

## Research Article

# Development of Gluten-Free Cupcakes Enriched with Almond, Flaxseed, and Chickpea Flours

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Received 9 August 2021; Revised 8 November 2021; Accepted 14 May 2022; Published 7 June 2022

Academic Editor: Anis Ali

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**Background.** The mixing of cereals and legumes with nuts and seeds can produce various products that can be optimal for consumption with respect to their nutritional density. The use of legumes in gluten-free foods is becoming very prominent with increase in advancement in food engineering and technology. This study was aimed to develop a gluten-free product (cupcake) enriched with chick pea flour, almonds, and flaxseeds for celiac disease patients. **Materials and Methods.** The procured raw material including almonds, chickpea flour, and flaxseeds was mixed in appropriate amounts, and cupcakes were formulated following a specified recipe. The cupcakes were divided in five groups based on their recipe. First one was wheat based; 2nd one was corn and rice based; and next 3 were chickpea, almond, and flaxseed based, respectively. The cupcakes were checked for their proximate analysis parameters (carbohydrate, protein, and nitrogen-free extract), gluten and heavy metals (lead, chromium, and arsenic), bacterial load (*E. coli*, total coliforms, *salmonella*, yeast, and molds), fatty acid, texture parameters (firmness, cohesiveness, gumminess, and chewiness), and physical parameters (weight, height, volume, and specific volume). All these parameters were compared with wheat-based cupcakes to assess the product's quality. **Results.** The volume of intervention cupcakes was a bit low compared to wheat/rice-based cupcakes but the specific volume was comparable. In texture analysis, firmness, gumminess, and chewiness were enhanced in intervention cupcakes compared to control but cohesiveness was comparable also. The carbohydrate contents were significantly lower in intervention cupcakes 22–30% compared to control cupcakes. Protein, fiber, and fat contents were significantly higher 11–14, 8–10, and 40%, respectively, with improvement (2–3 folds) in calcium, zinc, and iron contents in intervention cupcakes. All the safety parameters including heavy metals and bacterial and fungal load were found normal. The sensory attributes like size and color were found to be less acceptable but odor and taste were comparatively high in formulated products. **Conclusion.** The use of chickpeas, almonds, and flaxseeds in high concentration was first-time used to formulate a recipe of gluten-free cupcake. These cupcakes were more nutritious compared to available conventional recipes. Apparently, no health hazard was found in these cupcakes. Some sensory characteristics of cupcakes were slightly lower and comparable to wheat/rice-based cupcakes.

## 1. Introduction

With the development of economic zones, globalization, and urbanization, the health of majority of the people around the globe have deteriorated with an increase in the burden of noncommunicable diseases (NCDs), that is obesity, cancer, diabetes, stroke, coronary heart diseases, hypertension, etc [1].

Due to modernization and increased nutrition awareness, the mass population has also become more interested in the use of health-benefitting agents in specific functional foods. With the emergence of functional foods concept, the development of foods with various health benefits became prominent [2].

Celiac disease (CD) is condition of chronic inflammatory bowel secondary to the ingestion of gluten peptides [3] with

prevalence of 1-2% around the globe including Middle East and India [4]. The lifelong elimination of gluten from the diet is the only available treatment/management of CD. This elimination can be made by complete restriction of wheat, barley, rye, kamut, triticale (hybrids), and various other hidden/non-food gluten sources. The symptoms and inflammatory markers of intestinal mucosa often do not improve after a prolonged period of time. Compliance to gluten-free diet (GFD) can be very promising in reversion of histological lesions and clinical parameters related to CD [5]. The untreated patients have an increased risk of having non-Hodgkin's lymphoma (4-fold), intestinal adenocarcinoma (30-fold), and ending up in death (1.4-fold) [6]. It is without a doubt that GFD greatly benefits the CD patients in managing the symptoms of disease but it can be a great challenge for the patient to follow it. With increased processing technologies, a lot of foods can have hidden gluten sources and it also limits the person socially and affects the quality of life.

Gluten is made up of gliadin (prolamin) and glutenin (glutelin) [7]. The name gluten is derived from the Latin terminology with the meaning "glue" [8]; when mixed and blended with water, it provides specific three-dimensional properties, which are essential to various baking applications. Due to its binding and viscous nature, it is the most widely used food additive [9]. However, eliminating gluten from the food may decrease the quality of the product. Therefore, with the emergence of advanced technologies, food scientists all over the globe are trying to formulate the gluten-free products with technological/sensory properties (rheological) comparable to their gluten-containing counterparts [10]. In recent years, there has been an increasing interest on GF food products. The market for GF products is projected to grow at a compound annual growth rate (CAGR) of 10.4% from \$4.63 billion in 2015 to reach \$7.59 billion in 2020 [11]. To achieve valuable genetic modulation, blends of foodstuffs offering diverse bioactive constituents will be substantial [12]. Starches, refined flours, and rice are the major components of the gluten free (GF) formula. The majority of available GF products are nutritionally imbalanced with high glycemic indexes (83.3 and 96.1), low protein content, and increased fat [13]. This type of diet may lead to possible nutrient imbalance [14, 15].

The partial replacement of sorghum and chickpea flours is considered as a good source to formulate gluten-free bakery products for celiac patients. A study was designed to assess the effect of sorghum and chickpea substitution on rheological and sensory characteristics of rice flour-based cakes. Sorghum and chickpeas were incorporated at 20, 30, and 40% concentration. Results showed good (23%) crude protein and fiber (4.89%) contents in chickpea-based flour as compared to other flours. Positive trend was noticed in gelatinizing temperature, while viscosity was decreased with increasing sorghum and chickpea flour substitution from 20% to 40%, and no significant difference was observed in specific volume between standard rice cake. Color analysis showed that an increase in lightness with 40% sorghum flour and 20% chickpea flour retained natural yellow color. Acceptability analysis revealed high score of 90.7 and 87.5 from

sorghum and chickpea flour substitution, respectively [16]. Qualitative analysis of cakes (made from chickpea, millet, flaxseed, quinoa, and white rice) including minerals, texture, and sensory profile concluded 3% increased protein content. The concentration of Fe, zinc, and Mg was found to be 26, 20, and 34.2% of daily nutrient intake (DNI), respectively, as compared to control recipe, enabling it to be considered as a source of respective nutrients by hedonic scale confirmed "liked very much" (84% acceptance) [17].

