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Multi-objective development of novel egg free cakes using quinoa protein and its quality attributes

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Abstract:
This study explores the potential of utilizing quinoa protein as an egg substitute in bakery products for customers with health, culture/religion, or dietary restrictions.

Quinoa protein was prepared from quinoa seed by alkaline solubilization followed by isoelectric precipitation and drying. Four different formulations of egg-free cakes were prepared by incorporating quinoa protein in egg equivalents of 50 g (Formulation 1), 75 g (Formulation 2), 100 g (Formulation 3), and 150 g (Formulation 4). The research involved Fourier-transform infrared spectroscopy and revealed such functional properties as proximate composition, physical properties, color, texture, microstructure, and sensory characteristics for the batters and the cakes.

The incorporation of different quinoa protein concentrations significantly ($p < 0.05$) affected all the functional properties of the batters and the cakes. Such variables as crude protein and ash increased while moisture and fat contents decreased. The baking loss went down as the share of quinoa protein went up. The structural analysis showed an increase in gumminess and chewiness accompanied by a decrease in cohesiveness and elasticity. The analysis also revealed hardness and non-uniform changes. The lightness (L^*) and yellowness (b^*) of the cake surface and crumb decreased while the redness (a^*) increased.

The cakes prepared according to Formulation 4 with the greatest share of quinoa protein had a high nutritional value with reasonable concentrations of essential amino acids in general and a high level of lysine in particular. The same sample also received the highest score for overall sensory properties. The sensory assessment proved that quinoa protein could meet consumer expectations of egg-free cakes.

Keywords: Quinoa protein isolate, functional cake, egg-free products, microstructure, functional and physicochemical attributes, amino acid composition

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INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) and its products have been gaining more and more scientific attention since 2013, which was proclaimed the international year of quinoa by the Food and Agriculture Organization. As a result, production and consumption of quinoa increased exponentially worldwide [1]. This pseudocereal grows on marginal soils, tolerates salinity as well as drought, and adapts well to extreme or changeable weather conditions [2]. Quinoa is richer in protein than other cereals and boasts a better distribution of essential amino acids. In addition, quinoa protein contains more lysine (5.1–6.4%) than cereals and more methionine (0.4–1.0%) than legumes [2, 3]. Quinoa protein has a balanced amino acid profile and can be used as an alternative to milk or egg proteins in bakery products [4].

Eggs are an indispensable part of cake formulation and affect the quality of the final product. Eggs give the cake its soft texture while their foaming and emulsifying properties provide moistness. Egg proteins assist in entrapping air during mixing, thus improving aeration [5]. The gelling properties of egg protein are responsible for the cake volume while egg lipoproteins provide good emulsification [6]. In addition, eggs contribute to the color and aroma [7]. For customers who suffer from egg allergy, the availability of egg-free products can be quite a challenge. Vegetarianism, religious reasons, or personal lifestyle have also increased the demand for egg-free cakes [4]. Quinoa proteins with their diverse bioactive components, good functional properties, and low anti-nutritional content may offer a solution to the abovementioned problems [2].

A lot of studies introduce proteins of whey, soya, pea, and lupine as an egg substitute [7–10]. Other baking additives include xanthan gum, soya lecithin baking powder, mono- and di-glycerides, or combinations of two protein concentrates, e.g., lupine and whey [10, 11]. Unfortunately, the quality of egg-free cakes is almost always inferior to traditional samples. For example, the replacement may result in such undesirable changes as low cake volume, coarse structure, or poor foaming stability [12].

Quinoa protein concentrate in bakery and cakes represents a novel hypoallergenic egg substitute and creates niche products with unique sensory characteristics that meet contemporary consumer expectations and needs. Our study responds to the growing demand of egg-free products by exploring quinoa proteins as an egg substitute in egg-free cakes with a conventional sensory profile. This work explored the potential benefits of utilizing quinoa protein as part of formulation of egg-free cakes. This multi-objective formulation was developed so as to improve nutritional quality, preserve sensory properties, and respond to the growing demand for allergen-free bakery products. In addition, quinoa proteins could contribute to a more environmentally friendly and sustainable food industry.

STUDY OBJECTS AND METHODS

Materials. Cake ingredients involved all-purpose wheat flour, eggs, milk, oil, sugar, vanilla, and baking powder, all purchased from a local market. The quinoa seeds were obtained from the Desert Research Center (Egypt). Other reagent-grade chemicals came from the Network of Central Laboratories and Centers of Excellence.

Methods. ***Preparing quinoa flour.*** The flour was defatted by shaking for 12 h with hexane to the ratio of 1:4 (w/v), filtered, and air-dried at 40°C for 8 h. The defatted flour was stored in a polyethylene film bag at 4°C until further use.

Preparing quinoa protein concentrate. The quinoa protein concentrate was prepared according to the method described in [13]. The defatted quinoa flour was suspended in water 1:20 (w/v). We adjusted its pH to 11 using 2N NaOH, stirred it for 150 min, and centrifuged at 4500 g at 36°C for 30 min. After bringing the pH down to 4.0 with 1N HCl, we centrifuged the mix at 4500 g for 20 min to precipitate the protein. The precipitates were resuspended in water, neutralized to pH 7.0, dried, and stored at −20°C until further use. The protein concentration was measured by the micro-Kjeldahl method in line with method 920.152 developed by the American Association of Cereal Chemists (%, N×6.25).

