoa									
0 (Control)	34.21±0.42 ^a	36.54±0.41 ^a	39.14±0.59 ^a	11.46±0.04 ^d	10.51±0.05 ^d	10.06±0.09 ^d	0.31±0.01 ^d	0.23±0.01 ^e	0.19±0.01 ^d
25	23.00±0.37 ^e	24.01±0.24 ^e	26.39±0.29 ^d	15.26±20 ^c	13.19±0.14 ^c	11.56±0.07 ^c	0.35±0.02 ^d	0.30±0.01 ^d	0.24±0.01 ^c
50	23.88±0.15 ^d	27.54±0.12 ^d	33.45±0.42 ^c	16.78±19 ^b	13.01±0.07 ^c	11.34±0.14 ^c	0.41±0.01 ^c	0.34±0.01 ^c	0.26±0.01 ^c
75	26.82±0.23 ^c	31.24±0.25 ^c	35.02±0.50 ^c	17.15±21 ^a	14.21±0.15 ^b	12.58±0.12 ^b	0.44±0.01 ^b	0.37±0.01 ^b	0.29±0.01 ^b
100	30.91±0.27 ^b	34.17±0.33 ^b	37.23±0.35 ^b	16.78±17 ^b	14.61±0.17 ^a	12.89±0.09 ^a	0.47±0.01 ^a	0.40±0.01 ^a	0.34±0.01 ^a
Steamed quinoa									
0 (Control)	34.21±0.42 ^a	36.54±0.41 ^a	39.14±0.59 ^a	11.46±0.04 ^d	10.51±0.05 ^d	10.06±0.09 ^d	0.31±0.01 ^d	0.23±0.01 ^e	0.19±0.01 ^d
25	19.13±0.39 ^e	20.60±0.46 ^e	21.68±0.37 ^d	11.81±0.11 ^c	11.49±0.14 ^b	10.70±0.07 ^c	0.32±0.01 ^d	0.25±0.01 ^c	0.22±0.01 ^c
50	22.27±0.54 ^d	24.23±0.35 ^d	26.29±0.41 ^c	12.79±0.09 ^b	12.34±0.07 ^a	11.15±0.06 ^b	0.34±0.01 ^c	0.26±0.01 ^{bc}	0.24±0.01 ^{bc}
75	24.25±0.60 ^c	25.15±0.54 ^c	26.49±0.26 ^c	13.29±0.14 ^a	12.18±0.28 ^a	11.78±0.11 ^a	0.36±0.01 ^b	0.29±0.01 ^{ab}	0.26±0.01 ^b
100	25.60±0.40 ^b	27.39±0.57 ^b	28.94±0.26 ^b	13.31±0.14 ^a	12.41±0.23 ^a	11.24±0.08 ^b	0.40±0.01 ^a	0.32±0.01 ^a	0.29±0.01 ^a

Values are means±SD (n=3) and different superscript letters in the same column, for each treatment separately, are significantly different at p≤0.05. Substitution level: 0, 25, 50, 75 and 100% are (100% corn and 0% quinoa), (75% corn and 25% quinoa), (50% corn and 50% quinoa), (25% corn and 75% quinoa), and (0% corn and 100% quinoa), respectively.



Fig. 5. Photographs of the produced gluten-free cupcakes with various quinoa flour substitution levels.

Alkaline Water Retention Capacity (AWRC, %) of gluten-free cupcakes

The freshness of cupcake was assessed using the AWRC test. Cupcakes with a higher AWRC value are considered to be fresher. Along with water migration, crystallization of starch and lipid of the cake crumb are the main factors affect the cake freshness during storage (Hesso et al., 2015). The AWRC (%) of gluten-free cupcakes prepared with corn flour (control) and those substituted with quinoa flour which stored at room temperature for 0, 4, and 8 days are displayed in Table 8. Results showed that quinoa flour significantly

improved cupcakes freshness across all storage durations. Cupcakes prepared with all quinoa flour substitution maintained a higher level of freshness than the control (100% corn flour) due to the higher WHC, and OHC of quinoa flour than corn flour (Fig. 2) as well as the higher moisture content (Fig. 3) of quinoa-based cupcake relative to control cupcake. Noticeably, AWRC values of cupcakes steadily decreased with longer storage periods and the cupcakes substituted with different levels of quinoa flour were more fresh rather than the control (100% corn flour) during storage (zero, 4 and 8 days) at room temperature ($25\pm 2^{\circ}\text{C}$).

