

# Research Article

# Preparation of a Low-Calorie, Gluten-Free All-in-One Cake Mix, Containing *Bacillus Coagulans* Using Quinoa and Inulin Functionality

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Received 7 August 2021; Accepted 28 March 2022; Published 23 April 2022

Academic Editor: Muhammad Faisal Manzoor

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Celiac patients must follow a strict gluten-free diet along the life that leads to many nutritional deficiencies. This study aimed to produce an "all-in-one gluten-free cakemix based on quinoa flour" for celiac patients and other gluten-sensitive people. Nine treatments were provided with quinoa flour (25, 27.5, and 30% of total formula), inulin as a prebiotic, fat replacer and natural sweetener (2.4, 3.2, and 4% of total formula). The content of oil was reduced using oil powder consisting of sunflower oil and wall materials (resistant starch and maltodextrin 50:50). Bacillus coagulans was added as a probiotic bacteria. A commercial cakemix was considered as a control sample. The nutritional and chemical properties of cake mixes (percentage of moisture, protein, mineral, carbohydrate, crude fiber, fat, and calorie) and physical and textural properties of cakes (springiness, specific volume, porosity, chewiness, browning reaction, moisture, and water activity) were tested. Data were analyzed by https://graphpad.ir/prism/software using one way analysis of variance (ANOVA). After sensory evaluation, the treatment number 5 was selected as the most acceptable cake among all treatments. Its amino acid profile, fatty acid profile, and peroxide index were determined. The minerals, protein, and fiber of quinoa cake mixes were significantly higher, and the fat, carbohydrate and calorie were lower than control. Addition of quinoa reduced springiness, specific volume, porosity, chewiness, and increased the browning reaction. But these changes did not have a very negative effect in general. The moisture, water activity, and bacteria count changes were followed during four days of cakes preservation in refrigerator. The reduction trend of quinoa cakes moisture and water activity was slower than control. The number of bacteria was enough to be considered as a probiotic product after 4 days. Quinoa flour could improve the nutritional and functional properties of glutenfree cake mixes.

### 1. Introduction

Celiac is a genetic disease which causes many difficulties in nutrients absorption through damage intestinal mucosa. Currently, the only effective treatment for this disease is to follow gluten-free (GF) diet throughout the lifetime [1].

However, this kind of diet can be accompanied with some disadvantages including a negative impact on quality of life, psychological problems [2], nutritional deficiencies, metabolic syndrome, cardiovascular diseases, and often severe constipation [3]. According to the mentioned problems, nowadays the importance of producing GF products has increased.

Quinoa is a GF grain with high levels of protein, essential fatty acids, essential amino acids, minerals, vitamins, dietary fiber, and carbohydrates that have positive hypoglycemic effects [4]. So its application in cakes and other bakery formulations can lead to functional products [5].

Nowadays, you can make your favorite cake at home using ready-made cake premix powders that are available in

stores [6]. The point of this study was the production of a functional GF cakemix based on quinoa flour for glutensensitive patients and other people who need to follow a lowcalorie diet with lower amounts of oil and sugar, high protein, and high fiber percent.

The oil was used in powder form. Resistant starch and maltodextrin were used as wall materials in oil encapsulation. Wheat is replaced completely by different percentages of roasted quinoa flour. Inulin powder acts as a fat replacer, natural sweetener, and prebiotic at the same time. In addition, use of probiotic bacteria *Bacillus coagulans* turned this snack into a symbiotic product. All the ingredients including whole egg powder, whole milk powder, emulsifier, flavor, sugar and other additives were used to make an all-in-one cake premix; therefore, it is possible that gluten-sensitive people be able to provide a suitable snack in a short time just by adding water.

The novelty of this product is that in addition to the functional properties and being gluten-free, this product has low calorie due to its low percentage of fat and sugar so can also be used in slimming, cardiovascular, and diabetic diets.

The aim of the present research was to evaluate the effects of above materials on the nutritional properties of cake premixes, physical, and sensory characteristics of the resulting cakes and their final acceptability. It is assumed that these homemade cakes could be refrigerated for a maximum of four days after baking. After sensory evaluation by semitrained panelists, one of the nine treatments was selected as the most acceptable and its amino acid profile, fatty acid profile, and peroxide index was determined.

#### 2. Material and Method

2.1. Source of Materials. Whole egg powder was purchased from Golpowder Company in Gonbad Kavos, Golestan province, Iran. Inulin powder, maltodextrin, and resistant starch were purchased from Helmi Company in Tehran province, Iran. Emulsifier was purchased from Pishgaman Chemistry Company, Esfahan province, Iran. Ready-made "Parsilact" bacterial powders were purchased from Pardis Roshd Mehregan, Shiraz province, Iran. Quinoa seeds, ready-made cake mix, sunflower oil, whole dry milk powder, sugar, vanilla, salt, and baking powder were purchased from city stores.

2.2. Preparation of Quinoa Flour. Quinoa seeds were cleaned and were washed in order to clean up the dust and saponin layer completely. After drying in room temperature, they were roasted on a metal plate heated to  $177^{\circ}$ C for 30 min. They were ground after cooling into ( $125-180 \,\mu$ m) particles and were stored in closed containers in a dry, cool place [7].

2.3. Preparation of Oil Powders. Resistant starch, maltodextrin, water, emulsifier, and various percentages of sunflower oil were mixed to preparation of nine different emulsions (Table 1) [8]. The emulsions were spray dried in condition: atomizer spin: 1400 rpm, inlet temperature 140°C, out let temperature 70°C, spray dryer capacity: 25 L/min [9]. The oil amounts in final oil powders were ranged from 32.4 to 40.4%. The quality of produced oil powders, including surface oil, encapsulated oil, total oil, and encapsulation efficiency were evaluated and confirmed. The appearance, particle flow (clumping and adhesion), and particle size were confirmed. The prepared oil powders were stored in special closed containers and were stored in the freezer  $(-20^{\circ}C)$  [10].