Determining functional properties of quinoa protein. The functional properties of quinoa protein to be tested included protein swelling capacity, bulk density, water-holding capacity, oil-holding capacity, emulsifying capacity, whippability, and foaming stability.

The protein swelling capacity was determined according to the method described by Robertson *et al.* and reflected the ease with which quinoa proteins increased in volume under water excess [14]. This variable was calculated as X mL of water retained per 1 g of dry sample for 18 h.

We measured the bulk density in line with the method suggested by Wani *et al.* [15]. We put a sample of 50 g in a 100-mL graduated cylinder and tapped 20–30 times. To calculate the bulk density, we divided the weight of the sample by its volume (g/mL).

The water-holding capacity and the oil-holding capacity were determined based on the procedure proposed by Fallah-Delavar & Farmani and calculated as the difference between the weight of the sample before and after we added water/oil by gram [16].

To determine the emulsifying capacity, we turned to the methodology described by Shahidi *et al.*, who expressed it as the volume of the emulsified layer vs. the total volume [17].

The procedure for foam stability followed the protocols described by Shao & Kao, who expressed it as the difference between the initial and the final foam volumes measured after settling for 30 min [18].

The whippability was calculated as the percent increase in volume [18]. For each test, the measurements were conducted in triplicates.

Fourier-transform infrared spectroscopy (FTIR). The study involved a Fourier-transform infrared spectrometer coupled to an attenuated total reflectance (ATR) accessory (FTIR-ATR Bruker Vertex 80v). We placed the powdered samples on the surface of the ATR crystal and pressed with a flat-tip plunger. An average of 32 scans were performed between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}. The analysis was carried out at room temperature.

Preparing batter and cake. The cake batter samples were made according to the formulations represented in Table 1. They included wheat flour (100 g), sugar (100 g), oil (40 mL), milk (90 mL), whey protein (6.6 g), and baking powder (1.1 g). The control contained a liquid whole egg (50 g) while the test samples included 2.87, 4.30, 5.74, and 8.61 g of quinoa protein. These ratios were derived from a set of preliminary experiments conducted in our lab. After mixing wheat flour, sucrose, and baking powder with eggs/quinoa protein, milk, and oil, we whipped the ingredients to avoid lumps. The obtained smooth and uniform batter was poured in aluminum cake molds and baked at 200°C for 20 min. After baking, the cakes were removed from molds, allowed them to cool at room temperature for 30 min, and wrapped in polyethylene bags for further analysis.

Determining physical properties of batter. The batter density in the finished cakes was tested in line with the method proposed by Özhamamci *et al.*, who divided certain weight of batter by volume [19]. The specific gravity was measured and calculated by dividing the weight of a certain batter volume by the weight of the same volume of distilled water. The viscosity of each batter sample was measured at room temperature using a Brookfield digital viscometer (USA) equipped

Table 1 Batter formulations: control vs. egg-free cakes

Ingredients	Batter composition, %				
	Control	Formulation 1 (50 g quinoa protein in egg equivalent)	Formulation 2 (75g quinoa protein in egg equivalent)	Formulation 3 (100 g quinoa protein in egg equivalent)	Formulation 4 (150 g quinoa protein in egg equivalent)
Wheat flour	100	100	100	100	100
Liquid whole egg	50	–	–	–	–
Quinoa protein	–	2.87	4.30	5.74	8.61
Whey protein	–	6.6	6.6	6.6	6.6
Sugar	100	100	100	100	100
Milk	90	90	90	90	90
Oil	40	40	40	40	40
Emulsifier	–	1.75	1.75	1.75	1.75
Vanilla	0.1	0.1	0.1	0.1	0.1
Baking powder	1.1	1.1	1.1	1.1	1.1

with a So4 spindle. The samples were subjected to shear rates of 0–30 s^{-1} [19].

Determining physical properties of cake. The weight loss was represented as the difference between the weight of cake batter in each cake mold and the weight of finished cake after 4 h of cooling at room temperature. The weight loss, %, was calculated using the following Eq. (1):

$$\text{Weight loss} = \\ = (\text{weight}_{\text{batter}} - \text{weight}_{\text{cake}})/\text{weight}_{\text{batter}} \times 100 \quad (1)$$

The density, g/cm^3, was calculated as cake weight divided by cake volume:

$$\text{Density} = \text{weight}_{\text{cake}}/\text{volume}_{\text{cake}} \quad (2)$$

The specific volume, cm^3/g, of cake samples made according to different formulations was evaluated by the seed replacement method and calculated using the following Eq. (3):

$$\text{Specific volume} = \text{volume}_{\text{cake}}/\text{weight}_{\text{cake}} \quad (3)$$

To measure the cake volume index (B+C), we cut it into equal halves and made cross-sectional tracings. The symmetry index (2C – B – D) and uniformity index (B–D) were calculated from the height at the center and at the distance between the center and each edge according to method 10-91 described by the American Association of Cereal Chemists (2000) [20].