Rice, a very common ingredient in GF formula, is contaminated with an inorganic form of arsenic [18]. There is a need of security and nutritional adequacy in gluten-free diets (GFDs). However, the assessment of nutritional composition of GFDs among adults, adolescents, and children showed unbalanced proportions of various nutrients [19]. Composite flour-based products have great potential to improve the nutritional value such as essential amino acids and carbohydrate contents. Counterfeit natural gluten-free grains are considered as good source of vitamins such as riboflavin, niacin, thiamin, and vitamins C and E [14]. Gluten can be replaced by nuts, legumes, and seeds known as pseudo-cereals (e.g., sorghum, millet, chickpeas, quinoa, amaranth, flaxseeds, and linseeds). Chickpea (*Cicer arietinum*) is a legume belonging to Fabaceae family. It is an important food protein source as well as minerals,  $\beta$ -carotenes, and unsaturated fatty acids. Chickpea protein can provide good blending characteristics to gluten-free baked products to improve the volume [20]. Flaxseeds (*Linum usitatissimum*) belonging to Linaceae family contains approximately 534 kcal, 10.9 g of carbs, and 18.5 g of proteins in 100 g. One tablespoon of flax per day can help to meet daily nutritional requirements aiding in weight loss, good fiber, and good protein along with omega-3 fatty acids which help in lowering lipid level [21].

In Pakistan, available manufactured gluten-free foods are limited. The imported foods are unaffordable and at-least two folds expensive than their regular counterparts. Nutritional quality of GFD can be improved by pseudo-cereal substitution [22]. Cakes are the most consumed bakery product and increasing worldwide by about 1.5% per year [23]. Therefore, it is a need of time to develop nutrient-rich GF foods from locally available natural resources. Based on combinations of almond, flaxseed, chickpea, sorghum, and guar gum flours, this study was conducted to formulate gluten-free cupcakes that are low in carbohydrates, high in protein, and dense in calories and to evaluate their nutritional and safety properties as well as their taste as compared to gluten-containing cupcakes. If acceptability, availability, and affordability of gluten-free foods will be improved, it will ensure the compliance with a gluten-free diet.

## 2. Materials and Methods

This study was a product development trial specially designed for CD focusing on nutrient density and gluten absence, and all the nutritional, food safety, and qualitative trials were performed to ensure the quality and safety of the product.

**2.1. Procurement of Raw Material.** Sorghum, chickpeas, flaxseeds, and almonds rich in healthy nutrients were purchased from the local market. All these ingredients were ground into fine powder using uncontaminated grinder. Different flour blends were prepared by substituting wheat flour with sorghum, chickpea, flaxseed, and almond flours at different proportions.

**2.2. Formulation for Flour Blends.** Formulation for cupcake flour blends (Table 1) is adjusted to provide >20% of daily value of nutrient requirements of target population [24].

**2.3. Preparation of Nutrient-Enriched Cupcakes.** Before preparing the dough, the surrounding temperature of the laboratory was adjusted to  $24 \pm 2^\circ\text{C}$  via air conditioner (AACC, 2002).

- (i) The eggs were beaten for 2 minutes and then mixed with other ingredients, that is butter and milk.
- (ii) Afterwards, the dry ingredients, for example flour, honey, and baking powder, were mixed in a separate bowl.
- (iii) The cupcake batter was prepared by combining the mixture of first two steps.
- (iv) Butter paper was placed in cupcake pans and then batter was poured.
- (v) Each pan filled with 1/2 to 2/3 of batter.
- (vi) Afterwards, pans were placed in the preheated oven ( $270^\circ\text{C}$  for 30 min.) and baked at  $175^\circ\text{C}$  for 25–30 minutes.
- (vii) After baking, cupcakes were cooled at room temperature.

TABLE 1: Formulation and ingredients of various gluten-free cupcakes and control doughs.

Ingredients	T0	T1	T2	T3	T4
Wheat flour	100	0	0	0	0
Rice flour	0	75	0	0	0
Corn starch	0	25	0	0	0
Almond flour	0	0	60	48	40
Flaxseed flour	0	0	15	17	15
Chickpea	0	0	20	27	40
Sorghum	0	0	5	8	5
Guar gum	0	0.1	0.1	0.1	0.1

- (viii) For later use, cupcakes were packaged in polyethylene bags and stored at appropriate temperature.

#### 2.4. Various Quality and Safety Analysis of Cupcakes

**2.4.1. Gluten Test.** The gluten fraction in product was determined by the method of AOAC 2012. According to Codex Alimentarius Standards, it should be <20 ppm in gluten-free products [25].

**2.4.2. Moisture Content.** The moisture content of the cupcakes was determined by an electric drying oven (Type-DL-102A, Serial No. 0378, China). A sample of 3 g was weighed properly and was taken in clean, dried china dish and then kept in hot air oven for 24 hours. It was then cooled in desiccator for half an hour and weighed till constant weight is obtained.

$$\text{Moisture (\%)} = \left[ \left\{ \frac{(\text{weight of original sample} - \text{weight of dried sample})}{\text{weight of original sample}} \right\} \times 100 \right]. \quad (1)$$

**2.4.3. Crude Protein.** The protein contents in the cupcakes were determined using Kjeltac Apparatus (Model: D-40599, Behr Labor Technik GmbH, Germany) following the method as given in AOAC (2006). The sample (2 g) was first digested with 25 ml concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) and 1 digestion tablet. The apparatus was then settled and started and was left was 190 min or until light green color appeared. The

mixture is then taken out and diluted to make a total volume of 250 ml. Then, 10 ml of diluted material and 10 ml of 40% NaOH in were taken in a distillation apparatus. The ammonia obtained was collected in 4% boric acid solution containing methyl red indicator. Finally, the distillate was titrated using 0.1 N HCl solution until light pink color is obtained. The nitrogen content was calculated by the following formula:

$$\text{Nitrogen (\%)} = \left[ \left\{ \frac{(\text{volume of H}_2\text{SO}_4 \times 0.0014 \times 250)}{(\text{weight of sample} \times \text{volume of sample taken})} \right\} \times 100 \right]. \quad (2)$$

The crude protein percentage was determined by multiplying nitrogen with a factor 6.25, that is crude protein = nitrogen (%)  $\times$  6.25.