TABLE 7. Sensory attributes of gluten-free cupcakes

Substitution level (%)	Raw quinoa	Sprouted quinoa	Roasted quinoa	Steamed quinoa
Appearance (9)				
0 (Control)	8.00±0.61 ^a	8.00±0.61 ^b	8.00±0.61 ^b	8.00±0.61 ^b
25	8.36±0.35 ^a	8.39±0.45 ^a	8.57±0.53 ^a	8.54±0.27 ^a
50	8.36±0.64 ^a	8.36±0.20 ^{ab}	8.68±0.31 ^a	8.71±0.27 ^a
75	8.18±0.24 ^a	8.32±0.41 ^{ab}	8.71±0.27 ^a	8.86±0.24 ^a
100	8.29±0.75 ^a	8.07±0.24 ^{ab}	8.50±0.41 ^a	8.79±0.39 ^a
Crust color (9)				
0 (Control)	7.86±0.24 ^b	7.86±0.24 ^c	7.86±0.24 ^c	7.86±0.24 ^b
25	8.18±0.24 ^{ab}	8.21±0.27 ^b	8.36±0.38 ^b	8.43±0.31 ^a
50	8.21±0.57 ^{ab}	8.36±0.24 ^{ab}	8.50±0.25 ^{ab}	8.57±0.35 ^a
75	8.25±0.25 ^{ab}	8.43±0.45 ^{ab}	8.61±0.24 ^{ab}	8.64±0.13 ^a
100	8.29±0.39 ^a	8.54±0.17 ^a	8.68±0.19 ^a	8.71±0.22 ^a
Crumb color (9)				
0 (Control)	7.71±0.39 ^c	7.71±0.39 ^c	7.71±0.39 ^b	7.71±0.39 ^b
25	8.18±0.12 ^b	8.29±0.22 ^b	8.57±0.12 ^a	8.61±0.13 ^a
50	8.29±0.27 ^{ab}	8.43±0.12 ^{ab}	8.64±0.38 ^a	8.71±0.27 ^a
75	8.39±0.13 ^{ab}	8.57±0.12 ^a	8.71±0.27 ^a	8.82±0.19 ^a
100	8.46±0.22 ^a	8.61±0.13 ^a	8.75±0.20 ^a	8.86±0.20 ^a
Odor (9)				
0 (Control)	8.32±0.24 ^a	8.32±0.24 ^a	8.32±0.24 ^a	8.32±0.24 ^b
25	8.04±0.20 ^{ab}	8.04±0.20 ^{ab}	8.50±0.25 ^a	8.68±0.24 ^a
50	7.89±0.25 ^{bc}	8.11±0.41 ^a	8.36±0.38 ^a	8.50±0.41 ^{ab}
75	7.79±0.31 ^{bc}	7.71±0.25 ^b	8.43±0.12 ^a	8.54±0.37 ^{ab}
100	7.64±0.38 ^c	7.28±0.30 ^c	8.50±0.24 ^a	8.61±0.13 ^{ab}
Taste (9)				
0 (Control)	8.39±0.48 ^a	8.39±0.48 ^a	8.39±0.48 ^b	8.39±0.48 ^b
25	8.32±0.19 ^a	8.43±0.20 ^a	8.46±0.27 ^{ab}	8.54±0.09 ^{ab}
50	7.64±0.31 ^b	8.29±0.45 ^a	8.57±0.24 ^{ab}	8.64±0.12 ^a
75	7.14±0.50 ^c	8.04±0.40 ^a	8.61±0.40 ^{ab}	8.68±0.24 ^a
100	6.75±0.22 ^d	7.39±0.49 ^b	8.71±0.20 ^a	8.71±0.48 ^a
Overall acceptability (9)				
0 (Control)	8.06±0.26 ^{ab}	8.06±0.26 ^{bc}	8.06±0.26 ^b	8.06±0.26 ^c
25	8.21±0.28 ^a	8.27±0.41 ^a	8.49±0.20 ^a	8.56±0.09 ^b
50	8.08±0.23 ^{ab}	8.31±0.24 ^a	8.55±0.19 ^a	8.63±0.17 ^{ab}
75	7.95±0.11 ^{bc}	8.21±0.50 ^{ab}	8.61±0.29 ^a	8.71±0.15 ^a
100	7.89±0.24 ^c	7.98±0.51 ^c	8.63±0.20 ^a	8.74±0.14 ^a
Acceptability index (%)				
0 (Control)	89.52±2.30 ^{ab}	89.52±2.30 ^{bc}	89.52±2.30 ^b	89.52±2.30 ^c
25	91.27±1.57 ^a	91.90±4.54 ^a	94.37±2.22 ^a	95.08±0.96 ^b
50	89.76±2.60 ^{ab}	92.30±2.71 ^a	95.00±2.08 ^a	95.87±1.92 ^{ab}
75	88.33±1.22 ^{bc}	91.27±5.56 ^{ab}	95.71±2.27 ^a	96.75±1.69 ^a
100	87.62±2.66 ^c	88.65±5.65 ^c	95.87±2.23 ^a	97.06±1.59 ^a