Proximate analyses. The proximate composition analyses of cakes formulations were carried out according to the method described by the American Association of Cereal Chemists (2010) [21]. We also determined such variables as moisture content, crude protein, ash, and crude fiber. The carbohydrate amount was represented as the difference between 100 and the sum of protein, lipids, ash, fiber, and moisture content.

Color assessment. We used the HunterLab scan XE and the CIELAB color scale to define the color of the four samples [22]. The white and black tiles of HunterL ab color standards served as equipment standardization, after which we evaluated the lightness (L^*), redness (a^*), and yellowness (b^*) of every sample. The total color difference (ΔE) was calculated according to the following Eq. (4):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (4)$$

where $\Delta L = L_{\text{sample}} - L_{\text{control}}$; $\Delta a = a_{\text{sample}} - a_{\text{control}}$; and $\Delta b = b_{\text{sample}} - b_{\text{control}}$.

Texture profile analysis of cake crumb. We used a TA-CT3 Brookfield texture analyzer (USA) to determine the texture parameters, i.e., hardness, springiness, cohesiveness, gumminess, and chewiness of cake samples according to method 74-09 developed by the American Association of Cereal Chemists (2000) [20]. The samples were cut into 25-mm cubic pieces of cake crumbs. All the experiments were performed in triplicates; the results were expressed as mean ± SD values.

Microstructural analysis. After cutting the cake samples into 0.5×0.5 cm cubes, we froze them in liquid nitrogen and freeze-dried. After drying, the surface of the sample was sputter-coated with a thin layer of gold palladium. The microstructure of the sample was scanned with a QUANTA FEG250 Scanning Electron Microscope.

Sensory properties. The sensory profile included taste, color, softness, flavor, cell size uniformity, and overall acceptability. The panelists evaluated the samples in 6 h after baking using the nine-point hedonic test, i.e., from 1 (most disliked) to 9 (most liked). The panel consisted of seven men and eight women, who were selected randomly. The samples were served in white plastic containers in random order. The panelists were provided with drinking water to wash their mouths between samples. Each panelist signed a consent approved by the National Research Council ethical committee in 2022.

Statistical analysis. We used the Duncan test to identify significant differences between the control and the egg-free samples. The experiment involved a one-way analysis of variance (ANOVA) and a SPSS Statistics 20.0 package for Windows (SPSS Inc., USA). The

alpha level was 0.05 ($p < 0.05$). All the experiments were performed in triplicates, except for the sensory evaluation. The results were presented as mean ± standard deviation (SD). Statistically significant differences were indicated by superscripts.

RESULTS AND DISCUSSION

Proximate analysis and functional properties of quinoa protein. Table 2 shows the approximate composition of quinoa protein prepared using the alkaline precipitation technique. The protein content was 72.21%, which confirmed the data published in [5–7]. Quinoa protein showed water holding capacity of 2.9 g/g and oil holding capacity of 2.05 g/g. Foaming capacity was 71.12%. Foaming stability reached 94.61% after 30 min whereas whippability was 63.33%. These results reflected the functional properties of quinoa protein that are linked to its physicochemical properties, i.e., those that govern the behavior of protein in foods and affect the choice of protein to be used in an industrial process. Emulsifying capacity and emulsion stability are two important functional properties of proteins that affect the structure of adhesives [23]. Emulsion capacity and stability can affect tension in the water-and-oil interface and help prevent coalescence [24]. Proteins stabilize emulsions due to the membrane matrix that surrounds the oil drop and prevents coalescence [25].

Quinoa protein showed good foaming properties that suggested it could be used as an egg replacer in food processing. In other publications, the foaming capacity of egg albumin ranged between 156 and 200% while the foaming stability was 33–54%, which makes it an excellent foaming agent [26]. Consequently, quinoa protein demonstrated poorer foaming properties compared to egg albumin but exhibited a good foaming stability. Dakhili *et al.* compared the foaming stability of quinoa protein to soybean protein and egg white protein: it was similar or significantly higher in foaming stability than soybean protein but lower than egg white protein [27]. Such results support its use in bakery products [28].

Amino acid profile of quinoa protein. The amino acid profile of quinoa protein (Table 3) confirmed previous results where quinoa protein proved to be a complete protein. Unlike some other plant proteins, quinoa protein contained all nine essential amino acids that human body cannot synthesize on their own. From highest to lowest mean content, the most abundant essential amino acids (n = 8) were lysine, isoleucine, tryptophan, leucine, histidine, methionine valine, and phenylalanine (Table 3). The most abundant non-essential amino acids (n = 8), from highest to lowest, were alanine, aspartic acid, glutamic acid, tyrosine, serine, proline, arginine, and glycine.