2.4. Preparation of Cake Premixes. Nine cake premix powders were produced with various formulations. The variable components of cake premix powders including quinoa powder, inulin, and nine types of oil powders (number 1 to number 9) are represented in Table 2. The constant ingredients included are following: whole egg powder: 6%, whole milk powder: 5%, sugar: 20%, flavors: 0.4%, emulsifier: 0.35%, bacteria:  $10^{10}-10^{11}$  cfu/g, baking powder: 2.2%, salt: 0.1%. The ingredients were weighed by a laboratory balance (Sartorius Entris model 4202-1s) into a jar and were mixed well by a Kitchen-Aid mixer (model Elektra) and were stored in a cool and dry place until performing chemical tests. The control cake powder ingredients are presented in Table 3.

*2.5. Cake Baking.* The control cake was baked according to its factory instruction exist on the label, after adding three eggs, sunflower oil: 125 g, milk: 166 ml. The quinoa premixes were baked according to the instruction below:

The dry cake premixes were mixed at a low-speed Kitchen-Aid mixer (model Elektra) into a bowl for one minute to ensure uniform blending of the ingredients. A 60% of water was added at low speed in 30 seconds. The rest of water was added in four steps after mixing and scrapping down the batter for 6 min. Cake batter was placed into a small tin mold ( $80 \text{ mm} \times 60 \text{ mm} \times 40 \text{ mm}$ ) and baked in an oven (Prima convection oven model Axis COA1003) and heated at 180°C for 45 min. All batches were prepared in triplicate. After cooling at room temperature, cakes were stored overnight into polypropylene bags [11].

2.6. Cake Mixes Analysis. The prepared quinoa cake mixes and control cake mix were examined for nutritional and chemical properties. The total fat, total protein, total ash, total carbohydrates, crude fiber, and moisture percentages were determined for cake mixes according to Association of Official Analytical Chemists method (AOAC), American association of cereal chemist (AACC 10–90) method, using three trials. The calorie content for each treatment was determined by bomb calorimeter (Model-https://archiwum. allegro.pl/oferta/kalorymetr-ika-kalorimeter-c400-adiabatis ch-i7247720712.html) [12].

In order to correct the comparison of chemical factors between quinoa cake mixes and control sample (such as protein and fat), the values related to these parameters were calculated and were added to control cake mix values.

2.7. Baked Cake Analysis. The physical and sensorial properties of cakes were analyzed based on methods below.

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Oil powder number	In emulsion	ns before spry drying			After spry drying
	Resistant starch/maltodextrin (50:50)	Sun flower oilin emulsions	Water	Emulsifier	Oil contents in oil powders
1	25	13.6	61.4	_	32.4
2	25	12.8	62.5	—	33.8
3	25	12	63	—	34.7
4	22.5	13.6	63.9	—	35.2
5	22.5	12.8	64.7	—	36.2
6	22.5	12	65.5	—	37.5
7	20	13.6	66.4	_	37.6
8	20	12.8	67.2	0.3	39
9	20	12	68	0.3	40.4

TABLE 1: Components of oil powders Emulsions (%).

Spray drying conditions: Atomizer spin: 1400 rpm, inlet temperature 140°C, out let temperature 70°C, spray dryer capacity: 25 L/min (paola, 2014).

 TABLE 2: Variable ingredients of the cake powders (%).

 Quinoa
 Type of cil nounder

Treatment number	Quinoa	Inulin	oil powder	Oil powder
1	25	2.4	No. 1	41.9
2	25	3.2	No. 2	37.9
3	25	4	No. 3	34.6
4	27.5	2.4	No. 4	38.6
5	27.5	3.2	No. 5	35.35
6	27.5	4	No. 6	32
7	30	2.4	No. 7	36.2
8	30	3.2	No. 8	32.8
9	30	4	No. 9	29.7

TABLE 3: Control cake formula.

Ingredient	%
Flour	55.95
Sugar	32
Modified starch	3
Lactose	3
Baking powder	2.2
Whey protein	3
Emulsifier	0.35
Salt	0.1
Flavor	0.4
Sum	100

2.7.1. Moisture. Moisture content of cake samples were measured through general methods of (AACC 200044–16) by electric oven (model Shimaz CHF) [13].

2.7.2. Water Activity. Water activity (aw) of cakes was measured on homogenized samples with an electronic hygrometer (model Aqua Lab v.2.0; Decagon Devices, Inc., Pullman, WA, USA) at  $25 \pm 0.2$ °C. Samples were collected from the middle section of each cake [14].

*2.7.3. Specific Volume.* The volume and specific volume of the cake samples were measured by a method of millet seeds replacement [13].

2.7.4. Texture Analysis. The textural properties including springiness, chewiness of cakes were determined using a Texture Analyzer (model CT3 American-made Brookfield).

Cubes of 2.5 cm were gently cut out of the center of each cake to expose the crumb for texture measurement. The resulting force-time curve was used to determine the mentioned properties [15].

2.7.5. Porosity. Image processing technique was used to evaluate the porosity of the cakes' crumbs. For this purpose, the slices of samples were imaged by a scanner (model 2400 Scanjet) made in China with a resolution of 300. The provided images were analyzed by a software (Image J). The light and dark points were calculated as an indicator of the porosity of the samples [6].

