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NUTRITIONAL AND SENSORY EVALUATION OF GLUTEN-FREE MUFFINS PREPARED BY USING MAIZE, SORGHUM, AND CHICKPEA

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ABSTRACT

Healthy and nutritious food is a basic need of humans; therefore, the food industry is striving to develop healthy food products with fewer calories and improved nutritional quality. Nowadays, one of the major challenges to the food industry is developing novel wheat-free products, especially for celiac patients, as people are gluten intolerant to this disease. Celiac disease is spreading day by day, and it affects 1-2% of the population all over the world. Thus, the food industry needs to use new sustainable wheat alternatives for the development of bakery products. Therefore, the present study is designed to develop gluten-free muffins from plant-based sustainable raw materials such as maize, sorghum, and chickpea. Maize flour (50, 60, and 70%), sorghum flour (20, 25, 30, 40, and 50%), and chickpea flour (20, 25, 30, 40, and 50%) were utilized to prepare composite flour. The composite flour was then analyzed for proximate composition and rheological characteristics. The findings revealed that different flour blend compositions had significantly affected flour's nutritional profile and rheological properties. Afterward, gluten-free muffins were developed and examined for proximate composition, mineral, color, texture, and sensory assessment. The results showed a significant increase in protein (30.8%), fiber (5.31%), ash (2.93%), Cu (0.35 mg/L), Mg (1.23mg/L), Fe (1.30mg/L) content and decrease in moisture (9.20%), NFE (15.9%), and Cd (0.10mg/L) content of gluten-free muffins as compared to wheat muffins. Moreover, during the storage study, an increase in texture hardness (30.9 to 34.3%) value and a decrease in moisture (9.72 to 6.59%) value for developed muffins was observed from day 1 to day 21. The crust color (l*, a*, b*) values and sensory score decrease significantly as compared to control. Conclusively, maize (60%), sorghum (20%), and chickpea (20%) act as appropriate combinations of flour to prepare gluten-free muffins with excellent nutritional value, color attributes, and consumer acceptance.

Keywords: Coeliac disease, Gluten-free muffins, Composite flour

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1. INTRODUCTION

Gluten-related disorders are prevailing these days especially celiac disease which is induced by the consumption of gluten protein. It is characterized by malabsorption and abdominal discomfort and comes under auto-immune response. It is a chronic disorder that causes inflammation of the inner walls of the small intestine as a result its ability to absorb nutrients decreases. Due to the damage to the inner surface of the small intestine, the condition of malnourishment may occur over time. Other symptoms shown in celiac patients are depression, weight loss, insomnia, liver and the nervous system also affected (Felber et al., 2014). Pakistan is an agricultural country and a good producer of wheat, which is utilized as a staple food, especially in Punjab. About 1-3% the population of Pakistan is affected with celiac disease. The only effective strategy for celiac patients is to exclude gluten from the diet and follow a gluten-free diet throughout life. Therefore, the demand for gluten-free products is now increasing in global food markets (Mohsin and Khan 2009).

Many studies have been carried out to test the potential ingredients that can be utilized for the production of gluten-free bakery products as similar as possible to wheat. Extensive previous research has been published in which different ingredients and technologies have been developed to prepare gluten-free pasta and bakery products with good nutritional composition and sensory characteristics (Gao et al., 2017). Gluten-free flour can be obtained from cereals (rice, corn, millet, teff, and sorghum), legumes (chickpea, soybeans, lentils, and peas), pseudocereals (quinoa, buckwheat, and amaranth) roots and tuber flour (potato), chia seeds and beans. To improve the quality of gluten-free products addition of starches, non-gluten protein, enzymes, emulsifiers, and hydrocolloids has been done in previous studies (Polo et al. 2020). Previous studies have shown that multigrain flour consisting of neglected cereals (maize,

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millet, and sorghum) has been used effectively for the development of gluten-free bakery products (Rohi et al. 2022).

Maize (*Zea mays L.*) is a good source of many nutritional compounds that provide numerous potential health benefits. It is nutritionally superior to other cereals because 72-73% of its grain weight is starch. For developing countries that rely on imported wheat, maize flour is a good alternative for preparing baked items (Zhang et al. 2014). Whole maize grains are a good source of phytochemicals that have antioxidative activities and help in preventing many diseases. Yellow varieties of maize contain carotenoids which cannot be synthesized in the human body thus reducing the risk of cancer in humans (Rouf Shah et al. 2016). Maize endosperm contains 39.4mg/100g of resistant starch which is helpful in preventing cecal cancer, obesity, diarrhea, and atherosclerosis. Consumption of resistant starch affects the microbial population in the digestive tract, has a positive effect on cholesterol metabolism, increases the production of short-chain fatty acids in the large intestine, and also improves insulin sensitivity in humans (Shen et al. 2009).

Sorghum (*Sorghum bicolor*), is the 5th significant crop globally and its common name is Jowar in Pakistan. It is considered a staple food, especially for poor people (Proietti et al. 2015). Nowadays its advantageous features are eye-catching as food products as its chemical composition includes protein (8.3g), fat (3.67%), starch (50g), fiber (13.8g), and ash (2.6%) per 100g (Hossain et al. 2022). Among cereals, it has been gaining importance as it is also a good source of bioactive flavonoids (Yang et al. 2015), slowly digestible starch (Simnadis et al. 2016), and vitamins (Egbujie and Okoye 2019). It can be used as a valuable ingredient for the bakery industry to fulfill the nutritional needs of the underprivileged due to its micronutrient potential and protein content (Hossain et al. 2022). Refined sorghum flour is used to produce gluten-free bakery items such as cookies, bread, cupcakes, and muffins (Serna-Saldivar 2016).

Regular consumption of cereal-based extruded snacks imparts a high glycemic index impact which contributes to obesity and several other disorders (Brennan et al. 2013). Mutual supplementation of cereals with legumes provides a complementary nutritional profile as well as a low glycemic index for consumers. Legumes contain good quality protein as compared to cereals, as they contain essential amino acids that are limited in cereal protein (e.g., lysine). However, legume protein often lacks sulphur-containing amino acids (e.g., methionine) which can be complemented by mixing cereal flour with legume flour (Liu et al. 2016).

