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# Properties and Qualities of Functional Gluten-Free Cupcake Fortification with Brown Rice and Red Beans Flour

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## ABSTRACT

This study aimed to evaluate gluten-free cupcakes made from white rice flour and formulations in which white rice flour was partially substituted with brown rice flour and red bean flour. The results showed that red beans had the highest protein and crude fiber content, while rice had the highest carbohydrate content. Meanwhile, brown rice and red beans exhibited higher antioxidant content and DPPH radical scavenging activity compared to white rice. Sensory evaluation revealed that all cupcake formulations provided the best crumb texture and crumb moistness. Additionally, differences in the color parameters of the cupcakes were observed, attributed to the characteristic pigments of brown rice and red beans. Regarding physical properties, a significant decrease in cupcake weight and volume was noted when red bean flour substitution reached 20% of the white rice flour. Color measurements indicated that gluten-free cupcakes made with brown rice and red beans had a darker color, while those made with white rice flour (control) had a lighter color. These findings were confirmed by peroxide value measurements during a four-week storage period. Based on the results, it can be concluded that white rice cupcakes and their formulations exhibited natural antioxidant activity and nutritional value. Moreover, substituting white rice with brown rice and red beans produced the best formulations. Therefore, the high levels of essential nutrients in the gluten-free cupcake formulations are strongly associated with health benefits.

## 1. Introduction

Due to growing consumer awareness of nutrition, food supplements have recently gained significant interest. One strategy to meet protein requirements is the supplementation of baked goods with beans, grains, and pulses. Protein-rich flours can be easily incorporated into cupcakes to create convenient meals that help boost nutrition and protein intake (Rebecca et al., 2016). Celiac disease (CD) and wheat allergy (WA) are the two most well-known human illnesses linked to gluten intake (Sapone et al., 2012). Celiac disease is characterized by small intestinal mucosal damage and nutritional malabsorption in genetically predisposed individuals after gluten consumption. The pathogenesis of CD involves an interplay of genetic, immunologic, and environmental factors (Kagnoff, 2005). The only safe and effective treatment for CD is lifelong adherence to a gluten-free diet. Another therapeutic approach includes modifying dietary components (Tack et al., 2010). The growing prevalence of gluten-related illnesses has drawn significant attention and increased the demand for gluten-free diets. This rising interest is fueled not only by individuals with gluten-related disorders but also by those who prioritize healthy lifestyles and choose gluten-free diets. Over the past five years, there has been a remarkable expansion in the availability of gluten-free bakery products. Gluten is typically found in traditional wheat-based foods such as breads, pastas, crackers, and baked goods. However, numerous gluten-free alternatives using various grains and flours are now available. However, numerous gluten-free alternatives using various grains and flours are now available. The major challenge remains the development of novel gluten-free products with acceptable sensory qualities.

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Gluten-free flours are generally more nutrient-dense than conventional wheat flour, often containing higher levels of protein, fiber, vitamins, and minerals. Consequently, gluten-free bakery products may contain lower amounts of unhealthy fats, refined carbohydrates, and sugars, making them a healthier option for those seeking a balanced diet and lifestyle. Gluten-free alternatives offer a vital option for individuals with celiac disease or gluten intolerance. The baking industry is increasingly recognizing the importance of offering gluten-free products to meet a wide range of dietary needs. The expanding gluten-free baking market presents opportunities for technological innovation and adaptation to evolving consumer preferences. Thus, incorporating a variety of gluten-free flours into the diet can enhance nutritional diversity, ultimately benefiting overall health and nutrition (Dharani Raja Sree and Sindhu, 2024). Because brown rice flour contains the bran, germ, and endosperm, it is considered a whole grain flour. Brown rice contains 366kcal, 7.5g of protein, 76.2g of fat, 2.8g of carbohydrates, and 8 mg of sodium (Akriti Thakur and Sontakke, 2023). Brown rice flour has been used in making muffins, cakes, cookies, cupcakes, bread, and pancakes. The best cupcake performance was achieved using a blend of 30.71% tapioca starch, 9.29% potato starch, and 60% high-protein brown rice flour (Sukhmandeep and Kaur, 2017). Legumes contain approximately three times more protein than grains, making them a highly valued global source of protein. In the human diet, legumes are a significant source of calories, protein, vitamins, and minerals (Abdallah et al., 2017). Red beans (*Phaseolus vulgaris L.*), a nutrient-rich food with diverse applications, are an excellent source of vegetable protein and both soluble and insoluble fiber (Lichtenstein, 2017). Red kidney beans (Phaseolus vulgaris) are among the most widely consumed whole grains globally and offer health benefits such as a reduced risk of colon cancer and heart disease. They are also a rich source of dietary fiber, both soluble and insoluble (Stoin et al., 2019). According to Viti et al. (2016), the only effective therapeutic option currently available for celiac disease is strict adherence to a gluten-free diet. Red bean flour and white rice flour provide different qualities to soft cookie products. A higher proportion of rice flour results in cookies with a lighter white-brown hue and a crunchier texture. Conversely, a higher proportion of red bean flour stiffens the cookies, giving them a brittle texture, a reddish-brown color, and a strong red bean flavor and aroma (Nur Yudiastuti et al., 2025). As the number of individuals with gluten sensitivity continues to rise, there is an increasing need for a broader variety of naturally gluten-free baking ingredients. Composite flours, combining different types and quantities of ingredients like corn flour, offer a promising approach to utilizing underused food sources with diverse qualities and characteristics. Given the growing demand for products tailored to celiac disease patients, this study evaluated the substitution of white rice with brown rice and red beans to create novel functional gluten-free products with high nutritional content, such as cupcakes.