**2.4.4. Crude Fat.** The crude fat content was determined by using a Soxhlet following the method described in AOAC (2006). A filter paper-covered moisture-free sample was

placed in the Soxhlet apparatus (Model: HT2 1045 Extraction Unit, Hoganas, Sweden). The samples were run for 2 hours, and the fat was collected in the apparatus flask. Extra n-hexane was evaporated by adding the fat extract in drying oven. The crude fat was calculated using the following formula:

$$\text{Crude fat (\%)} = \left( \frac{\text{weigh of fat in sample}}{\text{weight of sample}} \right) \times 100. \quad (3)$$

**2.4.5. Crude Fiber.** The crude fiber was determined following the method as described in AOAC (2000). Cupcake sample after fat extraction was tested for crude fiber content. Only 2 g of fat-free sample was tested for crude fiber and digested with 200 ml of boiling with 1.25% H<sub>2</sub>SO<sub>4</sub> for 30 minutes, filtered through muslin, and washed thrice. The sample was again digested with 200 ml of boiling 1.25% NaOH for 30 minutes, filtered, and washed thrice. The resultant residue was then dried at 105°C for 2 hours and weighed. Dried residue was ignited at 600 ± 15°C, cooled, and reweighed. The crude fiber was calculated by the following formula:

$$\text{Carbohydrates (\%)} = [100 - \{\text{Moisture (\%)} + \text{Ash (\%)} + \text{Protein (\%)} + \text{Fiber (\%)} + \text{Fat (\%)}\}]. \quad (6)$$

**2.4.8. Mineral Analysis.** Mineral analysis was performed by digesting the samples followed by quantifying each mineral through injecting the sample in an atomic absorption spectrophotometer using calibrated standards of CRMC [26].

**2.4.9. Heavy Metals.** Products were analyzed for heavy metals by digesting the samples followed by quantifying each mineral through injecting the sample in an atomic absorption spectrophotometer [27]. According to Food and Drug Authority (FDA), lead should be <0.2 ppm.

**2.4.10. Texture Analysis.** The texture of cupcakes was measured using texture analyzer (Model TA-XT 2i) with software texture expert exceed (version 2.61).

**2.4.11. Storage Study.** The expiry of the preferred formulation was evaluated by storing it in polyethylene bags until physical deterioration of the product, and changes in sensory characteristics were studied during storage period at different intervals. Total plate count (TPC) was performed by using MFHPB-18 and it should be <50,000 cfu/g. Total coliforms should be <100 cfu/g, while yeast and mold should be <500 cfu/g.

**2.4.12. Sensory Evaluation.** Sensory evaluation through organoleptic tests conducted by experts is necessary to ensure the reliability of the product on small scale before

$$\text{Crude fiber (\%)} = \left( \frac{\text{weigh after ignition}}{\text{weight of sample}} \right) \times 100. \quad (4)$$

**2.4.6. Ash Content.** The ash content of cupcakes was determined following the method as described in AOAC (2004) in a muffle furnace (Gallenham, England). The weighed amount of sample is first charred to make the sample smoke free. Incineration of a weighed amount of a sample was done in a muffle furnace at 550°C for 5 hours until white ash was obtained. It is calculated by the following formula:

$$\text{Ash (\%)} = \left( \frac{\text{weight of ash}}{\text{weight of sample}} \right) \times 100. \quad (5)$$

**2.4.7. Carbohydrates.** Carbohydrate content in the product was determined by difference according to AOAC (2004) method with the following equation:

giving any product in market and to make adjustments in product if required.

#### 2.4.13. Procedure

- (i) Sensory evaluation of selected attributes of products was conducted through a 10-member panel with previous experience in food products evaluation.
- (ii) All evaluations were conducted under white incandescent light at room temperature (24 ± 1°C).
- (iii) The samples were presented in random order, and panelists were asked not to disturb the order of presentation.
- (iv) A 9-point hedonic scale was used to evaluate the product
- (v) On the day of evaluation, all the products were placed in covered disposable plates labeled with 3-digit random codes and were kept at room temperature before serving to the panelists.
- (vi) Panelists were provided with distilled water to clean their mouths between samples.
- (vii) Samples were presented in random order, and panelists were asked to rate their likeness for various sensory parameters of consumer acceptability on a 1 to 9 hedonic scale (1 = dislike a lot and 9 = like a lot
- (viii) The assessed attributes by difference test included appearance (surface color, crumb color, surface

smoothness, volume, air cell homogeneity), flavor (off flavor), taste (sweet), and texture (hardness, adhesiveness, springiness, chewiness, moistness, softness) (AOAC, 2000).

**2.5. Statistical Analysis.** Data obtained from the study were analyzed using statistical package for social sciences (SPSS) and expressed as mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was applied to assess any significant change in study parameters, and Duncan's multiple range test was applied to assess the groups' inter difference. The level of significance was 5%.

### 3. Results

This study was carried out to formulate novel gluten-free cupcakes with innovative recipe having nutrient density and other functional and nutraceutical properties. For this purpose, various doughs were made and were analyzed for their physical properties, texture analysis, proximate composition, safety analysis, and mineral composition. Microbiological and fatty acid analyses were also performed to ensure safety and nutrient density of gluten-free cupcakes.

Table 2 explains the physical properties of various gluten-free cupcakes as well as control cupcakes with 100% wheat flour (Table 2). There was no significant difference in volume and specific volume between controls *T0* and *T1* cupcake samples, while *T2*, *T3*, and *T4* samples have comparatively low volume and specific volume.

Table 3 elaborates the texture analysis of various gluten-free cupcakes as well as control cupcakes with 100% wheat flour. Maximum firmness was found in *T2* (8.630) and minimum firmness was reported in *T0* (4.53) which was for control cupcakes. A high level of cohesiveness was found in *T1* (1.58) with minimum cohesiveness reported in *T4* (0.74). Gumminess was maximum in *T3* (9.31), while *T0* (3.62) had the least gummy cupcakes. Maximum chewiness was reported in *T3* (4.47) with minimum chewiness in *T0* (1.79). Firmness, cohesiveness, and chewiness were reduced in *T4*. Overall, the treatment cupcakes were better than control cupcakes in texture analysis (Table 3).