Chickpea (*Cicer arietinum L.*) is one the major legumes that is widely used for human consumption. Chickpea seeds have a high protein digestibility, a good amino acid balance, and a high nutritional value (Kaur and Prasad 2021). The composition of chickpea includes carbohydrates (60.65g/100g), protein (19.30g/100g), fiber (17.4g/100g), fat (6.04g/100g), and ash (2.48g/100g). Chickpea has been recognized as a functional food due to its nutritional composition (Boye et al. 2010). Oligosaccharides such as raffinose, stachyose, and verbascose are present in chickpea which shows excellent prebiotic properties (Mathew et al. 2022). Chickpea flour is considered gluten-free and thus useful in making gluten-free food for persons suffering from wheat allergy and celiac disease (Bessada et al. 2019).

In Pakistan, muffins are known as popular breakfast and snack food item consumed by all age groups. They are less sweet than other bakery products and are usually baked in small portions like small cakes or cup-sized bread. They are convenient and nutritious breakfast cereal-based food especially when prepared by whole grain cereals. Muffins are usually characterized by porous structure, dense volume, and ready to eat nature (Singh et al. 2020).

In the bakery industry, there is an interest in developing ingredients to replace wheat flour due to consumer preference for healthy food products, especially after the COVID-19 pandemic. Current researchers are also striving to find wheat alternatives from sustainable sources to overcome disease rates in the population (Grand et al. 2020). The available range of gluten-free food products is limited in Pakistan and imported food products are expensive (Jabeen et al. 2022).

Therefore, the aim of the current study was to overcome this challenge by developing gluten-free muffins from sustainable plant-based sources (maize, sorghum, and chickpea). This study evaluates the effect of a novel combination of maize, sorghum, and chickpea on the nutritional, textural, and sensory characteristics of gluten-free muffins and also the effect of the storage period on the moisture and texture of muffins.

2. MATERIALS AND METHODS

2.1. Procurement of Materials

Maize, sorghum, chickpea, and Wheat (LU-26) were procured from MNS University of Agriculture Multan, Pakistan. Chemicals for proximate, mineral analysis were purchased from the Merck/Sigma–Aldrich, (Sigma-Aldrich Tokyo, Japan), (Merck KGaA, Darmstadt, Germany) and the Duksan pure chemicals (Kyungkido, Ansan, South Korea).

2.2. Preparation of Gluten-free Composite Flour

To produce whole grain maize flour, sorghum flour, wheat flour, and chickpea flour, grains were cleaned from any extraneous contaminants by sieving, then dehulled, and milled to flour using a Roller Mill (Bastak Instruments, Turkey) according to the procedure as described in AACC (2000). Gluten-free multigrain flour was prepared by using maize flour (50, 60, and 70%), sorghum flour (20, 25, 30, 40, and 50%), and chickpea flour (20, 25, 30, 40, and 50%)

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as illustrated in Table 1. Gluten-free composite flour was packed in polyethylene bags and kept in a moisture-free place at room temperature until used.

Table 1: Formulation of composite flours using maize, sorghum, and chickpea

Treatments	Maize flour (%)	Sorghum flour (%)	Chickpea flour (%)	Wheat flour (%)
T_0	_	_	_	100
T_1	50	0	50	
T_2	50	50	0	
T_3	50	25	25	
T_4	60	40	0	
T_5	60	0	40	
T_6	60	20	20	
T_7	70	0	30	
T_8	70	30	0	
T_9	70	15	15	

2.3. Compositional Analysis of Gluten-free Composite Flour

The oven drying method was used to determine moisture (method No. 44–15 A), the Soxhlet apparatus was used to determine crude fat (method No. 30–10), Kjeldahl's technique was used to determine crude protein (method No. 46–10), gravimetric method was used to determine crude fiber (method No. 32–10), and dry ashing technique was used to measure ash content (method No. 08–01) of wheat and composite gluten-free flours as described in AACC (2000). The Gluten content of wheat and composite flour samples was determined by performing a gluten hand-washing test as described in AACC (2000) approved Method No. 38-10.01. NFE content was then calculated as:

$$\text{NFE \%} = 100 - (\text{Crude fat \%} + \text{Ash \%} + \text{Moisture \%} + \text{Crude protein \%} + \text{Crude fiber \%})$$

2.4. Farinographic Study

Rheological characteristics of wheat and GF composite flour dough such as water absorption, dough development time, mixing tolerance index and dough stability were determined by using Farinograph (Bradender, Duisburg, Germany) according to the guidelines as stated in AACC 2000, method No. 54-21.02.

2.5. Preparation of Muffins

The preparation of control and gluten-free muffins was carried out according to a procedure described by Isık et al. (2022) with some modifications. First of all, a weighed quantity of ¾ cup sugar, ½ cup oil, and 2 eggs were whipped into a cream-like texture in a planetary mixer at maximum speed for 5-6min. Thereafter, 1½ cup gluten-free composite flour with different proportions of maize, sorghum, and chickpea according to the treatment plan as mentioned in Table 1 and 2 teaspoons baking powder were added and whipped at minimum speed for at least 2min. Almost 50g of this gluten-free cake batter was poured into muffin paper cups then placed in aluminum trays and baked in a pre-heated baking oven at 170°C for 20 min.