The mean values for such amino acids as histidine, isoleucine, lysine, sulfur amino acids, aromatic amino

Table 2 Functional properties of quinoa protein

Properties	Value
Bulk density, g/mL	0.77 ± 0.01
Swelling capacity, mL/g	1.31 ± 0.08
Water holding capacity, g/g	2.90 ± 0.13
Oil holding capacity, g/g	2.05 ± 0.05
Emulsifying capacity, %	71.12 ± 1.02
Foaming stability after 30 min, %	94.61 ± 1.47
Whippability, %	63.33 ± 2.08
Protein content, %	72.21 ± 1.78

Table 3 Amino acid content in quinoa protein

Amino acids	g/100 g dry weight	%	Amino acid requirements for adults, mg/kg of body weight/day (WHO/FAO)
Essential amino acids			
Histidine	3.17	4.96	
Isoleucine	6.39	9.97	9.5
Leucine	3.82	5.97	12.5
Lysine	7.40	11.55	9.4
Methionine	3.15	4.91	12.1 (methionine + cysteine)
Phenylalanine	2.93	4.57	12.1 (phenylalanine + tyrosine)
Tryptophan	3.97	6.21	2.9
Valine	2.97	4.63	10.7
Non-essential amino acids			
Alanine	7.81	12.19	
Aspartic	4.09	6.38	
Arginine	2.94	4.59	
Glycine	2.92	4.55	
Glutamic	3.16	4.94	
Proline	2.95	4.61	
Serine	3.10	4.84	
Tyrosine	3.23	5.05	
Total essential amino acids	33.84	52.81	
Total non-essential amino acids	30.23	47.18	
Total amino acids	64.07		

acids, threonine, tryptophan, and valine met the daily requirements set by the World Health Organization or the Food and Agriculture Organization (mg/kg of body weight/day). Quinoa protein isolates were reported as similar to casein and other milk proteins [29]. Quinoa protein is high in lysine, methionine, and threonine, which are the limiting amino acids in wheat and maize. Our results confirmed that quinoa protein has a good amino acid profile and can be used as a reliable source of protein [30, 31].

Fourier-transform infrared spectroscopy (FTIR). Fourier-transform infrared spectroscopy is a useful method that defines the secondary structure of certain proteins via the unique vibrations of their structural units. The infrared region showed three distinct absorption bands: amide I band, amide II band, and amide III band (Fig. 1). The secondary structure of proteins and peptides mainly contains three absorption bands in the infrared region, i.e., amide I band, amide II band, and amide III band. Amid I accounts for ≈ 80% of peptide links, i.e., C=O stretch [29]. Amid I band (1700–1600 cm^{-1}) is also considered the most sensitive spectral region of protein secondary structural components. This band appeared at 1630 cm^{-1} with C=O vibrations predominating, followed by C-N. The Fourier-transform infrared spectra of quinoa protein also showed some in-plane N-H bending contributed to amide I. Amide II band exhibited less sensitivity than amide I band and appeared at 1534 cm^{-1}. Amide III band was coupled with C-N stretching, as well as C-H and N-H deformation vibrations. It appeared at ≈ 1234 cm^{-1} and was associated with the N-H plane. Amide A band appeared at 3276 cm^{-1} and arose from N-H stretching. We also observed the presence of residual carbohydrates in the spectrum between 1158 and 1023–900 cm^{-1}.

Physical properties of control vs. quinoa protein cake batter. The list of physical properties to be tested included density, specific gravity, and viscosity. The increasing ratios of quinoa protein affected the physical profile of batter as presented in Table 4. The density of cake batter ranged between 0.8209 ± 0.0100 and 1.0856 ± 0.0000. Quinoa protein significantly decreased the density of the batter ($p < 0.05$). The density of cake batter usually depends on the air content. As a result, a lower density can be associated with a decrease in the air volume incorporated into the batter [26].

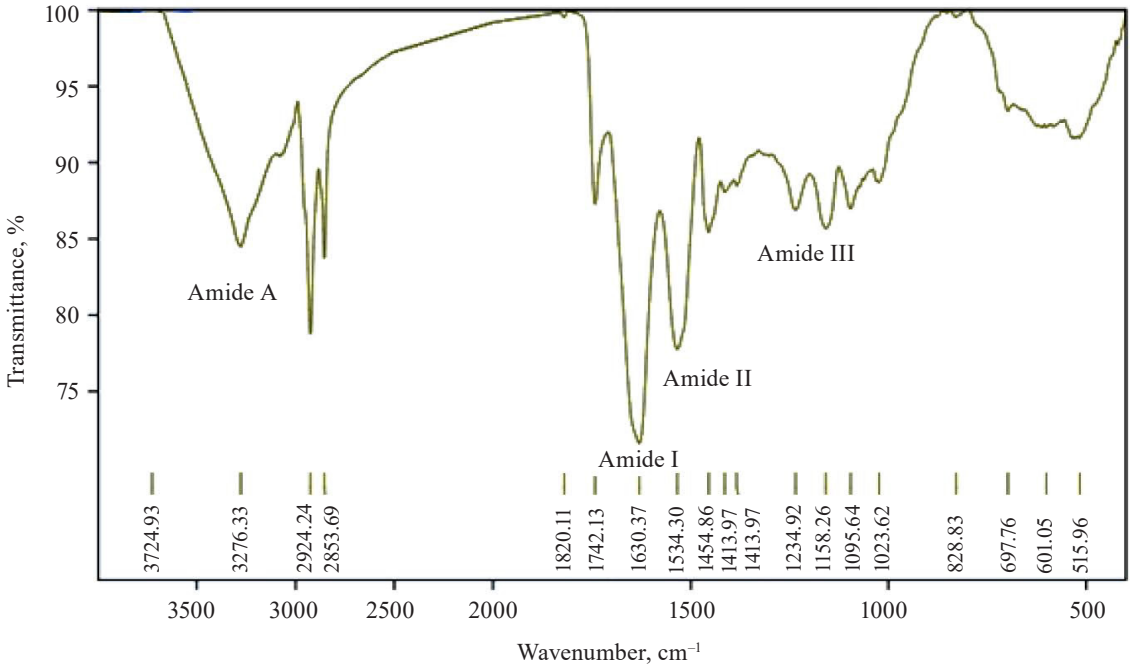

Figure 1 Fourier-transform infrared spectroscopy spectra of quinoa protein

Table 4 Physical properties of cake batter samples with different shares of quinoa protein