# 2. Materials and Methods

## Materials

Brown (*Oryza sativa L.*) and white rice were obtained from the Rice Breeding Section of the Field Crops Research Institute, Agricultural Research Center, Egypt. Red bean variety (*Phaseolus vulgaris L.)* was purchased from the Field Crops Research Institute, Agricultural Research Center, Egypt. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Corn starch, butter, milk, sugar, eggs, baking powder, and vanilla were procured from a local market. Grains were rinsed and then immersed in warm distilled water (approximately 60°C) at a grain-to-water ratio of 1:3. This soaking process was repeated twice for two hours each to eliminate anti-nutritional factors, followed by drying in an air oven at 50°C.

After drying the rice and red beans were ground at 25,000rpm using a WK-1000A grinder (Qing Zhou Machinery Co., Ltd.) and then sieved through a 60-mesh screen. The resulting flour samples were stored at room temperature in polyethylene bags until further use, following the method of Akubor and Eze (2012).

# Methods

## Chemical Composition of Raw Materials

The proximate composition of white rice, brown rice, and red beans—including moisture, crude protein, lipid content, crude fiber, ash, and total carbohydrates was determined in triplicate according to the procedures of AOAC (2019).

## Determination of Mineral Content

The mineral contents (Ca, Mn, Fe, Zn, Cu, and Mg) were measured using an atomic absorption spectrophotometer. Sodium (Na) and potassium (K) levels were determined using a flame photometer, following the AOAC (2019) methods.

## Antioxidant Content of Raw Materials

The total phenolic content (TP) in red beans, brown rice, and white rice was measured using the Folin-Ciocalteu reagent method, and results were expressed as milligrams of gallic acid equivalents per 100g of dry weight (mg GAE/100g), following Qawasmeh et al. (2012). The total flavonoid content (TF) was determined according to Eghdami and Sadeghi (2010) and expressed as milligrams of quercetin equivalents per 100g of dry weight (mg QE/100 g).

## DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity of water-soluble extracts was evaluated using the method described by Lim and Quah (2007). In brief, 250µL of a 0.5mM DPPH methanolic solution was added to 1 mL of each extract. The mixtures were incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517nm. Scavenging activity was calculated, and the results were expressed as the $IC_{50}$ value (mg/mL), representing the concentration required to inhibit 50% of DPPH radicals.

## Preparation of Cupcakes from Raw Materials

Cupcake formulations were prepared using white rice, brown rice, and red beans according to the methods described by Rebecca et al. (2016) and Pathan et al. (2019), with modifications as detailed in Table 1.

**Table 1. Cupcakegluten-free formulas**

| Ingredients | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| White rice flour | 100g | 90g | 80g | 90g | 80 g | 80g |
| Corn Starch | 20g | 20g | 20g | 20 g | 20 g | 20g |
| Brown rice flour | — | 10g | 20g | — | — | 10g |
| Red beans flour | — | — | — | 10g | 20g | 10g |
| Butter | 45g | 45g | 45g | 45g | 45g | 45g |
| Milk | 45g | 45g | 45g | 45g | 45g | 45g |
| Salt | 0.5g | 0.5g | 0.5g | 0.5g | 0.5g | 0.5g |
| Sugar | 100g | 100g | 100g | 100g | 100g | 100g |
| Baking powder | 8g | 8g | 8g | 8g | 8g | 8g |
| Egg | 2 | 2 | 2 | 2 | 2 | 2 |
| Vanilla essence | 4g | 4g | 4g | 4g | 4g | 4g |

WRF: White rice flour, BRF: Brown rice flour, RBF:Red beans flour

## Methodology for Preparing Cupcakes

- Preparation of Materials: Gather all necessary ingredients.
- Cream Butter and Sugar: Mix butter with sugar until creamy.
- Add Wet Ingredients: Incorporate eggs, vanilla, and milk into the mixture.
- Sift Dry Ingredients: Sift dry ingredients and add them to the mixture.
- Bake: Place the mixture in paper cups and bake at 180°C for 12–15 minutes.