Table 2: Mean value of compositional analysis of composite GF flours

Treatments	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	NFE (%)	Dry Gluten content
T_0	$11.10{\pm}0.51^{ab}$	$2.50{\pm}0.10^{e}$	$14.05{\pm}0.60^{b}$	$1.35{\pm}0.08^{g}$	$0.58{\pm}0.02^{e}$	$68.42{\pm}1.21^{a}$	9.90±0.41
T_1	$9.78{\pm}0.31^{c}$	$6.14{\pm}0.53^{a}$	$15.80{\pm}0.73^{a}$	$6.05{\pm}0.28^{a}$	$2.81{\pm}0.13^{a}$	$57.42{\pm}1.97^{e}$	0
T_2	$10.77{\pm}0.49^{abc}$	$5.06{\pm}0.35^{b}$	$10.32{\pm}0.47^{e}$	$2.61{\pm}0.12^{f}$	$2.05{\pm}0.09^{d}$	$67.19{\pm}1.53^{abc}$	0
T_3	$9.86{\pm}0.33^{c}$	$6.64{\pm}0.29^{d}$	$13.06{\pm}0.60^{bc}$	$4.32{\pm}0.20^{cd}$	$2.24{\pm}0.10^{cd}$	$61.88{\pm}1.52^{d}$	0
T_4	$10.85{\pm}0.39^{abc}$	$4.46{\pm}0.20^{cd}$	$10.02{\pm}0.46^{e}$	$2.52{\pm}0.12^{f}$	$2.27{\pm}0.10^{cd}$	$67.88{\pm}1.27^{ab}$	0
T_5	$9.95{\pm}0.48^{bc}$	$4.69{\pm}0.19^{bc}$	$14.40{\pm}0.66^{ab}$	$5.26{\pm}0.24^{b}$	$2.06{\pm}0.09^{d}$	$61.64{\pm}1.67^{d}$	0
T_6	$10.61{\pm}0.35^{abc}$	$5.09{\pm}0.22^{b}$	$12.21{\pm}0.56^{cd}$	$3.89{\pm}0.18^{de}$	$3.89{\pm}0.18^{d}$	$64.13{\pm}1.41^{bcd}$	0
T_7	$10.62{\pm}0.36^{abc}$	$4.02{\pm}0.26^{d}$	$13.01{\pm}0.60^{bcd}$	$4.48{\pm}0.21^{c}$	$2.60{\pm}0.12^{ab}$	$63.27{\pm}1.54^{cd}$	0
T_8	$11.32{\pm}0.39^{a}$	$3.98{\pm}0.65^{d}$	$9.72{\pm}0.54^{e}$	$2.42{\pm}0.11^{f}$	$2.15{\pm}0.10^{cd}$	$68.41{\pm}1.69^{a}$	0
T_9	$10.36{\pm}0.37^{abc}$	$4.27{\pm}0.18^{cd}$	$11.37{\pm}0.52^{de}$	$3.45{\pm}0.16^{e}$	$2.38{\pm}0.11^{bc}$	$66.17{\pm}0.95^{abc}$	0

Each value (mean±SD) is based on three observations. Values with different superscript letters in the same row differ significantly (P≤0.05). T0; Control sample, T1 = 50% Maize flour + 50% Chickpea flour, T2 = 50% Maize flour + 50% Sorghum flour, T3 = 50% Maize flour + 25% Sorghum flour + 25% Chickpea flour, T4 = 60% Maize flour + 40% Sorghum flour, T5 = 60% Maize flour + 40% Chickpea flour, T6 = 60% Maize flour + 20% Chickpea flour + 20% Sorghum flour, T7 = 70% Maize flour + 30% Chickpea flour, T8 = 70% Maize flour + 30% Sorghum flour, T9 = 70% Maize flour + 15% Sorghum flour + 15% Chickpea flour.

2.6. Compositional and Quality Analysis of GF Muffins

2.6.1. Chemical Composition of Gluten-free Muffins

Moisture, crude fat, crude protein, crude fiber, ash, and NFE content of control and nine samples of gluten-free muffins were determined following the procedures explained in AACC (2000). The mineral content (Cd, Cu, Fe, and Mg) of muffins was determined by using atomic absorption spectrophotometer according to the procedures described in AOAC (2018).

2.6.2. Color Analysis of GF Muffins

Color is a crucial parameter for initial product acceptability by consumer. Color analysis of gluten free muffins was performed by Chroma meter (Chroma Meter CR-400, Konica Minolta, Japan) according to the working protocol as mentioned in AACC (2000). The color values provided three parameters which were I* (whiteness/darkness), b* (yellowness/blueness) and a* (redness/greenness). Before performing experiment, chroma meter was calibrated with a white standard plate (Mitharwal and Chauhan, 2022).

2.6.3. Texture Analysis of GF Muffins

The texture profile of gluten-free muffin samples was analyzed by using an IMADA Digital texture analyzer (FRTS Series)/ TA-XT plus. This texture analyzer used a needle probe to check the hardness of muffin samples. It was operating at regular speed, with the knob and sphere probes moving at a rate of 2mm/s speed along with the trigger force was 0.05 N. Before the experiment, it was calibrated and each sample was subjected to 50% compression of its original height, with a rest period of 3 seconds between cycles (Herranz et al. 2016).

2.7. Storage Study

Gluten-free muffins were wrapped in polyethylene bags after preparation and kept at 25°C for 30 days. The hardness and moisture content of GF muffins were measured at the 1st, 7th, 14^{th,} and 21st day (Acosta et al. 2011).

2.8. Consumer Study

Sensory characteristics of gluten-free muffins were assessed as described by (Man et al. 2014) using a 9-point hedonic scale. The sensory panel consist of 15 people comprising of professors, faculty member and students of department of food science and technology, MNS University of Agriculture, Multan. The panelists assessed the gluten free muffins samples for various sensory characteristics such as taste, color, appearance, texture, aroma, flavor, firmness and overall acceptability. All samples were scored on a scale of 1–9 where 1 = dislike extremely to 9 = like extremely.

2.9. Statistical Evaluation of GF Muffins

All experiments were carried out in triplicate then statistically analyzed and the results were presented as mean ± standard deviation. For each parameter and property evaluated, one way analysis of variance (ANOVA) under completely randomize design was calculated using Statistics 8.1. Beyond ANOVA, Multiple comparison test was used to assess significant differences ($P<0.05$). For storage study of muffins, the one-way ANOVA under factorial design was calculated (Montgomery, 2017).

3. RESULTS AND DISCUSSION

3.1. Chemical Characterization of Gluten-free Composite Flour Blends

The chemical composition of control (wheat flour) and nine samples of gluten-free composite flour have been illustrated in Table 2. The results revealed that different proportions of maize, sorghum, and chickpea flour in gluten-free flour blends highly significantly ($P<0.01$) affected the moisture, fat, fiber, protein, ash, NFE, and dry gluten content. The moisture content of gluten-free composite flour was less as compared to T_0 (whole wheat flour) while fat, fiber, and ash content was significantly higher in all combinations of composite flour blends. The protein content was high in T_1 and T_5 as compared to T_0 while in all other combinations of flour blends, it was comparable to whole wheat flour. The NFE content of gluten-free flour blends was less as compared to the control sample. The moisture content of composite flour blends was less due to the addition of sorghum and chickpea flour as they contain less moisture. Similar results for moisture content were reported by Mishra et al. (2012) in which moisture content ranged from 9.37 to 11.94%.