2.7.6. Browning Index. The crusts of cake samples were tested with the colorimeter (model Hunter Color-Flex, CFLX 45–2, USA). After standardizing the colorimeter, the factors "L", "a," and "b" of the cakes crusts were measured. The "L" values indicate the opacity and brightness of the surface, "a" indicates the intensity of the red color, and "b" indicates the brightness of the yellow surface [1]. The browning index (BI) was also calculated for each sample using equations (1) and (2).

$$BI = \frac{100 \left(x - 0.31\right)}{0.17},\tag{1}$$

$$X = \frac{a + 1.75 \times L}{5.645 \times L + a - 3.012b}.$$
 (2)

2.7.7. Sensory Features of the Cake. In order to determine the sensory acceptability of quinoa cakes, to select the best one, a 5 point hedonic scale (very good, good, moderate, bad, and very bad), was performed for taste, odor, texture, color, and overall acceptance by semitrained panelists (n = 10) at the "Sanjesh Mizan Pasargad" laboratory in Science and Technology Park of Tehran University, Iran [16].

2.7.8. Determination of Probiotic Bacteria in Quinoa Cake Samples. The bacterial survival was determined in the quinoa cake samples during 4 days from baking trough enrichment in NYSM broth medium and culturing in NYSM agar medium through pour plate method, according to producer company protocol [17].

2.7.9. Determination of Amino Acid and Fatty Acid Profiles. The profile of amino acids was evaluated through HPLC method, and fatty acids profile was evaluated through gas chromatography (GC-FID) methods for the sample that received the highest score from the sensory panel judges among the nine samples.

*2.7.10. Peroxide Index.* Peroxide index were determined for the best sample in the case of sensory properties according to IUPAC 2.501, AOCS Cd 8b—90 (97) or ISO 3961:1998.

2.8. Statistical Analysis. All of the analyses were performed at least in three replications. The average of three parallel measurements was calculated in all the cases. Data were subjected to Analysis of Variance by https://graphpad.ir/ prism/ software using one way analysis of variance (ANOVA) at a 95% confidence level ( $p \le 0.05$ ). The significance of differences between average values was evaluated using Duncan's test.

#### 3. Results and Discussion

*3.1. Appearance of All Cake Mixes.* The appearance of all cake mixes was examined according to the type of oil powder which was used in them. They were uniform, homogeneous, and did not clump.

3.2. Chemical and Nutritional Parameters of Cake Mixes. Proximate composition of cake mixes is represented in Table 4. The nutritional parameters including total ash, protein, and crude fiber had a significant (p < 0.001) increase in quinoa cake powders in comparison with control sample.

3.2.1. Moisture. The results showed a significant difference (p < 0.001) in moisture contents between the treatments of quinoa and control cake mixes (Table 4). According to Codex Standard for wheat (CODEX STAN 199–1995), the moisture content of wheat flour (used in control treatment) is a maximum of 14.5. While being based on Codex Alimentarius Standard for quinoa (CXS 333–2019 Adopted 2019.Amended in 2020), the maximum humidity of quinoa

is 13. In this study the moisture content of quinoa grains determined before was about 8.83%. It seems that the moisture content differences between two cake mixes were related to their sources. In addition, the moisture difference between quinoa mixes is also significant (p < 0.001)) and with increasing of quinoa flour the moisture percent decreased gradually. Low moisture content in the quinoa treatments can make them more resistant to microbial, enzymatic, and chemical peroxidation activities.

3.2.2. Minerals. In general, all the mineral (ash) contents of gluten-free cakes significantly (p < 0.001)) were more than control sample. Due to the fact that the other components of quinoa cake mix contain minerals such as milk and eggs, and had the same amounts in all treatments and control, it can be concluded that most of these differences between quinoa treatments and control are related to quinoa, which increases in treatments 1 to 9. Quinoa has higher levels of minerals (sodium, potassium, magnesium, and calcium, moderate amounts of iron, zinc, and manganese, and small amounts of copper) in its outer layers of bran compared to other grains, and this lead to a higher percentage of ash in quinoa treatments. For this reason, this pseudocereal can meet part of the needs of celiac patients for minerals. The richest mineral composition was obtained at highest enrichment ratio (T9) (Table 4). This result is confirmed by other researches [16, 18]. It should be noted that the other components of quinoa and control cake powder that contain minerals such as milk and eggs had the same amounts in all treatments and controls.

It can be concluded that most of this difference in mineral percentage between treatments and control is related to quinoa, which increases with increasing percentage in treatments 1 to 9.

3.2.3. Protein. The amount of quinoa cake mixes protein was significantly (p < 0.001) higher than control cake mixes (Table 4). It should be noted that the other components of quinoa cake mix that contain protein such as milk and eggs had the same amounts in all treatments and control, so most of these differences between guinoa treatments and control are related to quinoa, which increases in treatments 1 to 9. Most of the gluten-free cereal-based foods are using refined flour or unenriched starch and are only high in carbohydrates and fats, while quinoa contains more protein than wheat flour. It helps in the compensation for the lack of protein that occurs for removing of wheat in GF-restricted diet [2]. Sándor Tömösközi results approved the high amounts of protein in quinoa cakes [18]. It should be noted that the other components of quinoa cake mixes do not contain a significant amount of protein, so most of these differences between quinoa treatments and control are related to quinoa, which increases in treatments 1 to 9.