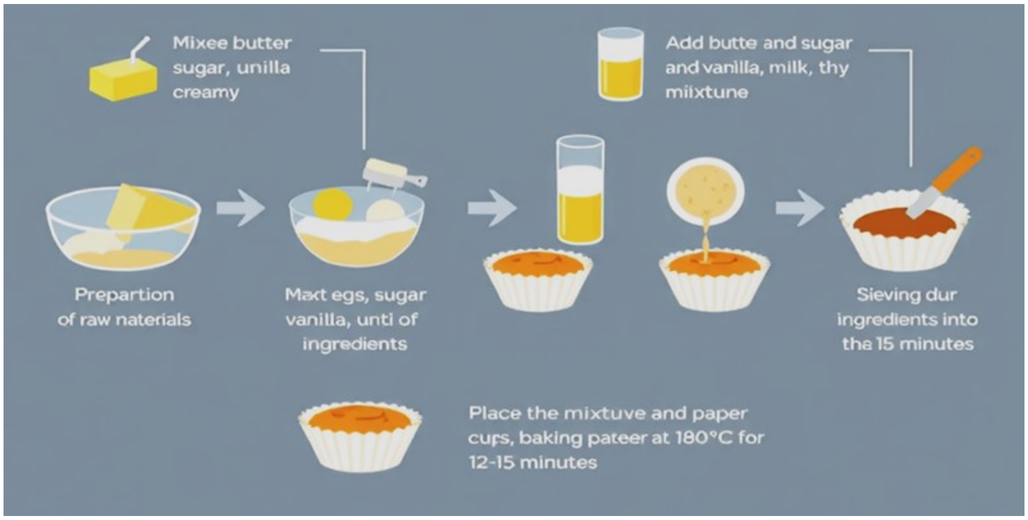


**Figure 1. Flowchart of the Cupcake Preparation Process**

## Nutritional Values of Cupcakes with Different Formulations

Cupcakes made from white rice, brown rice, and red beans were evaluated for their chemical composition, mineral content, antioxidant components (phenols and flavonoids), and antioxidant activity, as measured by DPPH assay.

## Sensory Evaluation of Gluten-Free Cupcakes and Their Formulations

According to Rosa et al. (2015), sensory qualities were evaluated using a 5-point hedonic scale. The aim of the sensory evaluation was to estimate the differences between each formulation and the control cupcake. Ten experienced employees from the Food Technology Research Institute, Agricultural Research Center, Egypt, evaluated the cupcakes and their formulations based on color, odor, crumb texture, moistness, taste, and overall acceptability.

## Physical Properties of Gluten-Free Cupcakes and Their Formulations

Moisture analysis was conducted following AOAC (2019) methods, with three independent measurements taken. Water activity (aw) of the products was measured using a Rotronic Hygrolab3 (CH-8303, Switzerland) according to Cadden (1988).

## Physical Characteristics of Gluten-Free Cupcakes and Their Formulations

According to Gaines (1991), the physical attributes of the cupcakes and their gluten-free formulations were assessed for weight (g), height ($cm^3$), and specific volume ($cm^3/g$). Specific volume was calculated as height divided by weight.

## Color Analysis of Gluten-Free Cupcakes and Their Formulations

The color of the cupcakes produced with different formulations was measured using a Hunter Lab color analyzer. A standard white tile/board was used for illuminant calibration. Color values were recorded as L* (0 = black, 100 = white), a* (+ = red, – = green), and b* (+ = yellow, – = blue) according to Saricoban and Yilmaz (2010).

## Instrumental Analyses of Gluten-Free Cupcakes and Their Formulations

Texture Profile Analysis (TPA) indices of the cupcakes and their formulations were determined using a Brookfield CT3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., MA 02346-1031, USA). Firmness, hardness, resilience, cohesiveness, springiness, chewiness, and gumminess were calculated from the force-time curve according to Gomez et al. (2007).

## Determination of Peroxide Value of Gluten-Free Cupcakes and Their Formulations

After being dry-blended into a powder, the samples were stored at 4°C in airtight bags. Oil was extracted from the samples using the Soxhlet method with petroleum ether (boiling point 40–60°C). The extracted oil was collected in 20 mL dark bottles. Throughout a 28-day storage period, the peroxide

(PV) was determined according to AOAC (2019) and expressed as mg KOH/g oil.

## Statistical Analysis

The collected data were evaluated using ANOVA, and all variables showed significant differences ($p < 0.05$). Mean comparisons were performed to determine the differences among samples, using the SAS System for Windows (SAS, 2008).