Table 4 explains the proximate analysis of multiple gluten-free cupcakes compared to control. *T1* (81.09%) was highest in dry matter and *T0* (73.92%) had lowest dry matter compared to all other treatments. On the other hand, maximum moisture was found in *T0* (26.08%) and minimum moisture was in *T1* (18.91%). *T1* (57.68%) was highest among the carbohydrate content and *T3* (22.68%) was lowest among the carbohydrates. *T0* (4.25%) had the lowest amount of proteins, while *T4* (14.64%) had the highest amount of proteins. Maximum fiber was found in *T4* (11.27%) and minimum fiber was present in *T1* (1.23%). Maximum fats were reported in *T4* (31.15%) and minimum were reported in *T0* (14.45%). Maximum ash contents were present in *T4* (2.53%) and minimum amount of ash was in *T1* (0.97%) (Table 4).

Table 5 elaborates the mineral composition of gluten-free cupcakes; only the treatment cupcakes were involved in this

TABLE 2: Physical properties of various gluten-free cupcakes and control.

Groups	Weight	Height	Volume	Specific Volume
<i>T0</i>	38.83 <sup>b</sup> $\pm$ 0.76	4.55 <sup>a</sup> $\pm$ 0.05	101.7 <sup>a</sup> $\pm$ 0.92	3.53 $\pm$ 1.66
<i>T1</i>	38.67 <sup>b</sup> $\pm$ 0.58	4.28 <sup>bc</sup> $\pm$ 0.03	94.7 <sup>b</sup> $\pm$ 0.56	2.45 $\pm$ 0.05
<i>T2</i>	40.17 <sup>a</sup> $\pm$ 0.29	4.32 <sup>b</sup> $\pm$ 0.03	89.17 <sup>d</sup> $\pm$ 0.38	2.41 $\pm$ 0.15
<i>T3</i>	40.33 <sup>a</sup> $\pm$ 0.58	4.18 <sup>c</sup> $\pm$ 0.08	81 <sup>c</sup> $\pm$ 0.56	2.07 $\pm$ 0.04
<i>T4</i>	38.5 <sup>b</sup> $\pm$ 0.87	4.38 <sup>b</sup> $\pm$ 0.08	91.23 <sup>c</sup> $\pm$ 0.61	2.35 $\pm$ 0.1
<i>p</i> value	0.013*	0.00*	0.00*	0.229

TABLE 3: Texture analysis of various gluten-free cupcakes and control.

Groups	Firmness	Cohesiveness	Gumminess	Chewiness
<i>T0</i>	4.53 <sup>c</sup> $\pm$ 0.03	0.89 <sup>d</sup> $\pm$ 0.01	3.62 <sup>c</sup> $\pm$ 0.03	1.79 <sup>c</sup> $\pm$ 0.02
<i>T1</i>	5.45 <sup>d</sup> $\pm$ 0.01	1.58 <sup>a</sup> $\pm$ 0.01	4.71 <sup>d</sup> $\pm$ 0.01	2.94 <sup>d</sup> $\pm$ 0.01
<i>T2</i>	8.63 <sup>b</sup> $\pm$ 0.02	1.12 <sup>c</sup> $\pm$ 0.02	7.47 <sup>b</sup> $\pm$ 0.01	3.25 <sup>b</sup> $\pm$ 0.03
<i>T3</i>	10.8 <sup>a</sup> $\pm$ 0.01	1.25 <sup>b</sup> $\pm$ 0.02	9.3 <sup>a</sup> $\pm$ 0.02	4.47 <sup>a</sup> $\pm$ 0.01
<i>T4</i>	8.12 <sup>c</sup> $\pm$ 0.01	1.11 <sup>c</sup> $\pm$ 0.01	7.32 <sup>c</sup> $\pm$ 0.01	3.17 <sup>c</sup> $\pm$ 0.01
<i>p</i> value	0.00*	0.00*	0.00*	0.00*

analysis, and wheat cupcakes (*T0*) and conventional recipe cupcakes were excluded (*T1*). The maximum level of calcium was found in *T2* (250 mg/100 grams) and the minimum amount of calcium was found in *T3* (175 mg/100 grams). Potassium level was maximum in *T4* (214 mg/100 grams), and *T2* (169 mg/100 grams) had the least amount of potassium. The maximum level of zinc was found in *T2* (4.62), and the lowest amount of zinc was found in *T4* (2.13). *T2* (18.7) had the maximum amount of iron (Fe), and *T4* (15.9) had the least amount of iron (Fe). Same trend was observed in the case of magnesium.

Table 6 elaborates the safety analysis of various gluten-free cupcakes and control involving amount of gluten and heavy metals. No gluten and heavy metals were found in any treatment except *T2* had a very low amount of chromium 0.02 mg/L (Table 6).

Table 7 elaborates sensory evaluation responses recorded at the baseline for all the gluten-free and control cupcakes. Crust color was highly rated in *T0* compared to all other cupcakes except *T1*. Thickness was significantly higher in *T0*, *T1*, and *T4* compared to all other groups. Size score of *T0* was higher to all other cupcakes. Uniformity was significantly higher in *T0* and *T1* compared to all other treatment cupcakes. Color of the cupcake was scored highest in *T0* and *T1* compared to all other groups. Softness was scored highest among *T0*, *T1*, and *T4* compared to all other groups. Tenderness was increased in *T2*–*T4* compared to *T0* and *T1*. Odor scores of *T0*, *T1*, and *T4* were comparable to other groups in sensory evaluation.

The microbiological analysis of gluten-free cupcakes was performed at 9<sup>th</sup> and 17<sup>th</sup> day of storage, and only *T4* group's analysis was performed as it was selected for efficiency trials. Only molds were found on the 17<sup>th</sup> day, otherwise no other microbial growth was detected in stored sample (Table 8).

TABLE 4: Proximate analysis of various gluten-free cupcakes and control.