*3.2.4. Crude Fiber.* A gluten-free diet reduces fiber intake in celiac patients [19]. As shown in Table 4, in this study crude fiber content in quinoa cake mixes was significantly

Treatment	Fat	Protein	Total ash	Carbohydrate	Moisture	Crude fiber	Calories (per100 g)
Control	$28.40 \pm 0.364^{a}$	$11.40 \pm 0.392^{a}$	$1.360 \pm 0.200^{a}$	$53.27 \pm 0.153^{a}$	$5.62 \pm 0.513^{a}$	$0.12 \pm 0.001^{a}$	$513.0 \pm 3.577^{a}$
1	$16.29 \pm 0.21^{b}$	$36.10 \pm 0.522^{b}$	$1.433 \pm 0.023^{a}$	$43.06 \pm 0.061^{b}$	$3.17 \pm 0.348^{b}$	$1.20 \pm 0.061^{ m b}$	$462.5 \pm 1.385^{b}$
2	$15.41 \pm 0.331^{bc}$	$36.76 \pm 0.317^{b}$	$1.420 \pm 0.200^{a}$	$43.33 \pm 0.176^{b}$	$2.71 \pm 0.276^{b}$	$1.42 \pm 0.314^{b}$	$458.0 \pm 2.314^{\mathrm{b}}$
3	$15.63 \pm 0.181^{ m b}$	$37.21 \pm 0.449^{b}$	$1.440 \pm 0.020^{a}$	$43.18 \pm 0.332^{b}$	$2.68 \pm 0.036^{ m b}$	$1.47 \pm 0.195^{b}$	$463.6 \pm 0.756^{\mathrm{b}}$
4	$15.58 \pm 0.236^{b}$	$35.45 \pm 0.392^{b}$	$1.703 \pm 0.059^{b}$	$44.60 \pm 0.621^{\circ}$	$2.71 \pm 0.025^{b}$	$2.91 \pm 0.118^{bc}$	$461.2 \pm 1.099^{b}$
5	$14.63 \pm 0.241^{\circ}$	$36.45 \pm 0.458^{b}$	$1.733 \pm 0.064^{b}$	$44.51 \pm 0.127^{\circ}$	$2.71 \pm 0.055^{b}$	$3.15 \pm 0.106^{\circ}$	$454.5 \pm 0.763^{\circ}$
6	$14.07 \pm 0.020^{\rm d}$	$36.90 \pm 0.036^{b}$	$1.723 \pm 0.119^{b}$	$44.67 \pm 0.074^{\circ}$	$2.65 \pm 0.015^{b}$	$3.27 \pm 0.053^{\circ}$	$451.9 \pm 0.186^{\circ}$
7	$14.15 \pm 0.175^{cd}$	$36.91 \pm 0.489^{b}$	$2.080 \pm 0.060^{\circ}$	$45.33 \pm 0.241^{cd}$	$2.48 \pm 0.070^{ m b}$	$3.37 \pm 0.111^{\circ}$	$453.3 \pm 0.975^{\circ}$
8	$13.87 \pm 0.044^{\rm d}$	$35.96 \pm 0.123^{b}$	$2.143 \pm 0.112^{\circ}$	$45.63 \pm 0.135^{d}$	$2.41 \pm 0.080^{b}$	$3.39 \pm 0.044^{\circ}$	$452.5 \pm 0.575^{\circ}$
9	$13.51 \pm 0.391^{d}$	$36.03 \pm 0.272^{b}$	$2.156 \pm 0.093^{\circ}$	$46.01 \pm 0.064^{d}$	$2.39 \pm 0.112^{b}$	$3.68 \pm 0.135^{d}$	$448.4 \pm 2.902^{\circ}$
	_						

Note: The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA. Different letters in columnsrepresent significant differences in nutritional properties between treatments at p < 0.05 probability level.

(p < 0.001)) higher than control sample. The increasing trend continued with increase of quinoa percentage. Quinoa is the only source of crude fiber among all formula's components. Also, the reports of some other studies confirm this point [20]. It has been shown that the enrichment of GF pasta by quinoa flour increased remarkably the contents of dietary fiber [21].

3.2.5. Carbohydrate. Despite the use of multiple sources of carbohydrates in quinoa treatments (such as resistant starch, maltodextrin, and inulin), the amount of carbohydrates in all quinoa cake mixes was less than the control significantly (p < 0.001)), and this reduced calorie intake, and they can be a good candidate in low calorie diets (Table 4). Between quinoa treatments, the amount of carbohydrates increased with increasing the quinoa and inulin percentages. Even though quinoa has a low glycemic index, but it is better to be eaten less on a low carbohydrate diet [22].

3.2.6. Fat and Calorie. In general, the function of fat in cakemaking is very important both from technological and the sensory properties point of view. On the other hand, fat has high calorie density and reducing of fat content is currently one of the primary trends in food product innovations.

As shown in Table 4, in this research, replacing part of oil by inulin and encapsulation of oil with resistant starch, decreased the final fat content significantly (p < 0.001)). Using of quinoa flour, reduction of sugar amount, and using inulin as a sweetener and high-fiber bulking agent, reduced calories received from final product (p < 0.001)) [23].

#### 3.3. Chemical and Physical Properties of Quinoa Cake Treatments and Control

3.3.1. Moisture Changing during 4 days Preservation. Generally, in this study what is more important, is moisture maintenance during preservation period (Figure 1). In sample 1, which contains 2.4% inulin and 25% quinoa, the moisture reduction trend had a lower slope than other samples and control sample. However, the amount of moisture and its reduction slope in all quinoa treatments was less than control sample. So the product retained its

moisture for a longer time. This may be due to the presence of soluble and insoluble fibers of inulin and quinoa that had more water absorption capacity because of their structures compared with other fibers and lost more water in the baking stage. The reports of Morris (2012) are consistent with the results obtained in this study [24].