Current findings revealed that the highest value of fat, fiber, protein, and ash content was observed for T_1 which contains 50% maize flour and 50% chickpea flour. Gluten-free composite flour showed good nutritional value as fat, fiber, protein, and ash content were quite high because individual compositions of chickpea and sorghum contain more ash, fat, fiber, and protein content as compared to whole wheat. Similar results were observed by Shatta et al. (2019).

The NFE content of composite flour was less, which means its consumption will impart a low glycemic index to consumers. This multigrain composite flour will not only be a suitable option for coeliac patients but also a healthy option for diabetic patients.

3.2. Rheological Characteristics of Gluten-free Composite Flour Blends

Farinograph analysis revealed the quality of the control and composite flour blends. As indicated in Table 3, different combinations of maize, sorghum, and chickpea in gluten-free composite flour blends are significantly affected by rheological properties such as water absorption, dough development time, dough stability, and mixing tolerance index. For all formulations of gluten-free composite flour, the mean value for water absorption, dough development time, dough stability, and mixing tolerance index fluctuated from 57.0±2.48 to 45.0±2.07, 2.0±0.05 to 4.20±0.19, 2.12±0.06 to 4.58±0.21 and 27±0.86 to 65±2.08, respectively. Gluten-free composite flour in all formulations showed less water absorption as compared to whole-grain wheat flour. According to the results, a combination of maize flour with sorghum flour showed less water absorption (48±2.21) as compared to a combination of maize flour with chickpea flour (51.0±2.08) due to a reduction of gluten content. The water absorption capacity of flour was more as the concentration of chickpea flour increased in composite flour due to an increase in protein content. A similar trend was reported in the findings of Gadallah et al. (2017) who observed that when wheat flour was replaced with 20% sorghum flour, water absorption was 57% as compared to wheat flour when replaced with 20% chickpea flour had 64.10% water absorption. The dough stability time of GF composite flour decreased as compared to whole wheat which had a dough stability time of 6 min as mentioned in Table 3. Similar results were reported by Krishnaiya et al. (2016) in which stability time decreased for composite flour of water chestnut and wheat flour. The current findings are in harmony with the findings of Gadallah et al. (2017) who reported 1 to 2.50 min of dough development time for composite flour.

Table 3: Farinograph parameters of composite flour containing different levels of maize, sorghum, and chickpea flour

Treatments	Water absorption (%)	Dough development time (min)	Dough stability (min)	Mixing tolerance index (BU)
T_0 (100% WWF)	62.0±2.85^{a}	5.83±0.27^{a}	6.00±0.28^{a}	30.0±0.96^{g}
T_1 (50% MF + 50% CF)	51.0±2.08^{bcde}	3.52±0.16^{c}	4.58±0.21^{b}	27.0±0.86^{g}
T_2 (50% MF + 50% SF)	48.0±2.21^{de}	3.20±0.15^{cd}	4.4±0.20^{bc}	36.0±1.15^{ef}
T_3 (50% MF + 25% SF + 25% CF)	57.0±2.48^{ab}	3.0±0.14^{d}	3.20±0.15^{d}	44.0±1.41^{d}
T_4 (60% MF + 40% SF)	46.0±2.12^{e}	3.15±0.14^{cd}	3.5±0.16^{d}	50.0±1.60^{c}
T_5 (60% MF + 40% CF)	49.0±2.35^{cde}	3.21 ±0.15^{cd}	3.33±0.15^{d}	39.0±1.25^{ef}
T_6 (60% MF + 20% SF + 20% CF)	55.0±2.62^{bc}	4.20±0.19^{b}	4.00±0.18^{c}	35.0±1.12^{f}
T_7 (70% MF + 30% CF)	48.0±2.21^{de}	01.09±0.05^{e}	01.18±0.05^{e}	59.0±1.89^{b}
T_8 (70% MF + 30% SF)	45.0±2.07^{e}	1.0±0.05^{e}	1.22±0.06^{e}	65.0±2.08^{a}
T_9 (70% MF + 15% SF + 15% CF)	54.70±2.53^{bcd}	3.50±0.16^{c}	3.50±0.11^{d}	40.0±1.28^{de}

For treatment detail, refer/check footnote of Table 2.

It was observed that composite flour samples which recorded low dough stability, had higher tolerance index scores. These findings are in conformity with results of Sibt-e-Abbas et al. (2014) in which MTI ranged from 20.00±2.00 to 50.00±2.52 for flour blends made of peanut protein isolates. So, it was concluded that weak flour has a low development time and high tolerance index while strong flour has more time for dough development and less tolerance index as indicated in T_4 and T_6 respectively when both are compared.

3.3. Chemical Composition of Resultant GF Muffins

Gluten-free muffins were prepared by using different proportions of maize, sorghum, and chickpea as mentioned in the treatment plan, after preparation the proximate and mineral values were tested. These values are indicated in Table 4 and 5. The results found that different proportions of maize, sorghum, and chickpea in all experimental treatments had significantly affected the moisture, protein, fiber, fat, ash, NFE, Cu, Cd, Fe, and Mg contents. The moisture content of gluten-free muffins ranges from 9.20±0.40 to 14.79±0.61, indicating that it was less or comparable to the moisture content of control muffins. The low moisture content leads to prolong shelf life thus gluten-free muffins could be stored for a long period of time as compared to wheat muffins.

Protein, fat, fiber, and ash contents almost increased in all experimental treatments as compared to control muffins. The mean value for protein, fat, fiber, and ash content ranged from 24.72±1.14 to 30.80±1.42, 27.41±1.26 to 34.01±1.56, 2.07±0.07 to 5.31±1.57 and 2.05±0.09 to 2.93±0.09, respectively. The highest values of protein and fiber were observed in T_1 while the highest values of fat and ash were observed in T_3 and T_7 respectively. The highest values of protein, fat, fiber, and ash content of gluten-free muffins ascribe to a good nutritional profile as compared to wheat muffins. It was observed that as the level of chickpea increases, leads to an increase in the percentage of protein and fiber.