Sample	Batter density, g/cm³	Specific gravity	Viscosity, CPs				
			5	6	10	20	30
Control	1.0856 ± 0.0000[a]	1.1428 ± 0.0100[b]	29.000	19.500	15.300	11.640	10.570
Formulation 1 (50 g quinoa protein)	0.8209 ± 0.0100[e]	1.5006 ± 0.3400[a]	93.000	70.800	46.800	33.900	28.400
Formulation 2 (75 g quinoa protein)	0.9076 ± 0.0100[d]	1.2341 ± 0.0200[ab]	158.000	100.800	84.700	72.000	65.200
Formulation 3 (100 g quinoa protein)	0.9315 ± 0.0200[c]	1.2783 ± 0.0400[ab]	172.400	139.700	113.700	86.900	76.800
Formulation 4 (150 g quinoa protein)	0.9730 ± 0.0100[b]	0.9878 ± 0.0100[c]	385.300	227.700	143.600	114.00	97.200

Values with different superscripts in the same line are significantly different at $p < 0.05$

The specific gravity in the control batter (1.14) was significantly lower than in the egg-free samples, except for Formulation 4, which contained the highest share of quinoa protein. We discovered the same trend for batter density. By incorporating quinoa protein into the control cakes, we obtained the highest specific gravity value of 1.50, which suggested heavier batter without proper aeration. As the quantity of quinoa protein kept increasing in Formulations 2, 3, and 4, the specific gravity of the egg-free cake batter went down as 1.23, 1.28, and 0.99, respectively. The specific gravity measures revealed the total air holding capacity. It was inversely proportional to the air holding capacity, i.e., high values indicated less air incorporated into the batter and vice versa. However, if the batter entraps a lot of air bubbles, it provides a better cake structure.

Figure 2 shows that viscosity and the flow properties of the control and the experimental samples remained within the 0.01–100 s^{-1} shear rate. When quinoa protein was incorporated, it resulted in an inverse relationship, where a greater quinoa protein share corresponded to a lower viscosity value. This effect can be explained by the fact that the incorporation of quinoa protein decreased the batter density whereas the high viscosity obstructed air incorporation during mixing [27]. The increase in the viscosity of the batter could be related to the quantity of available water in the system absorbed by the flour, which is known to depend on the quantity of proteins [26]. For instance, Mu *et al.* recommended to keep high viscosity as a favorable factor for batter stability and quality of the final product [28].

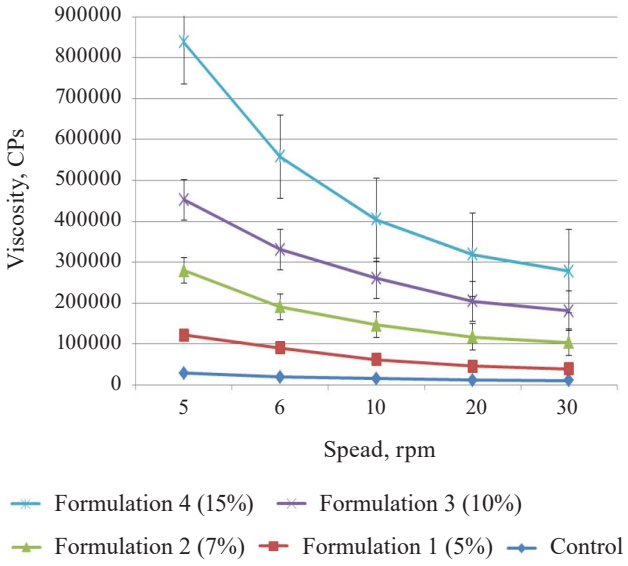

Figure 2 Rheological properties of cake batters; flow ramp curves of control and egg-free batter samples (curves are represented as a mean of at least two replicates)

Proximate compositions of control and quinoa protein cake samples. Proximate compositions for cakes include moisture, ash, crude protein, crude fat, and crude fiber. We determined these variables according to the recommendations given by the American Association of Cereal Chemists (2010). The total carbohydrates content was estimated as the following difference: total carbohydrate = 100 – (moisture + ash + crude protein + crude fat). The moisture contents of the egg-free samples decreased as the share of quinoa protein increased (Table 5). Harisha *et al.* reported the same trend: when they incorporated pea protein isolate, the moisture content went down [30]. We also detected a significant incremental increase in the crude protein (6.45–7.90%) and ash (0.46–0.96%) in the egg-free samples. When the concentration of quinoa protein in the cake formulations increased, the crude fat decreased from 13.01 to 9.56% whereas the crude fiber content showed no significant differences. The total carbohydrate calculated by differences increased together with the share of quinoa protein.