## 3. Results and Discussion

Table 2 presents the proximate analysis of the selected rice samples. Overall, carbohydrate content was the highest in both white and brown rice (exceeding 80%), as carbohydrates are the primary component of rice. Moisture content was the second most abundant component in both types, ranging from 11.15% to 11.91%. Moisture plays a crucial role in determining the shelf life of rice. Protein and ash contents were the lowest in both white and brown rice. Fat content ranged from 1.57% to 1.98%. Additionally, rice was found to be a good source of fiber, with the highest fiber content recorded between 4.07% and 4.38%. Regarding the mineral and chemical composition of the raw materials, the removal of the pericarp reduces the levels of lipids, protein, fiber, and ash, which in turn decreases the total sugar and carbohydrate contents, as well as trace amounts of vitamins, free amino acids, and free fatty acids (Zhou et al., 2002). The nutrient profile of red beans was also analyzed, including protein, fat, fiber, carbohydrate, and mineral contents. The gross chemical composition of red bean powder is shown in Table 2. The results showed that protein, fat, fiber, ash, carbohydrate, and moisture contents were 22.39, 1.40, 10.53, 4.28, 61.40, and 10.15g/100g, respectively. Compared to white and brown rice, red beans had higher levels of protein, crude fiber, and ash. However, white and brown rice contained higher fat and carbohydrate contents than red beans. Legumes, including red beans, are widely utilized worldwide due to their potential to improve the nutritional quality of diets, particularly for low-income populations. They are also considered a key source of protein in Egyptian diets (Ahmed et al., 2020). Red beans (*Phaseolus vulgaris L.*) are nutritionally valuable as they are rich in protein, crude fiber, carbohydrates, folic acid, and minerals such as iron, potassium, phosphorus, and manganese. However, the presence of anti-nutritional factors can reduce the bioavailability of these nutrients, thereby diminishing the overall nutritional quality of red beans (Kamboj and Nanda, 2018). The same table shows that the mineral contents (K, Na, Ca, Mn, Fe, Zn, Cu, and Mg) were lower in white rice, with values of 95.36, 101.68, 266.12, 21.14, 42.61, 36.26, 0.41, and 26.52mg/100g, respectively, compared to brown rice, which contained 105.24, 136.85, 390.25, 31.65, 51.43, 42.31, 0.80, and 35.36mg/100g, respectively. In particular, calcium, sodium, and potassium contents were significantly higher in brown rice (390.25, 136.85, and 105.24mg/100g, respectively) compared to white rice (266.12, 101.68, and 95.36 mg/100g). Similarly, the levels of iron, zinc, magnesium, and manganese were higher in brown rice (51.43, 42.31, 35.36, and 31.65mg/100g) than in white rice (42.61, 36.26, 26.52, and 21.14mg/100g), respectively. These findings are consistent with those of Lamberts et al. (2007), who reported that brown rice contains greater amounts of dietary fiber, proteins, lipids, vitamins, and minerals compared to white rice. According to Liang et al. (2008), the mineral content in rice products follows the descending order: rice bran > brown rice > white rice, primarily due to their distribution within the kernel and the impact of processing. White rice is essentially brown rice with the bran and germ removed, making it deficient in minerals, lipids, fiber, B vitamins, antioxidants, and small amounts of protein. Consequently, many types of white rice are fortified to compensate for the nutrient loss during processing. Rice, in general, is well tolerated and easy to digest. Both brown and white rice are naturally gluten-free. Interestingly, when rice is cooled after cooking, the amount of resistant starch a type of beneficial fiber increases, even if the rice is reheated later. This type of fiber may promote intestinal health. Although brown rice contains more total fiber, both white and brown rice exhibit increased resistant starch levels after cooling (Khalua et al., 2019). The analysis method used in this study did not detect lead in any of the rice varieties tested, indicating that the rice samples analyzed are safe for consumption. Lead and cadmium are known to be harmful to human health. The mineral contents of red beans are also presented in Table 2. Red beans were found to be particularly rich in potassium, calcium, sodium, and magnesium. Among the minerals measured, potassium was the most abundant, with a value of 708.25mg/100g, followed by calcium at 464.79mg/100g. Potassium is essential for maintaining pH balance and supporting healthy protein and carbohydrate metabolism (Onibon et al., 2007). Red beans, therefore, serve as an excellent source for meeting daily potassium requirements. According to Beto (2015), calcium is a macro-element critical for tooth and bone development, as well as hormonal regulation. The magnesium content of red beans was 272.22mg/100g, while the sodium content measured 115.76mg/100g. Manganese, iron, zinc, and copper contents were 45.26, 8.63, 7.47, and 2.53mg/100g, respectively. According to Kwaimgoin et al. (2018), magnesium is essential for bone development and helps maintain electrical potential in nerve cells. It also plays a vital role in insulin secretion and activity. Although sodium occurs naturally in foods, it is typically consumed as sodium chloride (table salt). Nevertheless, sodium levels in beans remain within recommended dietary limits when no additional salt is added (Wardlaw et al., 2004). Iron plays a key role in blood formation and in transporting oxygen and carbon dioxide between tissues. Iron deficiency, particularly in children, can result in behavioral issues, learning difficulties, and anemia (Mananga et al., 2021). According to Brigide et al. (2014), zinc is crucial for maintaining healthy hair and is essential for the proper function of taste and smell. Copper, another essential mineral found throughout the body, supports the immune system, maintains nerve cells, and aids in red blood cell production. As an antioxidant, copper also helps minimize DNA and cellular damage caused by free radicals (Yumei et al., 2022). Furthermore, red beans are recognized as a rich source of plant-based protein, soluble and insoluble fibers, minerals, and vitamins, all of which are essential for maintaining healthy body cells (Mullins and Arjmandi, 2021).