Groups	Dry matter	Moisture	Carbohydrates	Proteins	Fiber	Fats	Ash	Calories
T0	74 <sup>d</sup> ± 1.38	26.15 <sup>a</sup> ± 1.38	44.64 <sup>b</sup> ± 1.38	4.33 <sup>c</sup> ± 1.38	2.09 <sup>b</sup> ± 1.38	23.53 <sup>c</sup> ± 1.38	1.68 ± 1.38	386.83 <sup>d</sup> ± 3.82
T1	81.06 <sup>a</sup> ± 1.53	18.89 <sup>d</sup> ± 1.53	54.65 <sup>a</sup> ± 1.53	3.92 <sup>c</sup> ± 1.53	1.21 <sup>b</sup> ± 1.53	21.4 <sup>c</sup> ± 1.53	0.95 ± 1.53	402.83 <sup>c</sup> ± 3.82
T2	77.41 <sup>bc</sup> ± 1.38	21.74 <sup>bc</sup> ± 1.38	22.75 <sup>d</sup> ± 1.38	13.68 <sup>ab</sup> ± 1.38	9.88 <sup>a</sup> ± 1.38	39.74 <sup>a</sup> ± 1.38	2.1 ± 1.38	483.5 <sup>a</sup> ± 4.82
T3	76.96 <sup>c</sup> ± 1.12	23.19 <sup>b</sup> ± 1.32	25.18 <sup>d</sup> ± 1.38	11.51 <sup>b</sup> ± 1.18	10.52 <sup>a</sup> ± 1.38	38.51 <sup>a</sup> ± 1.48	2.02 ± 1.38	468 <sup>b</sup> ± 5
T4	79.78 <sup>ab</sup> ± 1.28	20.25 <sup>cd</sup> ± 1.28	30.49 <sup>c</sup> ± 1.28	14.66 <sup>a</sup> ± 1.28	8.29 <sup>a</sup> ± 1.28	34.17 <sup>b</sup> ± 1.28	2.55 ± 1.28	476 <sup>ab</sup> ± 5
<i>p</i> value	0.01*	0.01*	0.00*	0.00*	0.00*	0.00*	0.70	0.00*

TABLE 5: Mineral analysis of various gluten-free cupcakes and control (mg/100 grams).

Groups	Sodium	Calcium	Potassium	Zinc	Iron	Magnesium
T0	185.99 <sup>c</sup> ± 0.07	108.29 <sup>d</sup> ± 0.07	295.49 <sup>a</sup> ± 0.07	2.94 <sup>c</sup> ± 0.06	5.4 <sup>d</sup> ± 0.07	32.19 <sup>a</sup> ± 0.07
T1	196.19 <sup>b</sup> ± 0.07	96.69 <sup>c</sup> ± 0.07	156.29 <sup>c</sup> ± 0.06	1.33 <sup>c</sup> ± 0.07	2.63 <sup>c</sup> ± 0.07	24.69 <sup>d</sup> ± 0.07
T2	171.33 <sup>d</sup> ± 0.08	250 <sup>a</sup> ± 0.07	169 <sup>d</sup> ± 0.08	4.62 <sup>a</sup> ± 0.08	18.6 <sup>a</sup> ± 0.08	31.86 <sup>a</sup> ± 0.09
T3	131.99 <sup>e</sup> ± 0.07	174.99 <sup>c</sup> ± 0.07	206.99 <sup>c</sup> ± 0.07	3.12 <sup>b</sup> ± 0.08	17.29 <sup>b</sup> ± 0.07	27.41 <sup>c</sup> ± 0.08
T4	287.5 <sup>a</sup> ± 0.08	208.5 <sup>b</sup> ± 0.08	213.6 <sup>b</sup> ± 0.08	2.12 <sup>d</sup> ± 0.08	15.76 <sup>c</sup> ± 0.08	20.66 <sup>c</sup> ± 0.08
<i>p</i> value	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 6: Safety analysis of various gluten-free cupcakes and control (mg/L).

Safety Analysis	T1	T2	T3	T4
Gluten	nd	nd	nd	nd
Lead	—	—	—	—
Chromium	—	0.02	—	—
Arsenic	—	—	—	—

\*nd = not detected.

TABLE 7: Sensory evaluation of various gluten-free cupcakes and control (baseline).

Sensory evaluation	T0 mean ± S.D	T1 mean ± S.D	T2 mean ± S.D	T3 mean ± S.D	T4 mean ± S.D	<i>p</i> value
Crust color	8 <sup>a</sup> ± 0.8	7.6 <sup>ab</sup> ± 0.5	6.1 <sup>d</sup> ± 0.9	6.4 <sup>cd</sup> ± 0.5	7 <sup>bc</sup> ± 0.8	≤0.01*
Thickness	8 <sup>a</sup> ± 0.7	8 <sup>a</sup> ± 0.8	6.5 <sup>b</sup> ± 0.8	6.6 <sup>b</sup> ± 0.7	7.3 <sup>a</sup> ± 0.7	≤0.01*
Size	8.6 <sup>a</sup> ± 0.5	7.7 <sup>b</sup> ± 0.5	6.2 <sup>c</sup> ± 0.9	6.1 <sup>c</sup> ± 0.7	7.5 <sup>b</sup> ± 0.5	≤0.01*
Uniformity	8.4 <sup>a</sup> ± 0.5	8.3 <sup>a</sup> ± 0.7	7 <sup>c</sup> ± 0.7	7.1 <sup>c</sup> ± 0.7	7.6 <sup>b</sup> ± 0.5	≤0.01*
Color	8.3 <sup>a</sup> ± 0.7	7.8 <sup>ab</sup> ± 0.4	6.6 <sup>c</sup> ± 0.5	6.7 <sup>c</sup> ± 0.5	7.3 <sup>b</sup> ± 0.7	≤0.01*
Softness	7.1 <sup>ab</sup> ± 0.6	7.2 <sup>ab</sup> ± 0.6	6.5 <sup>b</sup> ± 0.8	6.6 <sup>b</sup> ± 1.2	7.8 <sup>a</sup> ± 0.8	0.08*
Tenderness	6.7 <sup>b</sup> ± 0.5	6.5 <sup>b</sup> ± 0.5	7.4 <sup>a</sup> ± 0.5	7.3 <sup>a</sup> ± 0.5	7.5 <sup>a</sup> ± 0.5	≤0.01*
Moistness	6.5 ± 0.5	6.5 ± 0.5	6.6 ± 1.1	7.1 ± 0.7	7.1 ± 0.9	0.19
Odor	7.1 <sup>ab</sup> ± 0.6	7.1 <sup>ab</sup> ± 0.3	6.4 <sup>c</sup> ± 0.5	6.7 <sup>bc</sup> ± 0.7	7.6 <sup>a</sup> ± 0.5	≤0.01*
Taste	6.9 <sup>b</sup> ± 0.9	6.5 <sup>b</sup> ± 0.5	6.4 <sup>b</sup> ± 0.7	6.6 <sup>b</sup> ± 0.5	8 <sup>a</sup> ± 0.8	≤0.01*