Physical property of cakes. Table 6 illustrates the impact of quinoa protein on the physical properties of cake, i.e., baking loss, density, specific volume, volume index, symmetric index, and uniformity index. The baking loss of the control cake (15.61%) was quite higher than that of the egg-free samples. As the percentage of quinoa protein increased, the weight loss increased

Table 5 Proximate compositions: quinoa protein samples vs. control

Sample	*Parameters						
	Moisture, %	Ash, %	Fat, %	Fiber, %	Protein, %	**Total carbohydrates, %	***Energy, kcal/100 g
Control	24.39 ± 0.27^{a}	0.46 ± 0.01^{b}	13.01 ± 0.52^{a}	3.46 ± 0.43^{a}	6.45 ± 0.07^{d}	52.22	365.81
Formulation 1 (50 g quinoa protein)	17.53 ± 0.30^{c}	0.90 ± 0.05^{a}	11.22 ± 0.27^{b}	3.05 ± 0.12^{a}	6.05 ± 0.01^{c}	61.25	383.18
Formulation 2 (75 g quinoa protein)	17.61 ± 0.18^{c}	0.98 ± 0.04^{a}	10.61 ± 0.54^{b}	3.34 ± 0.18^{a}	6.60 ± 0.02^{c}	60.85	378.65
Formulation 3 (100 g quinoa protein)	18.10 ± 0.43^{c}	0.97 ± 0.59^{a}	9.59 ± 0.33^{c}	3.16 ± 0.14^{a}	6.82 ± 0.06^{b}	61.36	374.91
Formulation 4 (150 g quinoa protein)	18.60 ± 0.49^{b}	0.96 ± 0.06^{a}	9.56 ± 0.42^{c}	3.50 ± 0.24^{a}	7.90 ± 0.07^{a}	59.47	369.88

*The values are mean ± SD; values marked by different superscripts in the same column are significantly different ($p \leq 0.05$);

**By difference; ***Calculated

Table 6 Physical properties of cakes with different shares of quinoa protein

Samples	Baking loss, %	Density, g/cm^3	Specific volume, cm^3/g	Volume index, mm	Symmetric index, mm	Uniformity index, mm
Control	15.61 ± 0.14^a	0.338 ± 0.000^b	2.96 ± 0.02^c	3.04 ± 0.78^{ab}	175.98 ± 0.21^e	0.857 ± 0.180^a
Formulation 1 (50 g quinoa protein)	13.02 ± 0.32^c	0.323 ± 0.000^c	3.10 ± 0.02^b	4.05 ± 0.40^a	184.45 ± 0.37^c	0.053 ± 0.070^d
Formulation 2 (75 g quinoa protein)	13.92 ± 0.15^b	0.343 ± 0.000^a	2.92 ± 0.01^c	2.58 ± 0.43^b	176.73 ± 0.51^d	0.403 ± 0.010^b
Formulation 3 (100 g quinoa protein)	12.86 ± 0.14^c	0.321 ± 0.000^c	3.11 ± 0.02^b	3.80 ± 0.33^a	190.88 ± 0.35^b	0.357 ± 0.040^c
Formulation 4 (150 g quinoa protein)	12.94 ± 0.17^c	0.313 ± 0.000^d	3.20 ± 0.03^a	2.60 ± 0.58^b	194.42 ± 0.24^a	0.347 ± 0.030^c

Table 7 Texture analysis of cakes with different shares of quinoa protein

Sample	Hardness, g	Cohesiveness	Resilience, mm	Gumminess, g	Chewiness, g
Control	657.00 ± 0.00^b	0.60 ± 0.02^b	8.75 ± 0.07^a	369.62 ± 0.22^c	3,283.16 ± 2.70^c
Formulation 1 (50 g quinoa protein)	716.00 ± 4.24^a	0.61 ± 0.03^b	8.30 ± 0.14^{ab}	443.06 ± 3.87^a	3,451.68 ± 2.61^a
Formulation 2 (75 g quinoa protein)	636.00 ± 4.24^c	0.82 ± 0.06^a	8.20 ± 0.42^{ab}	402.91 ± 5.82^b	3,417.35 ± 3.13^b
Formulation 3 (100 g quinoa protein)	605.00 ± 1.41^d	0.56 ± 0.06^b	8.20 ± 0.14^{ab}	351.14 ± 0.49^d	2,888.23 ± 8.62^d
Formulation 4 (150 g quinoa protein)	512.50 ± 0.71^e	0.58 ± 0.01^b	8.10 ± 0.14^b	283.06 ± 3.92^e	2,286.41 ± 4.45^e

Means with different superscripts in the same column are significantly different at $p \leq 0.05$

accordingly. The foaming capacity and the stability of quinoa protein can explain the reducing baking loss in the egg-free samples. The density decreased as the share of quinoa protein grew; the lowest value was reported for Formulation 4 with the highest quinoa protein concentration. However, Formulation 3 had a higher density value than the control. The specific volume significantly improved as the proportion of quinoa protein grew larger.