3.3.2. Water Activity Changing during 4 Days Preservation. Water activity (aw) changes of the cake samples during 4 days after baking are shown in Figure 2. In this study, all treatments and control cakes had gradual reduction in aw. But replacement of wheat flour with quinoa flour caused a significant lower aw in all quinoa cake samples. The lowest aw was related to sample number 9 which had the highest amounts of quinoa and inulin. In treatments number 7 and 8 also, the low aw led to freshness and delay in staleness. It seemed that aw changes in quinoa cakes were following the trend of moisture changes. It is clear that using of moistureabsorbing materials such as salt, sugar, inulin, and crude fiber in foods causes decreasing of aw. Lack of gluten also may lead to aw reduction. This results are conformed by other researchers [25]. The significant decreases in aw was reported in GF muffins made with rice flours in Singh study. Because of gluten absence, water-binding capacity of quinoa flour is less than wheat flour so quinoa cakes showed less aw [26]. The positive point is that low aw decreases the microbial activity probability and increase the shelf life.

3.3.3. Springiness. As shown in Table 5, the springiness of quinoa cakes was significantly reduced by replacing wheat flour with quinoa flour and adding inulin (p < 0.01)). After the control sample, the highest and lowest amounts of springiness were related to treatment 1 and treatment 5 with 25 and 27.5% of quinoa and 2.4% and 3.2% of inulin, respectively. But the difference between the samples of quinoa cake itself was not significant. Elasticity property and gas storage capacity depend on gluten content. Another cause of reduced springiness may be due to resistant starch that is used in the oil powder. Rostami et al. reported a decrease in the springiness of gluten-free cake samples using formulated resistant starch [27].

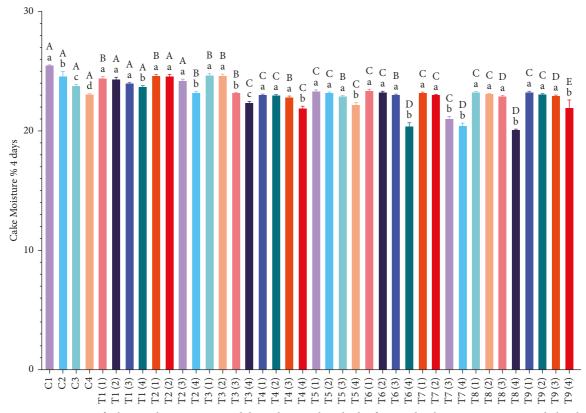


FIGURE 1: Moisture content of cake samples were measured through general methods of AOAC by electric oven, using weight loss before and after heating of 2 grams of the samples at  $103 \pm 2^{\circ}$ C to reach a constant weight. The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA. The different lowercase letters indicate significant changes of a treatment's moisture during the four-day period at *p* < 0.05 probability level. Different capital letters indicate the significant difference between treatments' moisture in the same day compared to each other at *p* < 0.05 probability level.

3.3.4. Specific Volume. Specific volume is one of the most important quality factors considered by the consumer. The specific volume of the cake is a good factor to check how much air bubbles enter the dough and how much air is retained during mixing. The more the dough is able to hold the air bubbles, the larger cake volume and the better its rheological properties [28]. As shown in Table 5, the specific volume of treatments 1, 2, 3, 4, and 5 was not significantly different from the control sample. However, in treatments 6 (p < 0.01), 7, 8, and 9, the specific volume of quinoa cakes decreased gradually with increasing quinoa percentage (p < 0.01). Treatments 1, 2, and 3 contained the same amounts of quinoa (25%) and variable amounts of inulin (2.4, 3.2, and 4%). One of the effective factors in increasing the volume of dough is the presence of gluten network, which increases the specific volume of the cake with its gas storage capacity. However, due to the fact that the quinoa cakes don't have structural gluten network, other factors such as insoluble and soluble fibers of quinoa and inulin can keep the cake volume in the absence of gluten by absorbing water [29]. As can be seen in Table 5, an increase in cake volume was observed at constant values of quinoa with increasing amount of inulin, and other studies have also shown the independent effect of inulin on increasing the volume of the cake [20]. Inulin and maltodextrin are both bulking agents. Maltodextrin was one of the wall materials

used in the preparation of oil powder. The used emulsifier could also help cake volume increasing. During cake baking, the interaction between high amounts of quinoa proteins can also be effective in creating the structure of the cake [29].

3.3.5. Porosity. Porosity has an opposite relationship with density and hardness. As shown in Table 5, the porosity of quinoa cakes was reduced significantly with increasing of quinoa amounts in comparison of control cake. This reduction is due to absence of gluten which has the main role in trapping and preserve of gas and size of bubbles [6].

3.3.6. Chewiness. As shown in Table 5, the chewiness of quinoa cakes was significantly lower than control sample, but with the increasing of quinoa percentage, this index improved and became closer to control (p < 0.01). The best chewiness was related to samples 7, 8, and 9 which had the highest amount of quinoa (30%). They had the same amounts of quinoa and the variable amounts of inulin (2.4, 3.2 and 4%). It seems that this improvement in chewiness was mostly related to changes of quinoa amounts and its high protein and insoluble fiber contents. Resistant starch also was used as wall material for encapsulation of oil and previous studies showed the positive effect of adding resistant starch on chewiness of GF cake samples [27].