**Table 2. Proximate chemical and minerals content of White rice flour, Brown rice flour and red beans flour**

| Raw Martial / Chemical Composition (g/100g) | WRF | BRF | RBF |
|---|---|---|---|
| Moisture | 11.15±0.81$^{a}$ | 11.91±0.92$^{a}$ | 10.15±0.73$^{b}$ |
| Protein | 6.11±0.42$^{b}$ | 6.59±0.43$^{b}$ | 22.39±0.41$^{a}$ |
| Fat | 1.57±0.02$^{b}$ | 1.98±0.02$^{a}$ | 1.40±0.02$^{b}$ |
| Fiber | 4.07±0.38$^{b}$ | 4.35±0.31$^{b}$ | 10.53±0.76$^{a}$ |
| Ash | 1.68±0.01$^{b}$ | 1.83±0.01$^{b}$ | 4.28±0.12$^{a}$ |
| Carbohydrates | 86.57±4.27$^{a}$ | 85.25±4.39$^{a}$ | 61.40±4.22$^{b}$ |
| **Raw Martial / Minerals content (mg/100g)** | **WRF** | **BRF** | **RBF** |
| K | 95.36±3.21$^{c}$ | 105.24±4.86$^{b}$ | 708.25±10.28$^{a}$ |
| Na | 101.68±5.32$^{c}$ | 136.85±5.42$^{a}$ | 115.76±4.68 b |
| Ca | 266.12±7.96$^{c}$ | 390.25±9.35$^{b}$ | 464.79±10.28$^{a}$ |
| Mn | 21.14±0.09$^{c}$ | 31.65±0.08$^{b}$ | 45.26±0.09$^{a}$ |
| Fe | 42.61±1.02$^{b}$ | 51.43±1.05$^{a}$ | 8.63±0.08$^{c}$ |
| Zn | 36.26±1.07$^{b}$ | 42.31±1.04$^{a}$ | 7.47±0.04$^{c}$ |
| Cu | 0.41±0.02$^{c}$ | 0.80±0.04$^{b}$ | 2.53±0.07$^{a}$ |
| Mg | 26.52±1.05$^{c}$ | 35.36±1.07$^{b}$ | 272.22±7.26$^{a}$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$)
WRF= White rice flour BRF = Brown rice flour RBN= Red beans flour

## Antioxidant Activity of Raw Materials

Table 3. shows that the polyphenolic content in brown rice was 225.27mg GAE/100g, whereas in white rice, it was 149.03mg GAE/100g. Thus, the polyphenolic content in brown rice was approximately 1.5 times higher than that in white rice. The flavonoid contents of brown and white rice were also significantly different, as indicated in the same table. Brown rice contained 157.86mg QE/100g, while white rice contained 60.57mg QE/100g. Additionally, the phenolic and flavonoid contents in red beans were determined and are also reported in Table 3. The results showed that red beans contained 228.29 mg GAE/100 g of phenolic compounds and 178.39 mg QE/100 g of flavonoids. Phenolic compounds, also known as secondary plant metabolites, play important biological roles. Flavonoids, one of the major families of phenolic compounds, consist of 15 carbon atoms arranged in two aromatic rings linked by a three-carbon chain. They are the most common anthocyanidins and are largely responsible for the red, pink, and purple colors in plants, which help attract pollinators and seed dispersers. The phenolic content in grains is associated with pericarp color, with red and black pericarp grains typically containing higher levels. According to Goffman and Bergman (2004), grains with a dark purple pericarp contain more anthocyanins and polyphenols than those with red-brown pericarps. Key parameters analyzed included proximate composition, anthocyanin content, and antioxidant activity. The antioxidant capacity was assessed using the DPPH assay, and results were expressed as $IC_{50}$ values (the concentration required to inhibit 50% of DPPH radicals). A lower $IC_{50}$ value indicates higher antioxidant activity. As shown in Table 3, brown rice exhibited the highest scavenging activity, with an $IC_{50}$ value of 25.76±0.95mg/mL, followed by white rice at 55.79±1.36mg/mL. Since a lower $IC_{50}$ signifies stronger antioxidant activity, brown rice was richer in antioxidants than white rice. These results were higher than those reported for sorghum, which showed a maximum antioxidant activity of 21.02±5.17mg/g Trolox equivalent (Rao et al., 2018). However, the $IC_{50}$ value for Bangladeshi rice cultivars was reported to be between 6.01–14.47mg/mL in a previous study by Dutta et al. (2012), which is lower than the values observed in this study. This variation may be attributed to the use of methanol as the extraction solvent and differences among rice cultivars. The DPPH assay was also employed to assess the antioxidant potential of red bean extracts. A strong correlation was observed between phenolic content and free radical scavenging activity. Zhao et al. (2014) also reported significant variation in antioxidant activity and phenolic content across different bean extracts. The $IC_{50}$ value of red bean extract was found to be 24.59 mg/mL. The anthocyanin and phenolic contents of rice are generally linked to their antioxidant properties. Higher levels of these compounds correspond to increased antioxidant activity. According to Nam et al. (2006), grains with red and black pericarps tend to show greater antioxidant activity compared to those with light brown pericarps. In a study by Lee (2010), the antioxidant efficiency of white and brown rice extracts was evaluated using the DPPH assay. The results showed a dose-dependent increase in DPPH scavenging activity, indicating that higher extract concentrations resulted in greater antioxidant effects.