Table 4: Mean value of proximate analysis GF muffin samples

Treatments	Moisture (%)	Fat (%)	Protein (%)	Fiber (%)	Ash (%)	NFE (%)
T_0	13.30±0.61^{abc}	30.78±1.42^{abc}	13.24±0.61^{d}	2.19±0.07^{d}	1.62±0.07^{e}	33.60±5.59^{a}

T_1	9.720±0.42^{ef}	31.20±1.44^{abc}	30.80±1.42^{a}	5.31±1.57^{a}	2.86±0.63^{a}	18.110±3.47^{c}
T_2	9.20±0.40^{f}	30.01±1.38^{abc}	25.32±1.16^{c}	2.07±0.07^{d}	2.38±0.11^{c}	29.020±3.02^{ab}
T_3	10.94±0.44^{de}	34.01±1.56^{abc}	28.06±1.29^{abc}	4.04±0.13^{b}	2.71±0.12^{ab}	18.240±2.68^{c}
T_4	11.74±0.54^{cd}	27.41±1.26^{c}	25.02±1.15^{c}	2.29±0.07^{d}	2.05±0.09^{d}	29.49±3.12^{ab}
T_5	13.23±0.63^{abc}	29.17±1.34^{bc}	29.40±1.35^{ab}	5.16±0.17^{a}	2.69±0.12^{ab}	18.350±3.59^{c}
T_6	13.31±0.68^{abc}	30.29±1.28^{c}	27.21±1.25^{bc}	3.27±0.10^{c}	2.73±0.13^{ab}	21.190±3.44^{bc}
T_7	13.76±0.61^{ab}	33.01±1.52^{ab}	28.01±1.29^{abc}	4.37±0.14^{b}	2.93±0.09^{a}	15.930±3.60^{c}
T_8	12.72±0.82^{bc}	32.05±1.47^{ab}	24.72±1.14^{c}	2.21±0.07^{d}	2.48±0.08^{bc}	23.820±3.17^{bc}
T_9	14.79±0.61^{a}	27.83±1.28^{c}	26.37±1.21^{bc}	3.26±0.10^{c}	2.70±0.09^{ab}	23.050±3.36^{bc}

For treatment detail, refer/check footnote of Table 2.

Table 5: Mean values for mineral content (Cu, Cd, Mg, and Fe) of GF muffins

Treatments	Cu (mg/1000g)	Cd (mg/1000g)	Mg (mg/1000g)	Fe (mg/1000g)
T_0	0.180±0.008^{e}	1.060±0.048^{a}	0.970±0.044^{b}	0.871±0.004^{ef}
T_1	0.340±0.0156^{ab}	0.094±0.0025^{cd}	1.230±0.056^{a}	0.236±0.0109^{d}
T_2	0.320±0.0147^{abcd}	0.091±0.0042^{cd}	0.168±0.0077^{f}	0.213±0.0098^{d}
T_3	0.286±0.0132^{d}	0.069±0.0032^{d}	0.125±0.0057^{f}	0.117±0.0054^{e}
T_4	0.290±0.0133^{cd}	0.094±0.0043^{cd}	0.168±0.0077^{f}	0.243±0.0112^{d}
T_5	0.326±0.0150^{abcd}	0.107±0.0049^{c}	0.462±0.0212^{d}	0.172±0.0079^{de}
T_6	0.354±0.0163^{a}	0.108±0.0050^{c}	0.263±0.0121^{e}	1.302±0.0599^{a}
T_7	0.330±0.0152^{abc}	0.076±0.0035^{d}	0.453±0.0208^{d}	1.025±0.0472^{b}
T_8	0.308±0.0141^{bcd}	0.080±0.0037^{d}	0.471±0.0216^{d}	0.025±0.0012^{f}
T_9	0.325±0.0150^{abcd}	0.087±0.0040^{cd}	0.813±0.0374^{c}	0.364±0.0167^{c}

For treatment detail, refer/check footnote of Table 2.

Current findings are in resemblance to the outcomes of Alhassan et al. 2019; Jabeen et al. 2022; Kaur et al. 2022; Rebecca et al. 2016, who reported 7.26±0.35 to 22.44±0.51 protein content, 21.4±1.53 to 39.74±1.38 fat content, 3.25± 0.07 to 7.15 ±0.13 fiber content and 9-21% moisture content of gluten-free cupcakes and biscuits, respectively.

Control muffins and gluten-free muffins showed significant variations ($P<0.01$) in the mineral content of (Cd, Cu, Mg, and Fe) as indicated in Table 5. The mean value of Cd, Cu, Mg, and Fe content fluctuated from 0.069±0.003 to 0.108±0.005 mg/kg, 0.28±0.01 to 0.35±0.01mg/kg, 0.125±0.005 to 1.23±0.05 mg/kg, and 0.025±0.001 to 1.30±0.05mg/kg, respectively. The highest values of Cd, Cu, and Fe contents were observed in T_6 while the highest value of Mg content was observed in T_1. These observations of mineral content are in accordance with those reported by Hassan et al. (2020) and Yeşil and Levent (2022).

3.4. Color Attributes of Gluten-free Muffins

Generally, the crust color of muffin depends upon the ingredients that are used in the formulation and it is also affected by caramelization and Millard reaction during baking. According to results, mean values of crust color (l*, a*, b*) of GF muffins prepared from composite flour of maize, sorghum and chickpea showed highly significant difference ($P<0.01$) between treatments as presented in Table 6 and Fig. 1. The recent results of crust color (l*, a*, b*) for whole wheat-based muffins are 63.50±2.91, 8.25±0.37, and 34.76±1.59 respectively. While the mean values of crust color (l*, a*, b*) for gluten-free muffins ranges from 16.68±10.78 to 45.66±0.12, 7.38±0.09 to 13.07±0.42, and 26.96±1.87 to 47.28±1.14 respectively. Experimental treatment (T_6) showed better results of l*, a*, and b* which are comparable to control wheat muffins. The significant decrease in lightness value (l*) value might be due to the light color of maize and chickpea flour. It was observed that l* values increase in the treatments that contain an increased level of sorghum flour in the formulation. The significant increase in redness (a*), and yellowness (b*) of gluten-free composite flour muffins induced darker color might be due to the presence of pigments in flour (especially sorghum) as well as due to the high protein content and rich amino acid profile (chickpea) that catalyze Millard and caramelization during baking and imparts brown color. Similar results have been reported by Alvarez et al. (2017) for wheat flour replaced by chickpea flour in muffins. These results are in accordance with the study conducted by Mitharwal and Chauhan, (2022) which documented an increase in b* values for muffins developed by composite flour of finger millet flour, germinated black soybean flour, and wheat flour.