Data presented as mean ± SD, \*significant difference, one-way ANOVA, level of significance (0.05), All the superscripts in a row represents significantly different groups.

TABLE 8: Microbiological analysis of various gluten-free cupcakes (T4 only).

Microbiological Analysis (cfu/g)	Day 9	Day 17
<i>E. Coli</i>	nd	—
Total coliforms	—	—
<i>Salmonella</i>	—	—
Yeast	—	—
Molds	—	2.13

\*nd = not detected.

3.1. Experiment 2. Table 9 elaborates the scores of sensory evaluations at various intervals of storage study. At the baseline, T4 had significant scores compared to T2 and T3 on

majority of sensory evaluation parameters such as crust color, thickness, size, color, softness, odor, and taste. Same trend was observed on days 7 and 14 of sensory evacuation (Table 9). No difference was observed in uniformity, tenderness, and moistness of the prepared samples, while size and crust color scores were significantly reduced ( $p < 0.05$ ).

#### 4. Discussion

The gluten-free diet is the only possible management strategy for the CD, and all the gluten-containing foods and food products should be restricted for lifelong in this regard. But the main problem with the gluten-free diet is that it lacks many essential nutrients and long-term adherence can result

TABLE 9: Sensory evaluation of various gluten-free cupcakes and control (at various intervals).

Sensory Evaluation	Day 0				Day 7				Day 14			
	T2	T3	T4	p value	T2	T3	T4	p value	T2	T3	T4	p value
	mean $\pm$ S.D	mean $\pm$ S.D	mean $\pm$ S.D		mean $\pm$ S.D	mean $\pm$ S.D	mean $\pm$ S.D		mean $\pm$ S.D	mean $\pm$ S.D	mean $\pm$ S.D	
Crust color	6.1 <sup>b</sup> $\pm$ 0.9	6.4 <sup>ab</sup> $\pm$ 0.5	7 <sup>a</sup> $\pm$ 0.8	0.038*	6.2 $\pm$ 0.8	6.2 $\pm$ 0.8	6.9 $\pm$ 0.7	0.083	6.2 $\pm$ 0.8	6.2 $\pm$ 0.8	6.9 $\pm$ 0.7	0.083
Thickness	6.5 <sup>b</sup> $\pm$ 0.8	6.6 <sup>b</sup> $\pm$ 0.7	7.3 <sup>a</sup> $\pm$ 0.7	0.047*	6.5 $\pm$ 0.8	6.4 $\pm$ 0.8	7.2 $\pm$ 0.6	0.061	6.4 $\pm$ 0.7	6.4 $\pm$ 0.8	7.1 $\pm$ 0.6	0.056
Size	6.2 <sup>b</sup> $\pm$ 0.9	6.1 <sup>b</sup> $\pm$ 0.7	7.5 <sup>a</sup> $\pm$ 0.5	$\leq$ 0.01*	6.2 <sup>b</sup> $\pm$ 0.8	5.9 <sup>b</sup> $\pm$ 0.7	7.4 <sup>a</sup> $\pm$ 0.5	$\leq$ 0.01*	6.2 <sup>b</sup> $\pm$ 0.8	5.8 <sup>b</sup> $\pm$ 0.8	7.3 <sup>a</sup> $\pm$ 0.5	$\leq$ 0.01*
Uniformity	7 $\pm$ 0.7	7.1 $\pm$ 0.7	7.6 $\pm$ 0.5	0.104	6.9 $\pm$ 0.7	7.2 $\pm$ 0.6	7.6 $\pm$ 0.5	0.064	6.9 <sup>b</sup> $\pm$ 0.7	7.1 <sup>b</sup> $\pm$ 0.6	7.6 <sup>a</sup> $\pm$ 0.5	0.047*
Color	6.6 <sup>b</sup> $\pm$ 0.5	6.7 <sup>b</sup> $\pm$ 0.5	7.3 <sup>a</sup> $\pm$ 0.7	0.021*	6.7 $\pm$ 0.7	6.7 $\pm$ 0.5	7.3 $\pm$ 0.7	0.059	6.7 $\pm$ 0.7	6.6 $\pm$ 0.5	7.1 $\pm$ 0.7	0.209
Softness	6.5 <sup>b</sup> $\pm$ 0.8	6.6 <sup>b</sup> $\pm$ 1.2	7.8 <sup>a</sup> $\pm$ 0.8	0.008*	6.5 <sup>b</sup> $\pm$ 0.8	6.5 <sup>b</sup> $\pm$ 1.1	7.8 <sup>a</sup> $\pm$ 0.8	0.004*	6.5 <sup>b</sup> $\pm$ 0.8	6.5 <sup>b</sup> $\pm$ 1.1	7.8 <sup>a</sup> $\pm$ 0.8	0.04*
Tenderness	7.4 $\pm$ 0.5	7.3 $\pm$ 0.5	7.5 $\pm$ 0.5	0.684	7.4 $\pm$ 0.5	7.4 $\pm$ 0.5	7.6 $\pm$ 0.5	0.612	7.3 $\pm$ 0.7	7.4 $\pm$ 0.5	7.5 $\pm$ 0.5	0.743
Moistness	6.6 $\pm$ 1.1	7.1 $\pm$ 0.7	7.1 $\pm$ 0.9	0.376	6.7 $\pm$ 0.9	7.1 $\pm$ 0.7	7.1 $\pm$ 0.9	0.494	6.7 $\pm$ 0.9	7.1 $\pm$ 0.7	6.9 $\pm$ 0.7	0.554
Odor	6.4 <sup>b</sup> $\pm$ 0.5	6.7 <sup>b</sup> $\pm$ 0.7	7.6 <sup>a</sup> $\pm$ 0.5	$\leq$ 0.01*	6.5 <sup>b</sup> $\pm$ 0.5	6.7 <sup>b</sup> $\pm$ 0.7	7.8 <sup>a</sup> $\pm$ 0.6	$\leq$ 0.01*	6.7 <sup>b</sup> $\pm$ 0.7	6.7 <sup>b</sup> $\pm$ 0.7	7.7 <sup>a</sup> $\pm$ 0.7	0.03*
Taste	6.4b $\pm$ 0.7	6.6 <sup>b</sup> $\pm$ 0.5	8 <sup>a</sup> $\pm$ 0.8	$\leq$ 0.01*	6.5 <sup>b</sup> $\pm$ 0.7	6.7 <sup>b</sup> $\pm$ 0.7	7.9 <sup>a</sup> $\pm$ 0.7	$\leq$ 0.01*	6.6 <sup>b</sup> $\pm$ 0.8	6.7 <sup>b</sup> $\pm$ 0.7	7.9 <sup>a</sup> $\pm$ 0.7	0.01*