**Table 3. Antioxidant activity of white rice, brown rice and red beans flours**

| Test / Raw Martial | WRF | BRF | RBF |
|---|---|---|---|
| Total phenolics (mgGAE/100g, d.w.) | 149.03±8.38$^{c}$ | 225.27±10.15$^{b}$ | 228.29±7.17$^{a}$ |
| Flavonoids compounds(mg QE/100g,d.w.) | 60.57±1.29$^{c}$ | 157.86±5.49$^{b}$ | 178.39±2.64$^{a}$ |
| Antioxidant activityDPPH IC50 (mg/ml) | 55.79±1.36$^{a}$ | 25.76±0.95$^{b}$ | 24.59±0.87$^{c}$ |

Data are mean ± standard deviation (n = 10). Values with different superscript letters within a raw are significantly different ($p \leq 0.05$)

## Sensory Evaluation of Gluten-Free Cupcakes and Their Formulations

The sensory characteristics of cupcakes made from white rice flour and formulations incorporating brown rice and red bean flours at different levels were evaluated, and the results are presented in Table 4 and Figure 2. The findings showed no significant differences among the various formulations. Rice plays a crucial role in the development of gluten-free baked products. Depending on the proportions of brown rice and red beans added to white rice flour, the quality of the resulting cupcakes varied. The results indicated that mixing brown rice flour with white rice flour at levels of 10% and 20% yielded optimal crumb texture and moisture content. However, the 80:20 formulations (80% white rice, 20% brown rice or red bean) resulted in a deeper brown color and an unsatisfactory flavor, although the aroma remained similar to the control cupcake. Color is the first sensory attribute perceived by panelists, and visually appealing colors can influence liking even before tasting the product (Amalia and Auli, 2017). Taste is the most critical factor in determining product acceptance, especially when flavor is a key marketing attribute. Regardless of good aroma, texture, or appearance, a product will be rejected if the taste is not favorable (Deglas, 2018). Aroma, influenced significantly by the processing method, helps panelists determine whether to accept or reject a product (Deglas, 2018). Texture is another key sensory parameter that reflects product acceptability through the sense of touch and mouthfeel. It includes attributes such as moisture, dryness, hardness, smoothness, roughness, and oiliness (Noviyanti et al., 2016). Texture is influenced by the product's content of water, protein, carbohydrates, and fat (Deglas, 2018). The best results for crumb texture, moisture, and flavor were observed when 10–20% red bean flour was added to white rice flour. In this case, the cupcakes developed a glossy red hue, and the 80:20 formulation showed a deeper red color along with a slightly reduced fragrance. The intense brown and red colors in some formulations may be attributed to the high levels of flavonoids and phenolic compounds particularly anthocyanins present in red beans and brown rice (Furukawa et al., 2007). These compounds contribute to the pigmentation and antioxidant properties of the raw materials. Compared to the control cupcake, the formulation containing 80% white rice, 10% brown rice, and 10% red bean flours produced moderate results in sensory evaluation. The variations in color across the different formulations were primarily due to the natural pigments present in brown rice and red bean flours.

**Table 4. Sensory evaluation of gluten-free Cupcake formulas with Brown rice and Red beans flour**

| Sensory Attribute \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Color | 5.0±0.27$^{a}$ | 3.0±0.09$^{b}$ | 2.5±0.08$^{c}$ | 4.2±0.14$^{a}$ | 3.5±0.12$^{b}$ | 3.0±0.09$^{b}$ |
| Aroma | 4.9±0.21$^{a}$ | 4.0±0.12$^{a}$ | 3.5±.09$^{b}$ | 3.5±0.11$^{b}$ | 3.0±0.09$^{b}$ | 3.0±0.07$^{b}$ |
| Crumb texture | 4.8±0.23$^{a}$ | 4.0±0.12$^{a}$ | 4.0±0.12$^{a}$ | 4.2±0.16$^{a}$ | 4.0±0.15$^{a}$ | 3.5±0.11$^{a}$ |
| Crumb moistness | 4.8±0.21$^{a}$ | 4.0±0.13$^{a}$ | 3.7±0.09$^{ab}$ | 4.2±0.14$^{a}$ | 3.7±0.16$^{ab}$ | 3.5±0.12$^{b}$ |
| Tasted | 5.0±0.25$^{a}$ | 3.0±0.08$^{b}$ | 2.5±0.08$^{c}$ | 4.0±0.13$^{ab}$ | 3.5±0.14$^{b}$ | 3.0±0.09$^{b}$ |
| Overall acceptability | 4.9 | 3.60 | 3.24 | 3.62 | 3.54 | 3.20 |

Data are mean ± standard deviation (n = 10). Values with different superscript letters within a raw are significantly different ($p \leq 0.05$)