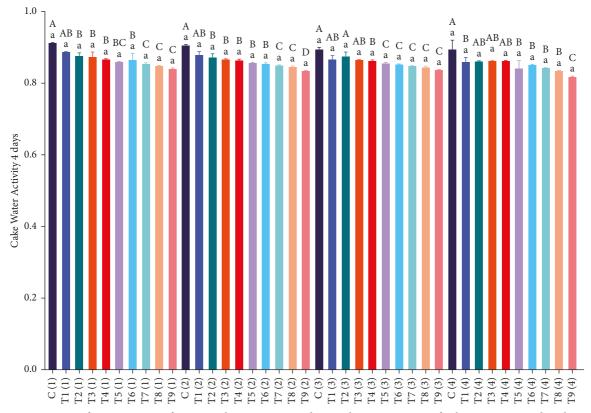


FIGURE 2: Comparison of water-activity of quinoa cake treatments and control. Water activity of cakes was measured on homogenized samples with an electronic hygrometer at  $25 \pm 0.2^{\circ}$ C. Samples were collected from the middle section of each cake. The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA. The same lowercase letters indicate no significant changes of a treatment's aw during four-day period at p < 0.05 probability level. Different capital letters indicate the significant difference between treatments' aw in the same day compared to each other at p < 0.05 probability level.

Treatment	Springiness (mm)	Specific volume (cm <sup>3</sup> /kg)	Porosity	Chewiness (g/mm)
Control	$9.277 \pm 0.409^{a}$	$2.733 \pm 0.067^{a}$	$26.56 \pm 0.230^{a}$	$11.33 \pm 0.208^{a}$
1	$7.657 \pm 1.605^{b}$	$2.718 \pm 0.065^{a}$	$24.44 \pm 0.208^{b}$	$7.800 \pm 0.265^{b}$
2	$7.253 \pm 0.153^{b}$	$2.624 \pm 0.014^{a}$	$24.42 \pm 1.215^{b}$	$7.807 \pm 0.133^{b}$
3	$7.057 \pm 0.232^{b}$	$2.620 \pm 0.045^{a}$	$23.54 \pm 0.081^{b}$	$7.497 \pm 0.150^{ m b}$
4	$6.923 \pm 0.172^{\mathrm{b}}$	$2.604 \pm 0.056^{a}$	$23.13 \pm 0.139^{b}$	$9.080 \pm 0.262^{\circ}$
5	$6.377 \pm 0.293^{\circ}$	$2.527 \pm 0.161^{a}$	$22.25 \pm 0.040^{b}$	$9.093 \pm 0.444^{c}$
6	$6.583 \pm 0.237^{\circ}$	$2.437 \pm 0.11^{ab}$	$22.28 \pm 0.031^{b}$	$9.127 \pm 0.577^{\circ}$
7	$7.107 \pm 0.266^{b}$	$2.326 \pm 0.05^{b}$	$21.25 \pm 0.015^{b}$	$10.28 \pm 0.017^{\rm d}$
8	$7.497 \pm 0.211^{ m b}$	$2.167 \pm 0.01^{b}$	$21.42 \pm 0.45^{b}$	$10.28 \pm 0.040^{ m d}$
9	$7.170 \pm 0.193^{\mathrm{b}}$	$2.110 \pm 0.04^{ m b}$	$20.22 \pm 0.225^{b}$	$10.333 \pm 0.153^{d}$

TABLE 5: Approximate values of Physical & textural properties of quinoa cake treatments.

Note: The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA.Different letters in columns represent significant differences of Physical & textural properties between treatments at p < 0.05 probability level.

3.3.7. Browning Index. Among the food physical properties, color is recognized as the most important visual feature that is related to ingredients reactions. In this study, the browning reaction in quinoa cake treatments was significantly (p < 0.001) higher than the control sample (Figure 3). However, with increasing quinoa and inulin levels, the intensity of reaction did not increase significantly between treatments. Simultaneous presence of large amounts of protein and carbohydrate in quinoa flour, egg powder, milk powder, inulin, maltodextrin, etc., in treatments increased

Maillard reaction. On the other hand, increasing the amounts of quinoa and inulin reduced the moisture content of the treatments and intensified the Maillard reaction. The maximum browning usually occur in medium humidity (20–40) such as cakes [15]. Another factor that affected the color of treatments was the pigments in the quinoa shells, which darkened the color of quinoa cake treatments. The results of this study about Maillard and caramelization were confirmed by the results of Bozgodan and Kumcuoglu (2019) [20].

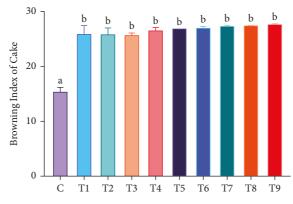


FIGURE 3: Comparison of Browning reaction of quinoa cake treatments and control. The crusts of cake samples were tested with the colorimeter. Increasing of quinoa flour enhances dark brown components. The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA.Different lowercase letters represent significant differences at p < 0.05 probability level.

3.4. Sensory Properties. Evaluation and comparing the sensory characteristics of quinoa and control cakes showed that the best sample in terms of taste, odor, color, texture, and overall acceptance was the control sample (Table 6). Among the quinoa cake treatments, the best sample in terms of taste was sample number 5, which had no significant difference with the control, followed by treatment 1 (p < 0.01), and other treatments were in the same range and less than them. In terms of odor, the first sample after control number was treatment 5 and then 6, and none of them had a significant difference with the control sample (ns). In terms of color, after the control sample, the highest score was for no. 5, and the next was no. 6, which were not significantly different from the control sample. In terms of texture, no 1 had a higher score than samples 5 and 6, but none of them had a significant difference with control. The highest score for overall acceptance was related to treatments 1, 5, 6, 7 and 8 with no significant differences, while treatments 2, 3, 4, and 9 were slightly different from the control sample (p < 0.05). The fact is that consumers are not familiar enough with the taste of quinoa and its products. The taste of quinoa was not well received by some panelists. The difference in acceptability of this product was not unexpected because the texture parameters have a significant effect on the sensory properties. Enricoise et al. (2003) also examined the effect of using mixed flour (wheat and quinoa) on product characteristics, stating that high levels of substitution reduced the sensory acceptance of samples [30].