Data presented as mean  $\pm$  SD. \*significant difference, one-way ANOVA, level of significance (0.05). All the superscripts in a row represents significantly different groups.

in various nutritional ailments. To avoid these consequences, nutrient dense and an acceptable product based on sensory properties should be introduced instead of typical gluten-free products. The objective of this study was to formulate gluten-free cupcakes enriched with chickpea, flaxseed, and almond flours. The study also aimed to explore the nutritional and sensory characteristics of these cupcakes in addition to various rheological properties.

Gluten basically plays an important role in binding for every baking product. It has unique baking properties as the glutenin are characterized for having mixing and elastic property, whereas gliadins play role in softening and giving viscosity to the product [28]. Novel gluten-free products are not easy to make considering the lacking of major ingredient required for proper making, some technical hurdles and organoleptic properties [29–31]. Gluten can be replaced by nuts, legumes, and seeds known as pseudo-cereals (e.g., sorghum, millet, chickpeas, quinoa, amaranth, flaxseeds, and linseeds).

The conventional gluten-free products are deficient in multiple vitamins and minerals, and following a strict GFD regime can alter the distributive proportion of macronutrients (carbohydrates, proteins, and fats), which can also result in obesity and many other NCDs [32]. In the study, three different categories including legumes (chickpeas), seeds (flaxseeds), and nuts (almonds) in combination were used for the first time to prepare gluten-free cupcakes. The proximate analysis of three different cupcakes showed similar moisture content between wheat flour and gluten-free cupcakes except rice flour *T1*, while carbohydrate (CHO) contents were  $22.67 \pm 0.02$ – $30.47 \pm 0.01$  with improvement in caloric content ( $T2, 480 \pm 3.85$ ) which are comparable with conventional cupcakes and many other available gluten-free recipes. The study conducted to make red kidney bean cupcakes reported 48% of carbohydrates with 27% of sugar [33]. In another study, different gluten-free products were prepared from composite flour comprised of  $49.45 \pm 2.9$ – $81.63 \pm 2.3$  CHO [34]. Defatted almond flour incorporation reduced CHO content in cakes  $46.8$  g/100 g as compared to wheat flour cupcakes  $67.3$  g/100 g [35]. The cupcakes formulated in this study are far healthier than the aforementioned ones.

On dry basis, the total replacement of wheat flour with gluten-free rice, almond, chickpea, and flaxseed flours improved the protein content of cupcakes 2–3 folds  $4.25 \pm 0.01$  in standard to  $14.64 \pm 0.01$  in experimental product. Similarly, fiber and ash content increased from  $2.01 \pm 0.01$  (*T0*) to  $10.44 \pm 0.005$  of *T3* and  $1.6 \pm 0.01$ , *T0* to  $2.53 \pm 0.01$  of *T4*. The values of fiber, ash, and protein content of gluten-free cupcakes were found to be in close agreement to that reported earlier by the addition of flaxseed in wheat flour. Fat content also increased with the addition of flaxseed and almond flour by 2 folds as in *T0*,  $23.45 \pm 0.01$ , to *T2* and  $39.66 \pm 0.00$ . Significant increase ( $p < 0.05$ , 59.9%) in lipids was observed in cakes prepared with almond flour [35, 36].

The use of cereals (rice, corn, and sorghum), minor cereals (teff, pearl millet, and jungle rice), pseudo-cereals (amaranth, buckwheat, and quinoa), and legumes (chickpea, lentil, soy-bean, and pea) has been documented in literature

for the development of gluten-free products [37]. In this study, prepared gluten-free cupcakes were comparable with standard wheat flour and rice flour-based cupcakes. Data regarding physical properties showed that incorporation of flaxseeds, chickpeas, and almonds effected weight and height of cupcakes. Gluten-free flours have poor water binding capacity and results in weight loss during baking as 38 g, 40 g, and 39 g for *T0*, *T3*, and *T4*, respectively. Similar trend was observed in gluten-free muffins baked with different flax concentrations, that is control 47 g, then decrease with the addition of gluten-free flour up to 39 g and further addition raised weight to 40 g. This inclination may be resulted from high crude fiber content in chickpea flour due to its water holding capacity [38].

The height of cupcake *T2* containing 20% of chickpea, 15% of flaxseed, and 60% of almond flours was in accordance with standard gluten-free cupcakes (4.3 cm), while *T4* (40% chickpea, 15% flaxseed, and 40% almond) was slightly higher 4.4 cm as compared to *T3* (4.1 cm). The height of cupcakes decreased as flaxseed, chickpea, and almond flour concentration increased. This decrease may be attributed to lesser air cells and poor capacity of baked items for the retention of air bubbles. As expected, highest volume was observed in wheat flour cupcakes  $100.9$  cm<sup>3</sup> followed by rice flour-based gluten-free cupcakes  $94.1$  cm<sup>3</sup>, then by 20%–40% chickpea flour in gluten-free cupcakes  $89.0$ – $91.08$  cm<sup>3</sup>. These results are in agreement with Herranz, et al., [39] as they reported lower expansion in cake volume with increasing chickpea level in flour. In this study, gluten free in combination showed similarity with wheat-based control cupcakes as specific volume for *T4* was  $2.36$  cm<sup>3</sup>/g near to *T0*  $2.62$  cm<sup>3</sup>/g. This increasing specific volume results are in harmony with 70% chickpea flour replacement for gluten-free mix [40] and 15% chickpea flour substitution in previous studies [41].