Specific volume affects consumer preference, which makes it one of the most important quality parameters for baked products. Table 6 shows that the control sample without quinoa protein exhibited the lowest specific volume, whereas the sample baked according to Formulation 4 had the highest specific volume. Samples with more quinoa protein had a greater specific volume ($p < 0.05$), which could be explained by the higher protein content. Thus, proteins increase the volume of cakes by increasing the viscoelasticity of batter and the time it takes the batter to become semisolid. This phenomenon is, in turn, related to the protein-starch interaction and transition [31]. Therefore, the cake volume depended not on the initial air quantity but on the capacity of retaining air during baking [32].

Texture analysis. Table 7 compares the texture quality of the control sample cake and the egg-free cakes with different shares of quinoa protein. The incorporation affected the hardness of the cake, its lowest value corresponding to Formulation 4 with the highest quinoa protein content (150 g). So, the degree of hardness depended on the protein content. Our findings contradict those reported in some previous publications, where, for instance, chickpea flour raised the initial firmness in cake [33]. Probably, the water binding capacity of quinoa protein made the cake softer and affected the crumb firmness as well. Regarding the cohesiveness values, we detected no significant difference between the control cake and the cake prepared with quinoa protein, the only exception being Formulation 2 (75 g quinoa protein), which exhibited the maximal value (0.61). Meanwhile, Formulation 4 showed significantly higher resilience compared to the other test samples and the control. In general, hardness and firmness had an impact on the cake structure and its compression resistance. These qualities are mirrored by the development of internal bonding in a three-dimensional protein network and affect consumer acceptance.

The egg-free samples demonstrated greater gumminess and chewiness that the control. Formulations 3 and 4 showed a more distinguish pattern where greater quinoa protein shares increased the gumminess and chewiness. Both values followed the same pattern as the one that was reported for hardness.

Color. The type of ingredients and interactions between them affect the final color of baked products. Color attributes include lightness (L^*), redness (a^*), and yellowness (b^*) of the surface and crumb (Table 8). By incorporating quinoa protein in different proportions, we changed the color of both crust and crumb. The lightness (L^*) and yellowness (b^*) decreased whereas the redness (a^*) increased. The minimal lightness and yellowness were registered for the cake baked according to Formulation 4 with the highest quinoa protein concentration. The redness grew together with the ratio of quinoa protein. As a rule, the increase in the redness in cake crust is associated with caramelization and the Maillard reaction. The larger amount of quinoa protein increased the protein content in the cakes and consequently stimulated the Maillard reaction, thus producing dark-brown components. Gallego *et al.* reported that protein increased the redness in muffin crust [34]. Our findings confirm those observed by Blanco Canalis *et al.*, who also reported an increase in redness (a^*) and significant reduction in lightness (L^*) as a result of increasing protein level in cake [35].

Table 8 Color parameters of cakes with different shares of quinoa protein

	Crust				Crumb			
	*L**	*a**	*b**	Total color difference	*L**	*a**	*b**	Total color difference
Control	47.10 ± 0.56^{c}	8.61 ± 0.05^{e}	22.16 ± 0.22^{b}		69.67 ± 0.01^{a}	0.50 ± 0.01^{c}	20.91 ± 0.02^{a}	
Formulation 1 (50 g quinoa protein)	51.08 ± 0.05^{a}	11.80 ± 0.01^{c}	23.62 ± 0.02^{a}	18.37 ± 0.06^{d}	68.47 ± 0.13^{b}	0.22 ± 0.02^{e}	17.95 ± 0.10^{e}	6.78 ± 0.02^{c}
Formulation 2 (75 g quinoa protein)	48.12 ± 0.01^{b}	12.86 ± 0.01^{a}	21.44 ± 0.01^{c}	22.11 ± 0.01^{c}	67.34 ± 0.05^{c}	0.29 ± 0.07^{d}	19.90 ± 0.11^{b}	6.54 ± 0.03^{d}
Formulation 3 (100 g quinoa protein)	43.10 ± 0.27^{d}	10.50 ± 0.05^{d}	20.00 ± 0.11^{e}	27.00 ± 0.03^{b}	63.42 ± 0.03^{d}	0.78 ± 0.01^{b}	18.43 ± 0.02^{d}	10.61 ± 0.02^{b}
Formulation 4 (150 g quinoa protein)	42.51 ± 0.17^{e}	12.34 ± 0.01^{b}	20.34 ± 0.08^{d}	27.52 ± 0.19^{a}	62.44 ± 0.04^{e}	0.94 ± 0.01^{a}	18.97 ± 0.01^{c}	11.30 ± 0.03^{d}

Values are represented as mean ± SD. Values with different superscripts in the same column are significantly different at $p \leq 0.05$