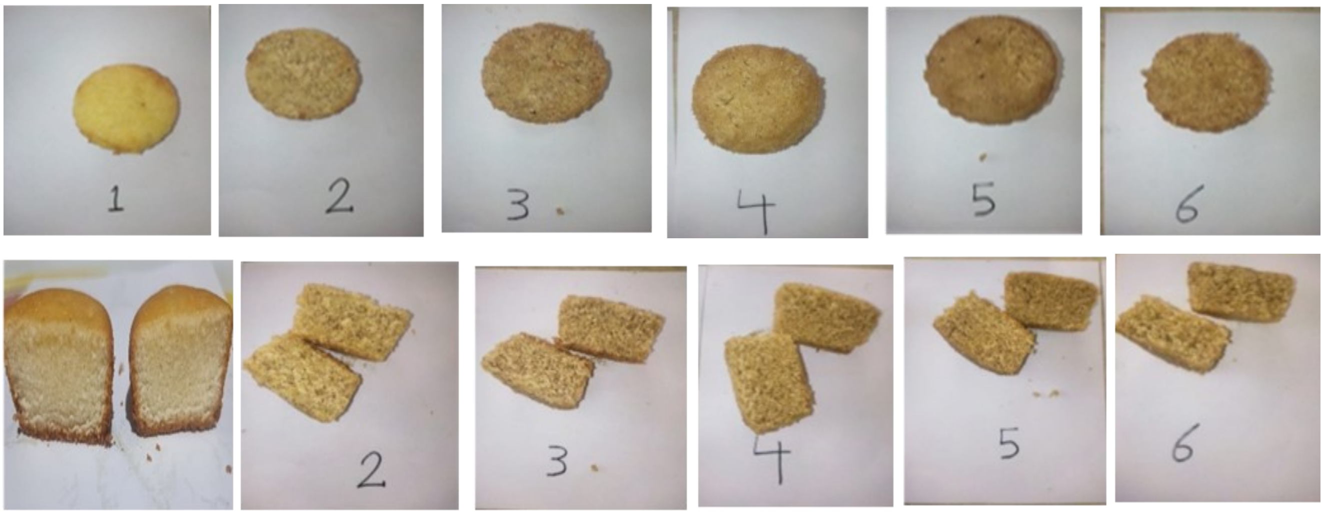


**Figure 2. Cupcake**

1= control white rice, 2 and 3= White rice and brown (90:10) and (80:20), 4 and 5= White rice and red beans (90:10) and (80:20) and 6 = White and brown rice and red beans= (8:10:10).

## Chemical compositions of cupcake and their formulas gluten free

## Nutritional value of cupcakes made from White rice and their formulations with brown rice and red beans

The nutritional values of cupcakes made from white rice flour and those substituted with brown rice and red beans at various levels are presented in Table 5. The results showed that the cupcake formula containing white rice and red bean flour in an 80:20 ratio had the highest protein content (9.36g/100g), followed by the mixed formula (white rice, brown rice, and red beans) with 7.45g/100g protein. The formulations containing white rice and red beans (90:10) and white rice with brown rice (80:20) had protein values of 7.95g/100 g and 9.36g/100g, respectively. Additionally, the white rice and brown rice (90:10) formulation and the control cupcake made from 100% white rice recorded protein contents of 7.95g/100g and 6.08 g/100g, respectively. These results suggest that the increased protein levels in the red bean and mixed formulations are due to the higher protein content of red beans (22.39g/100g, as shown in Table 2), compared to the lower protein contents of white and brown rice. Furthermore, crude fiber and ash contents increased in cupcake formulations as the fiber content of red beans increased. Conversely, total carbohydrate levels decreased as the fiber content rose in cupcakes containing brown rice and red beans. There was no significant variation in fat and moisture contents across all cupcake formulas, as the same amount of butter was used in each, and the raw materials (rice and red bean flours) were naturally low in fat. Red bean and rice flours provide diverse qualities to soft baked products. Products made with a higher proportion of rice flour tend to have a lighter color and a crunchier texture. In contrast, formulations richer in red bean flour result in a firmer, more brittle texture, a reddish-brown color, and a stronger red bean aroma and flavor (Yudiastuti et al., 2025). Fiber plays a vital role in maintaining digestive health. It slows the absorption of sugars in the intestines, helping to regulate blood sugar levels and reduce insulin spikes—factors associated with obesity and an increased risk of diabetes (Muthyala et al., 2022). The same table also includes measurements of water activity in the cupcake samples. The data indicated no significant difference in water activity between the formulations and the control. This consistency may be attributed to the starch characteristics of the rice used. The pasting properties of rice flour are influenced by the amylopectin content, which is capable of hydrogen bonding. Higher amylopectin content is positively correlated with peak viscosity, suggesting a greater water-holding capacity in the flour (Ye et al., 2016).