3.5. Hardness of GF Muffins Along with the Moisture Content during Storage

The moisture content and hardness of muffins are important indicators for physiochemical changes during storage. According to the results, treatment, days, and interaction between days and treatment had significant differences ($P<0.01$) on moisture and hardness of gluten-free muffins. The hardness of gluten-free muffins increased significantly from 7.70±0.35 to 30.91±0.42 for gluten-free muffins as compared to the hardness of control wheat muffins (11.30±0.51) as indicated in Table 7 and Fig. 1. The significant increase in the hardness of gluten-free muffins might be due to the absence of gluten content. The structure of the muffins was dense which reduced the number and

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size of air bubbles as depicted in Fig. 1, this result in an increased force of compression. Also, the high content of protein and fiber in gluten-free muffins contributes to more hardness as compared to control muffins. The muffin's texture was determined in terms of hardness at different day's intervals such as on day 1, day 7 day 14, and day 21. It was observed that the hardness of all experimental treatments increased from day 1 to day 7, day 14, and day 21 respectively. The current results regarding the hardness of gluten-free muffins are similar to the findings of Baixauli et al. (2008) those who found hardness (N) of muffins with 20% resistant starch was doubled over a storage period of 16 days.

Table 6: Mean values for color (l*, a*, b*) of GF muffins prepared from composite flour of maize, sorghum and chickpea

Treatments	Color (l*)	Color (a*)	Color (b*)
T_0	63.50±2.91^{a}	8.25±0.37^{de}	34.76±1.59^{bc}
T_1	44.39±0.25^{bc}	13.07±0.42^{a}	33.30±0.40^{bc}
T_2	43.67±0.88^{d}	10.02±0.17^{cd}	28.30±0.67^{bc}
T_3	43.67±0.88^{bc}	10.54±0.04^{bc}	30.17±0.10^{bc}
T_4	45.66±0.12^{b}	7.38±0.09^{e}	29.94±0.11^{bc}
T_5	23.08±1.60^{bc}	8.43±1.86^{de}	39.20±12.43^{bc}
T_6	41.97±2.49^{e}	8.90±1.96^{cde}	35.31±1.14^{ab}
T_7	16.68±10.78^{f}	12.24±0.41^{ab}	47.28±1.14^{a}
T_8	25.31±1.84^{e}	9.68±0.58^{cd}	26.96±1.87^{c}
T_9	41.14±0.33^{c}	12.95±0.33^{a}	31.29±0.18^{bc}

For treatment detail, refer/check footnote of Table 2.

Table 7: Mean values for hardness (N) of GF muffins prepared from composite flour of maize, sorghum and chickpea

Treatments	Day-1	Day-7	Day-14	Day-21	Mean
T_0	11.30±0.51^{no}	13.78±0.63^{mn}	17.57±0.24^{kl}	20.30±0.56^{jk}	15.73±3.99^{g}
T_1	30.91±0.42^{cde}	33.50±1.07^{bc}	35.80±1.14^{ab}	37.30±1.19^{a}	34.37±2.79^{a}
T_2	28.70±1.32^{de}	30.10±0.96^{de}	34.60±1.10^{ab}	36.90±1.18^{a}	32.57±3.83^{b}
T_3	22.20±1.02^{hij}	25.70±0.82^{fg}	28.40±0.90^{ef}	33.46±1.07^{bc}	27.44±4.75^{c}
T_4	15.60±0.71^{lm}	17.60±0.56^{kl}	22.80±0.72^{hij}	25.80±0.82^{fg}	20.45±4.68^{e}
T_5	12.02±0.55^{n}	16.80±0.77^{l}	20.60±0.65^{j}	22.40±0.71^{hij}	17.95±4.59^{f}
T_6	8.40±0.38^{p}	16.60±0.76^{l}	21.80±0.69^{ij}	24.78±0.79^{gh}	17.89±7.18^{f}
T_7	7.70±0.35^{p}	8.70±0.40^{op}	12.40±0.39^{n}	15.80±0.50^{lm}	11.15±3.70^{i}
T_8	15.40±0.70^{lm}	23.50±0.75^{ghi}	29.30±1.34^{de}	31.43±1.0^{cd}	24.90±7.17^{d}
T_9	6.60±0.30^{p}	11.80±0.54^{n}	15.80±0.50^{lm}	17.23±0.55^{l}	12.83±4.76^{h}
Mean	15.88±8.60^{d}	19.80±8.09^{c}	23.89±7.80^{b}	26.54±7.86^{a}	

For treatment detail, refer/check footnote of Table 2.

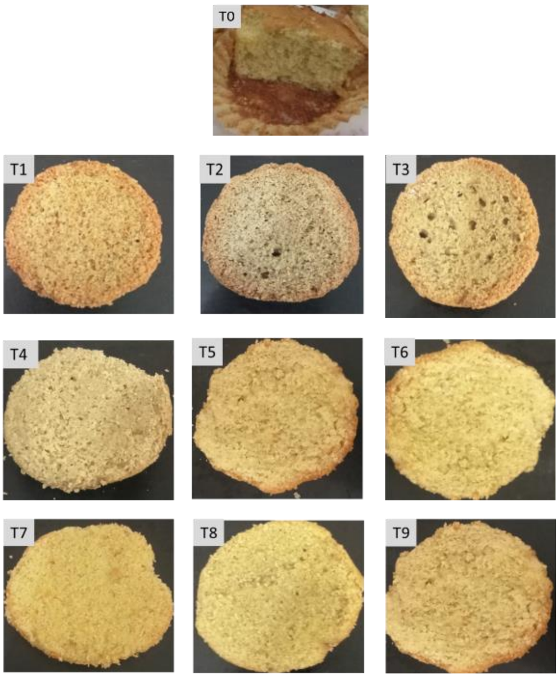

Fig. 1: Texture of various variants of developed muffins

Moisture content is an important parameter for storage study of bakery products such as muffins. From current findings, it was observed that moisture content of GF muffins decreases for all experimental formulations during

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storage. The Mean value of moisture content for all experimental treatments was high on day 1 and the lowest mean value of moisture was observed on day 21 in all formulations. As depicted in Table 8, the mean value for moisture content of GF muffins ranged from 9.20±0.40% to 17.72±0.82% on day-1 while a significant decrease in moisture was observed as it ranged from 7.46±1.13% to 14.34±0.62% on day-7. Similar results were reported by Acosta et al. (2011) who found moisture of bread crumb decreased within the first 5 days of storage. This might be due to low water retention capabilities which cause moisture loss during storage.