3.5. Bacterial Survival of B. coagulants in Cakes. The number of Bacillus coagulase spores in all quinoa cake treatments was determined in dilution  $10^7$  during 4 days of refrigeration (Figure 4). On the fourth day, this number varied from  $(4.12 \times 10^7)$  in treatment number 1 to  $(4.84 \times 10^7)$  in treatment number 6. Due to the fact that B. coagulants was used in the form of spores, changes in the physical, sensory, and tissue properties of the treatments cannot be attributed to its existence. But at the same time, it was a strength for the product that its spores' content was able to withstand the different baking process conditions and refrigerating temperature and after 4 days, it was still enough to be considered as a probiotic product.

3.6. Determination of Amino Acid Profiles. The results of comparing the amino acids' profile of the selected treatment from sensory tests (T5) and control cake showed that quinoa cake has a more valuable amino acids profile compared to the control sample and except for the histidine, other amino acids are superior to the control cake (Figure 5). According to the US National Nutrition Institute, quinoa contains nine essential amino acids. Quinoa is an excellent source of plant protein for vegetarians [31]. Due to the similar amounts of eggs and milk in the control and quinoa samples, the origin of these differences in the amino acids' profiles can only be related to the presence of quinoa. Its' essential amino acids profile can well meet the nutritional needs of the consumer.

3.7. Determination of Fatty Acid Profiles. Examination of the fatty acids profile of the treatment (T5) showed that its oleic acid and stearic acid contents was more than control cake and other fatty acids including linoleic acid, linolenic acid, and palmitic acid with a small difference, were less than the control sample (Figure 6). In general, due to the limited amount of fat in baked quinoa (2%), difference between the fatty acids content of quinoa cake and control is not so great [32]. The other reason for the difference in the quantity of fatty acids in the quinoa cake sample and control was the lower percentage of oil consumed in quinoa cakes (with 12.8% oil) compared to the control sample (with 25% oil).

3.8. Peroxide Index. The results of evaluation and comparison of peroxide index of selected treatment (T5) and the control sample showed that this index for T5 was significantly lower than control (Figure 7). However, the value for both the samples was less than the maximum allowed for the cake peroxide index (maximum 2 mEq/kg) according to ISIRI No. 2553. The low index of quinoa cake peroxide indicated the fact that the oil encapsulation temperature did not have any adverse effect on its health properties, and this

Treatment	Control	T1	T2	Τ3	T4	T5	Τ6	T7	Τ8	4D
Taste	$4.700 \pm 0.483^{a}$	$4.700 \pm 0.483^{a}$ $3.700 \pm 0.483^{ab}$ $3.300 \pm 10^{a}$	$3.300 \pm 0.483^{b}$	$3.300 \pm 0.483^{b}$	$3.300 \pm 0.675^{\rm b}$	$4.400 \pm 0.516^{a}$	$3.700 \pm 0.823^{\rm ab}$	$3.500 \pm 0.707^{\rm b}$	$0.483^{b}  3.300 \pm 0.483^{b}  3.300 \pm 0.675^{b}  4.400 \pm 0.516^{a}  3.700 \pm 0.823^{ab}  3.500 \pm 0.707^{b}  3.400 \pm 0.516^{b}  3.200 \pm 0.422^{b}  3.200 \pm 0.4$	$3.200 \pm 0.422^{b}$
Odor	$4.600 \pm 0.516^{a}$	$4.600 \pm 0.516^{a}$ $3.500 \pm 0.527^{b}$	$3.400 \pm 0.516^b  3.600 \pm 0.516^{ab}  3.700 \pm 0.483^{ab}  4.200 \pm 0.422^a  4.100 \pm 0.568^a  3.500 \pm 0.527^b  3.600 \pm 0.516^{ab}  3.800 \pm 0.422^{ab}  4.100 \pm 0.516^{ab}  3.800 \pm 0.516^{ab} $	$3.600 \pm 0.516^{ab}$	$3.700 \pm 0.483^{ab}$	$4.200 \pm 0.422^{a}$	$4.100 \pm 0.568^{a}$	$3.500 \pm 0.527^{\rm b}$	$3.600 \pm 0.516^{ab}$	$3.800 \pm 0.422^{ab}$
Color	$4.900 \pm 0.316^{a}$	$4.900 \pm 0.316^{a}$ $3.800 \pm 0.422^{b}$	$3.700 \pm 0.483^{b}$	$3.700 \pm 0.483^{\rm b}$	$3.400 \pm 0.516^{\rm b}$	$4.400 \pm 0.516^{a}$	$4.300 \pm 0.675^{a}$	$3.600 \pm 0.516^{\rm b}$	$0.483^b  3.700 \pm 0.483^b  3.400 \pm 0.516^b  4.400 \pm 0.516^a  4.300 \pm 0.675^a  3.600 \pm 0.516^b  3.500 \pm 0.527^b  3.600 \pm 0.516^b  $	$3.600 \pm 0.516^{\rm b}$
Texture	$4.900 \pm 0.316^{a}$	$4.900 \pm 0.316^{a}  4.500 \pm 0.707^{a}$	$3.700 \pm 0.483^{b}$	$3.400 \pm 0.516^{b}$	$3.800 \pm 0.422^{ab}$	$4.300 \pm 0.675^{a}$	$4.100 \pm 0.738^{a}$	$3.600 \pm 0.516^{\rm b}$	$0.483^b  3.400 \pm 0.516^b  3.800 \pm 0.422^{ab}  4.300 \pm 0.675^a  4.100 \pm 0.738^a  3.600 \pm 0.516^b  3.600 \pm 0.516^b$	$3.600 \pm 0.516^{\rm b}$
Overall acceptability $4.900 \pm 0.316^{a}$ $4.200 \pm 0.633^{a}$	$4.900 \pm 0.316^{a}$	$4.200 \pm 0.633^{a}$	$3.900 \pm$	$3.900 \pm 0.876^{ab}$	$3.900 \pm 0.738^{ab}$	$4.800 \pm 0.422^{a}$	$4.100 \pm 0.738^{a}$	$4.000 \pm 0.667^{a}$	$0.568^{ab}  3.900 \pm 0.876^{ab}  3.900 \pm 0.738^{ab}  4.800 \pm 0.422^{a}  4.100 \pm 0.738^{a}  4.000 \pm 0.667^{a}  4.000 \pm 0.667^{a}  3.900 \pm 0.738^{ab}  4.000 \pm 0.667^{a}  4.000 \pm 0.667^{a}  3.900 \pm 0.738^{ab}  4.000 \pm 0.667^{a}  4.000 \pm 0.738^{ab}  4.000 \pm 0.738^{ab}  4.000 \pm 0.667^{a}  4.000 \pm 0.000^{a}  4.000 \pm 0.000^{a}  4.000 \pm 0.000^{a}  4.000^{a}  4$	$3.900 \pm 0.738^{\rm ab}$
Note: The data are expressed as mean $\pm$ SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA. Different letters in the rows represent significant differences in sensory properties between treatments at $p < 0.05$ probability level.	ssed as mean ± SL een treatments at	) from at least three $p < 0.05$ probabilit	e separate experime y level.	nts. Inter-group co	omparisons were m	ade using one way	r ANOVA. Differei	nt letters in the rov	ws represent signifi	cant differences in