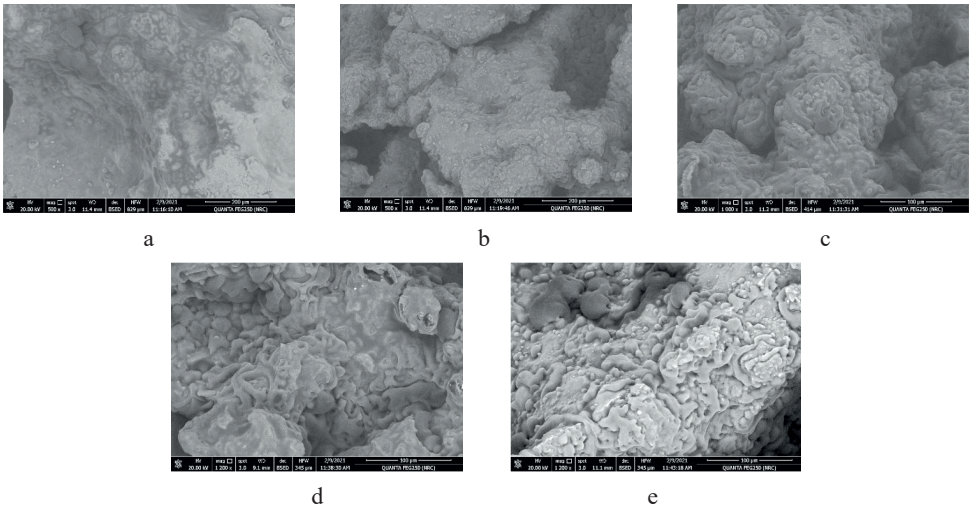

Figure 3 Scanning electron microscopy images (200×): a – control; b – Formulation 1 (50 g quinoa protein); c – Formulation 2 (75 g quinoa protein); d – Formulation 3 (100 g quinoa protein); e – Formulation 4 (150 g quinoa protein)

Table 9 Sensory scores of cakes with different shares of quinoa protein

Treatment	Taste	Color	Softness	Flavor	Cells size uniformity	Overall acceptability
Control	8.30 ± 0.95^{ab}	8.20 ± 0.92^{a}	8.80 ± 1.16^{a}	8.00 ± 0.94^{ab}	8.70 ± 0.82^{ab}	8.100 ± 0.744^{b}
Formulation 1 (50 g quinoa protein)	7.40 ± 0.84^{bc}	8.20 ± 0.92^{a}	8.20 ± 0.63^{ab}	7.90 ± 0.99^{abc}	8.20 ± 0.63^{bc}	7.70 ± 0.67^{b}
Formulation 2 (75 g quinoa protein)	7.90 ± 0.99^{abc}	9.00 ± 0.94^{a}	7.60 ± 1.43^{b}	7.40 ± 1.17^{bc}	7.90 ± 0.74^{c}	7.70 ± 0.95^{b}
Formulation 3 (100 g quinoa protein)	7.00 ± 1.41^{c}	7.20 ± 1.14^{b}	7.90 ± 1.52^{ab}	6.80 ± 1.40^{c}	7.80 ± 0.92^{c}	7.35 ± 1.38^{b}
Formulation 4 (150 g quinoa protein)	8.80 ± 1.135^{a}	6.70 ± 1.16^{b}	9.00 ± 0.94^{a}	8.70 ± 1.34^{a}	9.00 ± 0.94^{a}	9.00 ± 0.96^{a}

Values with different superscripts in the same line are significantly different at $p \leq 0.05$

Microstructure analysis. We appealed to scanning electron microscopy to study the microstructure of the control cake and the samples with different shares of quinoa protein (Fig. 3). The impact of the quinoa protein on the batter microstructure was determined in order to investigate the network or structure-forming potential of quinoa protein as a safe ingredient. The scanning electron microscopy revealed the preliminary structural properties of the egg-free cakes, which showed alterations in the microstructure. The experimental samples exhibited a distinct protein matrix with embedded starch granules. However, the size distribution of the microparticles was wide. This appearance was similar to that of the dough described in [36]. During mixing, the diverse bonds in the proteins started to interact with each other via hydrogen, ionic, hydrophobic, and covalent bonds, thus developing a cross-linked network [37]. Romano *et al.* reported gelatinized starch granules surrounded by a continuous protein matrix in a scanning electron microscopy image of cakes with quinoa protein [38].

Sensory properties. A descriptive sensory test made it possible to define and evaluate the sensory properties. Table 9 shows the sensory assessment of color, softness, flavor, cell size uniformity, and overall acceptance. The cakes with quinoa protein were significantly ($p < 0.05$) different from the control in all sensory aspects. The

color sensory score decreased as the share of quinoa protein grew. Formula 4 with the greatest quinoa protein content received the highest score for overall acceptability sensory.

CONCLUSION

Quinoa is a non-conventional source of protein rich in essential amino acids and with an excellent nutritional value. This study featured cakes with different shares of quinoa protein with improved nutritional properties and microstructure. Our results underscored the unique features of egg-free cakes, e.g., a higher protein value. The balanced amino acid profile could make quinoa protein a potential egg replacer in bakery products designed for people with allergy to eggs. The new product also demonstrated an acceptable overall sensory profile.

Quinoa protein proved to be a prospective nutritive source, a food supplement, and a functional food ingredient. With the rapidly developing processing technology and ingredient functionality, the mass production of quinoa protein can overcome the challenge of cost effectiveness as a competitive egg replacer and may become available in the near future. Yet, advanced research is needed to prove and improve its functional properties and versatility as a food ingredient. In addition, more work is required to better understand quinoa protein and its potential for the food industry in general, functional foods, and special dietary foods.

CONTRIBUTION

Rasha K. Mohamed and Safaa S. Abozed carried out the experiment, prepared the materials, and analyzed the obtained data. Zahra S. Ahmed aided in interpreting the results and drafted the manuscript. All the authors contributed to the study conception and design, as well as read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests regarding the publication of this article.

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