Hardness for the control cupcake was  $4.53 \pm 0.02$  N, and it increased with the addition of varying amount of flaxseed, chickpea, and almond flours from  $8.12 \pm 0.00$ ,  $8.63 \pm 0.01$ , and  $10.8 \pm 0.01$  for *T4*, *T2*, and *T3*, respectively. Hardness is inversely related to its volume. Fiber-enriched food upon dilution of gluten fraction provides harder texture and higher water absorption [42]. Cohesiveness for the control cupcake was  $0.89 \pm 0.01$  which increased up to  $1.58 \pm 0.01$  in *T1* gluten-free cakes but decrease in *T3* ( $1.25 \pm 0.01$ ), *T2* ( $1.12 \pm 0.01$ ), and *T4* ( $1.11 \pm 0.02$ ). This pattern is in accordance with similar studies which reported significant decrease ( $p < 0.05$ ) in cohesiveness with flaxseed (0.36–0.32) and almond substitution (0.6–0.4) [36]. Batter viscosity can be increased by soluble fibers which promotes starch-lipid and starch-protein complexes for batter stability during baking. Flaxseeds or gums form strong gel system as compared to rice flour [43]. Protein isolates decrease breakdown viscosity providing more starch granules and stability to product.

According to the nutrient database of the United States Department of Agriculture (USDA), chickpea, almond, and flaxseed flours are rich in proteins, essential fats, and many other vital micronutrients including calcium, iron, zinc, vitamin C, B vitamins, folic acid, and vitamin K [44–46]. The formulated products were found to be enriched with zinc,



iron, and multiple other minerals. Calcium (Ca<sup>+</sup>) concentration was high ( $250 \pm 1.00$ ) in sample containing maximum almond flour ( $T_2$ , 60%), while rice flour-based conventional gluten-free cupcakes exhibited lower Ca<sup>+</sup>,  $96.7 \pm 1.03$ .  $T_2$  was found to be rich in iron and zinc (Zn)  $18.6 \pm 0.1$  and  $4.62 \pm 0.01$ , respectively. On average, nutrient profile of  $T_2$  was good among all samples and can be labeled as high according to estimated average requirements (EAR). Micronutrient improvement (mild) with the addition of nuts, legumes, seeds, and cereals was also observed in previous studies, Ca<sup>+</sup> 170 mg/100 g, iron 6.88 mg/100 g [35]. This nutrient density can help to correct various nutritional deficiencies of CD patients and can also delay the progression of various NCD secondary to disturbed nutrients distribution and will reduce the use of refined ingredients [47].

Total plate count of gluten-free cupcakes was determined at days 9 and 17 at 30°C under stationary conditions. No total plate count was detected on all samples. Only molds 2.13 cfu/g were counted in samples at day 17 of storage. Viable count of gluten-free flour and products were determined at different intervals of storage on three different types of media (LB, PDA, and YEPDA). These results are in line with gluten-free food items prepared by researchers and no total viable count was found in sweet and salty biscuits at the storage of 15 days, whereas it ranged from  $1.0 \times 10^1$  to  $5.6 \times 10^1$  cfu/g within matthai, namakpara, noodles, macaroni, and gluten-free flour being minimum in matthai and maximum in flour and colony forming units increased with passage of time or long time storage. Maximum counts were observed at 45 days in all samples ranged  $1.0 \times 10^4$  to  $3.3 \times 10^4$ . Yeast colonies were also observed in samples of noodles, macaroni, and gluten-free flour at 45 days of storage. Fungi were not detected in any of the samples except in gluten-free flour at 45 days of storage period [34].

Chickpea protein provides good blending characteristics to gluten-free baked products to improve the volume [20] as normally the available products are low in protein content. Rice is a very common ingredient in GF formula [18]. Composite flour-based products have great potential to improve the nutritional value such as essential amino acids and carbohydrate contents as when studied it is noted that available products have higher glycemic index [48]. As counterfeit natural gluten-free grains are considered as good source of vitamins such as riboflavin, niacin, thiamin, and vitamins C and E [14], they are used to avoid multi-vitamins and mineral deficiencies.

Acceptability analysis showed that crust color was significantly ( $p < 0.05$ ) appreciable for  $T_0$  ( $8 \pm 0.8$ ) followed by  $T_1$  ( $7.6 \pm 0.5$ ) and then by  $T_4$  ( $7 \pm 0.8$ ). The size of the wheat-based cupcakes was highly acceptable ( $8.6 \pm 0.5$ ), while panelists liked uniformity of the rice-based cupcakes ( $8.3 \pm 0.7$ ) followed by  $T_4$  cupcakes ( $7.6 \pm 0.5$ ). There is no difference in moistness among all the prepared products as ( $p < 0.05$ ). Odor and taste of the formulated cupcakes were highly appreciated by the evaluation panelists and scored higher among all  $7.6 \pm 0.5$  and  $8 \pm 0.8$ , respectively. The sensory evaluation data were almost in-lined with [33] although our product had less

carbohydrates and free sugars compared to red kidney based rice.

## 5. Conclusion

The use of micro-cereals and legumes is becoming very prominent in the gluten-free market. The present study formulated and characterized protein-calorie rich gluten-free cupcakes with improved concentration of counterfeit grains/pseudo-cereals. Physical parameters including height, specific volume, and texture of formulated gluten-free cupcakes containing chickpeas, flaxseeds, almonds, and sorghum (40:15:40:5) were as good as that of standard wheat-based and previously prepared rice flour-based products. The organoleptic acceptability was found to be appreciably high, comparable to other gluten-containing/free products with safety for consumption, and could be a great improvement in mitigating undesirable characteristics in baking products.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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