Values are means±SD and different superscript letters in the same column, for each attribute separately, indicate significant differences between means ($p \leq 0.05$). Substitution level: 0, 25, 50, 75 and 100% are (100% corn and 0% quinoa), (75% corn and 25% quinoa), (50% corn and 50% quinoa), (25% corn and 75% quinoa), and (0% corn and 100% quinoa), respectively.

TABLE 8. Alkaline water retention capacity (%) of gluten-free cupcakes

Substitution level (%)	Storage periods		
	Zero time	4 days	8 days
Raw quinoa			
0 (Control)	91.40±0.40 ^e	71.43±0.33 ^e	61.17±0.40 ^e
25	98.52±0.45 ^d	81.90±0.40 ^d	76.06±0.33 ^d
50	106.31±0.10 ^c	94.32±0.29 ^c	84.97±0.15 ^c
75	109.54±0.71 ^b	102.67±0.76 ^b	91.83±0.46 ^b
100	127.11±0.84 ^a	114.00±0.85 ^a	105.03±0.61 ^a
Sprouted quinoa			
0 (Control)	91.40±0.40 ^e	71.43±0.33 ^e	61.17±0.40 ^e
25	101.10±0.64 ^d	86.12±0.82 ^d	76.56±0.46 ^d
50	104.99±0.12 ^c	90.92±0.62 ^c	83.94±0.12 ^c
75	107.97±0.20 ^a	104.93±0.20 ^b	89.80±0.42 ^b
100	115.77±0.21 ^a	113.93±0.35 ^a	101.81±0.73 ^a
Roasted quinoa			
0 (Control)	91.40±0.40 ^e	71.43±0.33 ^e	61.17±0.40 ^e
25	94.78±0.75 ^d	86.62±0.47 ^d	77.02±0.21 ^d
50	102.92±0.22 ^c	99.64±0.57 ^c	94.57±0.91 ^c
75	110.02±0.20 ^b	107.05±0.71 ^b	101.56±0.40 ^b
100	123.71±0.84 ^a	111.03±0.45 ^a	105.34±0.63 ^a
Steamed quinoa			
0 (Control)	91.40±0.40 ^d	71.43±0.33 ^e	61.17±0.40 ^e
25	91.98±0.08 ^d	76.82±0.45 ^d	68.72±0.19 ^d
50	114.17±0.46 ^c	89.51±0.68 ^c	82.38±0.28 ^c
75	122.01±0.21 ^b	94.82±0.65 ^b	88.37±0.70 ^b
100	129.68±0.26 ^a	116.37±0.27 ^a	110.98±0.68 ^a

Values are means±SD (n=3) and different superscript letters in the same column, for each treatment separately, indicate significant differences between means ($p \leq 0.05$). Substitution level: 0, 25, 50, 75 and 100% are (100% corn and 0% quinoa), (75% corn and 25% quinoa), (50% corn and 50% quinoa), (25% corn and 75% quinoa), and (0% corn and 100%

Conclusion

The results of the current study showed that the whole meal quinoa flour had higher protein, fat, ash and crude fiber while lower carbohydrate contents than corn flour indicating the nutritional benefits of quinoa flour in food preparation. Sprouting, roasting, and steaming of quinoa significantly reduced the anti-nutrients contents (phytates, tannins and saponins) while enhance the functional properties (WHC and OHC) compared to the untreated quinoa. It could be concluded that treated black quinoa flour improved the nutritional

and sensory characteristics of the functional gluten-free cupcakes compared to corn flour cupcake (control). Moreover, thermal treatments (roasting and steaming) of quinoa produced the most favorable cupcakes due to their caramel and roasted nut flavors as well as chocolate color. Additionally, roasted and steamed quinoa cupcakes showed higher scores in numerous sensory characteristics where they had the highest overall acceptability and acceptability index. Therefore, treated quinoa flour can be used as untraditional functional food ingredient for celiac disease patients.

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