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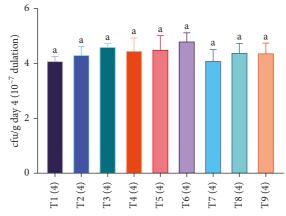
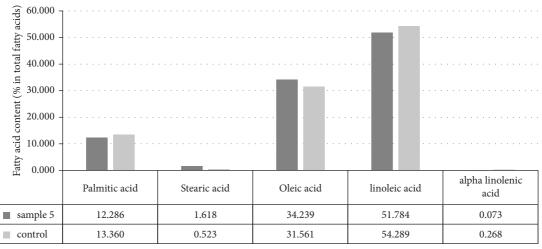


FIGURE 4: Comparison of bacterial survival of quinoa cake treatments after 4 days from baking through pour plate in NYSM agar medium. The cfu/g in quinoa cake treatments on day four were not different significantly from each other at p < 0.05 probability level. The data are expressed as mean ± SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA.

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	T5	8.25	17.2	6.62	5.13	1.23	4.90	2.23	4.00	5.48	2.75	2.67	0.67	0.00	2.41	5.14	3.47	2.68
	control	0.74	1.82	0.51	0.47	2.62	0.32	0.12	0.45	1.04	0.29	0.40	0.12	0.00	0.13	0.29	1.35	0.25

FIGURE 5: Comparison of amino acid profile of treatment No.5 (Selected treatment) with control sample. The amino acid profile was determined through HPLC method. The amounts of most of the essential amino acids were significantly more than control sample.



Fatty acid profile

FIGURE 6: Comparison of fatty acid profile of treatment No.5 (Selected treatment) with control sample. The fatty acid profile was determined through gas chromatography (GC-FID). There was not much positive differences between the amounts of T5 essential fatty acids with control sample.

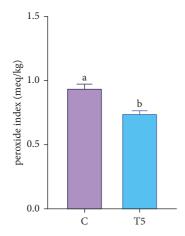


FIGURE 7: Comparison of Peroxide index of treatment No.5 (Selected treatment) with control sample. The data are expressed as mean  $\pm$  SD from at least three separate experiments. Inter-group comparisons were made using one way ANOVA. The different lowercase letters indicate the significant difference of peroxide index in treatments at p < 0.05 probability level.

process had a protective effect on the product [4]. Another reason for this difference could be the antioxidant properties of quinoa due to its polyphenol and plant flavonoids content compared to wheat flour [33]. Quinoa is also an important antioxidant due to its zinc and alpha-tocopherol content [34].

# 4. Conclusion

The results of this study showed that the use of quinoa flour significantly improves the nutritional properties of glutenfree cakes compared to wheat-based cake. Encapsulation of the consumed oil with the resistant starch and maltodextrin and the simultaneous use of inulin as a fat mimic reduced the percentage of oil and sugar consumed and decreased the calories received from this snack. Nutritional properties and its usefulness such as the high percentage of protein, minerals, and fiber can be a good reason for its production for gluten-sensitive patients and people with diabetes, cardiovascular patients, and fans of special foods. Use of Bacillus coagulant probiotic bacteria and inulin prebiotic together along with its health-promoting properties did not have a significant negative effect on the sensory properties of the product. Due to the different percentage of oil content in oil powders used for each treatment, it may be possible to attribute some rheological, physical, and textural behaviors of the resulting cakes to this issue, which requires more studies. This information opens the way for further investigation on the development of gluten-free products. Future studies will be undertaken to determine consumer acceptance among a group of celiac patients.

## **Data Availability**

All the data pertaining to this work have been provided within the text.

#### **Conflicts of Interest**

The authors declare that they do not have any conflicts of interest.

## Acknowledgments

The authors are grateful to Bioran Company for its cooperation in the production of oil powder and also thank Pardis Roshd Mehregan Company for providing the required probiotic bacteria.

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