Table 8: Mean values for moisture content (%) of GF muffins prepared from composite flour of maize, sorghum, and chickpea

Treatments	Day-1	Day-7	Day-14	Day-21	Mean
T_0	$13.30±0.61^{bcde}$	$10.91±0.6^{ghij}$	$9.02±0.39^{klmnopq}$	$8.76±0.27^{klmnopqr}$	$10.49±2.10^{cd}$
T_1	$9.72±0.42^{ijklmn}$	$7.99±0.37^{nopqrst}$	$7.02±0.30^{rst}$	$6.59±0.20^{st}$	$7.8±1.38^{f}$
T_2	$9.20±0.40^{jklmnop}$	$7.46±1.13^{opqrst}$	$6.93±0.30^{rst}$	$6.28±0.19^{t}$	$7.46±1.25^{f}$
T_3	$10.94±0.44^{ghijkl}$	$9.18±0.42^{jklmnop}$	$8.36±0.36^{mnozpqrs}$	$7.44±0.23^{opqrst}$	$8.98±1.22^{e}$
T_4	$11.74±0.54^{efgh}$	$10.20±0.44^{ghijklm}$	$9.63±0.41^{ijklmn}$	$7.93±0.25^{nopqrst}$	$9.87±1.57^{d}$
T_5	$13.23±0.63^{bcd}$	$11.32±0.49^{fghi}$	$9.88±0.42^{ijklm}$	$7.23±0.26^{qrst}$	$10.41±2.73^{cd}$
T_6	$13.31±0.68^{b}$	$12.82±0.55^{cdef}$	$9.45±0.41^{jklmn}$	$8.41±0.30^{mnopqrs}$	$10.99±2.95^{b}$
T_7	$13.76±0.61^{bc}$	$11.98±0.52^{defg}$	$9.23±0.40^{jklmno}$	$7.94±0.29^{nopqrst}$	$10.72±2.99^{bc}$
T_8	$12.72±0.82^{a}$	$10.34±0.62^{bc}$	$10.57±0.45^{ghijk}$	$8.49±0.31^{lmnopqr}$	$10.53±4.08^{a}$
T_9	$14.79±0.61^{bcde}$	$10.06±0.43^{hijklm}$	$9.46±0.41^{jklmn}$	$7.37±0.27^{pqrst}$	$10.42±2.46^{d}$
Mean	$12.27±2.64^{a}$	$10.27±2.12^{b}$	$8.95±1.19^{c}$	$7.64±0.81^{d}$	

For treatment detail, check footnote of Table 2.

Table 9: Mean value for sensory assessment of GF muffins prepared from composite flour of maize, sorghum and chickpea

Treatments	Appearance	Color	Flavor	Texture	Taste	Aroma	Firmness	Overall acceptability
T_0	$7.4±0.84^{a}$	$6.6±0.51^{ab}$	$7.1±0.87^{ab}$	$7.4±0.51^{a}$	$7.3±0.48^{a}$	$7.1±0.73^{a}$	$6.6±0.96^{abc}$	$7.2±0.78^{ab}$
T_1	$5.6±0.69^{bcd}$	$5.5±0.52^{bc}$	$5.7±0.67^{bc}$	$5.9±0.99^{c}$	$6.0±0.81^{abc}$	$5.8±0.78^{ab}$	$4.5±0.78^{d}$	$5.1±0.87^{cd}$
T_2	$4.6±0.96^{d}$	$4.1±0.73^{d}$	$4.8±0.91^{c}$	$5.1±0.87^{c}$	$4.8±0.78^{c}$	$6.1±0.73^{ab}$	$5.9±0.87^{abcd}$	$5.0±0.78^{d}$
T_3	$6.8±0.78^{ab}$	$6.5±0.70^{ab}$	$7.2±0.78^{a}$	$6.1±0.99^{bc}$	$7.3±0.94^{a}$	$6.0±0.81^{ab}$	$5.4±0.94^{ab}$	$6.0±0.81^{ab}$
T_4	$5.2±0.97^{cd}$	$5.5±1.08^{bc}$	$6.3±1.05^{ab}$	$5.7±1.05^{c}$	$6.0±0.66^{abc}$	$5.1±1.28^{b}$	$5.5±1.35^{bcd}$	$5.6±1.17^{cd}$
T_5	$6.2±0.91^{abc}$	$7.3±0.67^{a}$	$6.1±0.87^{abc}$	$7.0±0.67^{ab}$	$6.0±0.81^{abc}$	$5.3±1.16^{b}$	$5.8±0.91^{abcd}$	$6.2±0.78^{bc}$
T_6	$6.7±0.82^{ab}$	$6.4±0.69^{ab}$	$6.8±1.03^{ab}$	$7.3±0.82^{ab}$	$7.4±1.26^{a}$	$6.5±1.17^{ab}$	$6.0±0.94^{a}$	$7.0±0.94^{a}$
T_7	$5.5±0.65^{bcd}$	$5.9±1.10^{bc}$	$5.8±0.78^{abc}$	$6.2±0.91^{abc}$	$5.3±1.33^{bc}$	$5.3±0.67^{b}$	$5.7±0.91^{abcd}$	$5.6±0.78^{cd}$
T_8	$4.5± 0.70^{d}$	$4.9±0.73^{cd}$	$5.7±1.16^{bc}$	$5.2±0.78^{c}$	$5.4±1.43^{bc}$	$5.4±1.07^{b}$	$5.2±1.03^{cd}$	$5.5±0.97^{cd}$
T_9	$6.3±0.67^{abc}$	$6.2±1.03^{ab}$	$6.1±1.37^{abc}$	$5.8±0.78^{c}$	$6.7±0.94^{ab}$	$5.5±1.08^{b}$	$5.7±0.94^{abcd}$	$5.9±0.99^{ab}$

For treatment detail, check footnote of Table 2.

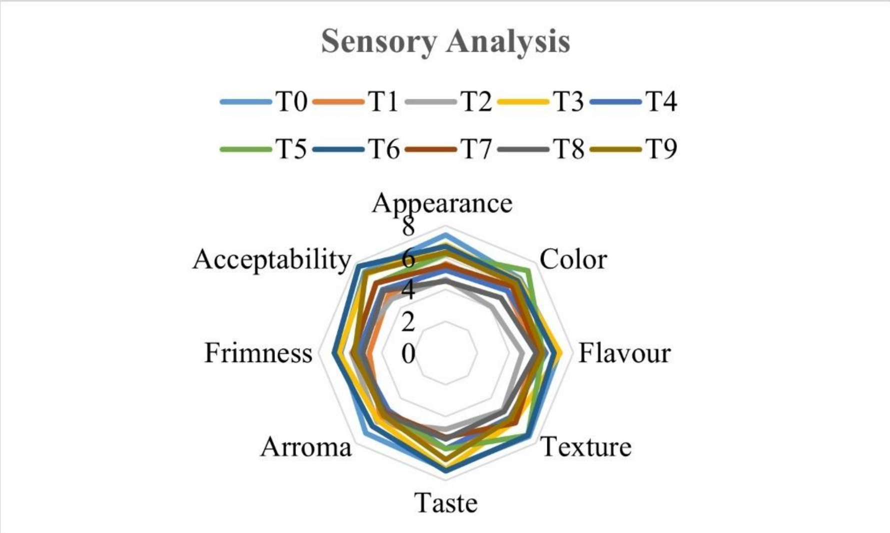

Fig. 2: Sensory Scores of gluten-free composite muffins

3.6. Sensory Assessment of Gluten-free Muffins

Sensory characteristics of gluten-free muffins were analyzed and the results were depicted in Table 9 and Fig. 2. There was a highly significant difference ($P<0.01$) in all samples of gluten-free muffins with control in terms of appearance, aroma, color, flavor, firmness, taste, texture, and overall acceptability. The appearance of gluten-free muffin samples had marginally lower scores ranging from 4.6±0.96 to 6.8±0.78 than that of wheat muffins 7.4±0.84. High sensory appearance scores of 7.0±0.14 to 7.4±0.22 were reported for gluten-free bakery products prepared by

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using quinoa (Kaur and Kaur, 2017). A decrease in color score of gluten-free muffins ranging from 4.1±0.73 to 6.4±0.69 was observed as compared to control muffins (6.6±0.51). This decrease in color score was due to the utilization of sorghum flour up to 50% which imparts darker color to muffins. Current color score of gluten-free muffins matched with the findings of Nazir et al. (2022) in which color score ranged from 6±0.75 to 7±0.82 for muffins containing water chestnut flour. The score for taste and texture of gluten-free muffins ranged from 4.8±0.78 to 7.4±1.26, and 5.1±0.87 to 7.3±0.82, respectively as compared to wheat muffins. They showed better results which are comparable to wheat which is the reason why panelists preferred gluten-free muffins. Similar findings have been reported by (Saliman and Malik, 2023) for gluten-free muffins containing rice flour and pumpkin powder. It was also observed that experimental treatments containing more proportion of sorghum flour give a moderately bitter taste. The flavor and aroma scores of gluten-free muffins ranged from 4.8±0.91 to 7.2±0.78, and 5.1±1.28 to 6.5±1.17 respectively as compared to the control (7.1). The findings of flavor of gluten-free muffins resemble the findings of (De Souza Nespeca et al. 2023) who reported flavor scores ranged from 6.77±0.2 to 7.84±0.16 for gluten-free orange-flavored cake. The firmness and overall acceptability scores of gluten-free muffins ranged from 4.5±0.78 to ±0.94, and 5.0±0.78 to 7.0±0.94 respectively as compared to wheat muffins (6.6±0.96, 7.2±0.78). Similar results have been documented by (Cervini et al. 2021) for gluten-free biscuits. The overall results indicate that T_6 showed the highest score of sensory properties which is close to control while T_2 showed the lowest score due to the addition of sorghum flour (50%) as the addition of a higher concentration of sorghum flour reduces elasticity and firmness as well as give unpleasant taste. The lack of gluten also resulted in a decrease in textural scores of multigrain gluten-free muffins.

4. CONCLUSION

The present study was conducted to develop gluten-free muffins by utilizing different concentrations of maize, sorghum, and chickpea flour. The aim of the study was to compare the traditional wheat-based muffins with the developed muffins. Significant variations in the nutritional and rheological properties of composite flour blends were observed. A significant increase in protein, crude fiber, ash, and mineral content and a decrease in NFE content was observed in the developed gluten-free muffins. This shows that a combination of maize (60%), sorghum (20%), and chickpea flour (20%) provides a good protein-energy balance for celiac patients. Further, a decrease in color (L*, a*, and b*) values and sensory scores was observed as compared to wheat muffins. Also, the developed muffins showed an increase in hardness during storage days (1^{st}, 7^{th}, 14^{th}, and 21^{st} days) while moisture content decreased. The overall findings suggest that the experimental treatment (T_6) had good mineral content, sensory score, and color values which are comparable to wheat muffins, indicating that this formulation is a suitable alternative to wheat flour for the bakery industry to develop gluten-free products. These results also encourage the utilization of underutilized cereals and legumes in developing gluten-free bakery products in order to close the nutritional gap.

Author Contributions

Mamoona Ahmad performed all research studies, Shabbir Ahmad designed the research study and supported in statistical analysis, Hira Jabeen and Ayesha Syed performed manuscript writing, Review and editing. The current research study presented in this manuscript is a part of the master thesis of the main/first author.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability Statement: The data regarding the findings of this research are available from the corresponding author upon reasonable request.

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