**Table 5. Physic-chemical analysis of Gluten-free Cupcake Fortified with Brown rice and Red beans flour on dry weigh (g/100g)**

| Chemical Composition \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Moisture | 10.35±0.84 $^{b}$ | 10.27±0.83$^{b}$ | 10.39±0.81 $^{b}$ | 11.05±0.92$^{a}$ | 11.38±0.96$^{a}$ | 11.78±0.94$^{a}$ |
| Total protein | 6.08±0.42$^{c}$ | 7.95±0.48 $^{b}$ | 9.36±0.67 $^{a}$ | 7.95±0.54 $^{b}$ | 9.36±0.71$^{a}$ | 7.45±0.62$^{b}$ |
| Fat | 10.24±0.86$^{a}$ | 10.19±0.83 $^{a}$ | 10.28±0.91$^{a}$ | 10.36±0.93$^{a}$ | 10.29±0.97$^{a}$ | 10.42±0.92$^{a}$ |
| Fiber | 4.00±0.05 $^{c}$ | 4.21±0.05$^{c}$ | 4.35±0.04$^{c}$ | 5.62±0.05$^{b}$ | 6.71±0.05$^{a}$ | 4.94±0.04$^{c}$ |
| Ash | 1.53±0.02$^{b}$ | 1.73±0.02$^{b}$ | 1.93±0.01$^{b}$ | 2.25±0.02$^{a}$ | 2.73±0.02$^{a}$ | 2.08±0.02$^{a}$ |
| Carbohydrates | 76.85±7.21$^{a}$ | 75.92±7.57$^{a}$ | 74.08±7.21$^{b}$ | 73.82±6.23$^{b}$ | 70.91±5.36$^{c}$ | 75.11±7.78$^{a}$ |
| Water activity | 0.854±0.04$^{a}$ | 0.863±0.03$^{a}$ | 0.849±0.04$^{a}$ | 0.853±0.04$^{a}$ | 0.843±0.03$^{a}$ | 0.839±0.04$^{a}$ |

Data are mean ± standard deviation (n = 10). Values with different superscript letters within a raw are significantly different ($p \leq 0.05$)

## Total Phenolic, Flavonoid Compounds, and Antioxidant Activity of Gluten-Free Cupcakes

Total phenolic content, flavonoid compounds, and antioxidant activity (measured via DPPH radical scavenging assay) were evaluated in cupcakes made from white rice flour and their formulations incorporating brown rice and red beans at various substitution levels. The results are summarized in Table 6. The findings revealed that the total phenolic content (expressed as mg GAE/100g dry weight) and flavonoid content (expressed as mg QE/100g dry weight) were comparable across the different cupcake formulations. This can be attributed to the naturally high levels of phenolic and flavonoid compounds in both brown rice and red beans. Moreover, antioxidant activity, measured by the DPPH assay and expressed as $IC_{50}$ (mg/mL), showed that cupcakes made with brown rice and red beans exhibited the highest antioxidant activity (i.e., the lowest $IC_{50}$ values). This strong antioxidant potential is consistent with the high phenolic and flavonoid contents in these raw materials.

It is well-established that phenolic compounds are potent antioxidants and effective scavengers of free radicals (Peng et al., 2017). Numerous studies have confirmed a significant positive correlation between the total phenolic content and antioxidant activity (Chen et al., 2015). Although other bioactive compounds such as phytosterols also contribute to antioxidant capacity, polyphenols remain the primary antioxidant agents in rice (Ragaee et al., 2014). According to Podio et al. (2017), these bioactive antioxidants play a preventive and protective role against chronic diseases associated with oxidative stress and excessive free radical generation in the body.

**Table 6. Total phenolic, Flavonoids and Antioxidant activity blends of Cupcake**

| Test \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Totalphenolics (mgGAE/100g,d.w.) | 138.86±7.25$^{c}$ | 156.64±5.49$^{b}$ | 164.43±6.25$^{a}$ | 156.93±4.68$^{b}$ | 167.09±5.89$^{a}$ | 159.38±6.14$^{b}$ |
| Flavonoids compounds (mgQE/100g,d.w.) | 62.59±0.86$^{c}$ | 69.71±0.85$^{c}$ | 79.58±0.68$^{b}$ | 71.82±0.71$^{b}$ | 83.63±0.76$^{a}$ | 74.21±0.68$^{b}$ |
| Antioxidant activity DPPH IC50 (mg/ml) | 45.28±0.32$^{a}$ | 40.12±0.31$^{a}$ | 34.25±0.27$^{c}$ | 39.51±0.30$^{b}$ | 33.19±0.28$^{c}$ | 30.24±0.27$^{c}$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$)

Table 7 presents the physical characteristics of the gluten-free cupcake formulations. The data indicate that the control sample made from 100% white rice flour exhibited the highest specific volume among all tested formulations. In comparison, cupcakes with red bean flour substitution showed lower specific volume values, while those formulated with brown rice flour had specific volumes closer to the control. A notable decrease in both weight and volume was observed as the substitution level of red bean flour increased to 20%. Similarly, the specific volume showed a slight but consistent reduction with increasing levels (10% and 20%) of brown rice flour replacing white rice flour. Specific volume is an important indicator of baked product quality, as higher values are typically associated with a softer and more aerated crumb structure (Capriles and Arêas, 2013). The presence of proteins in red beans may have contributed to the batter's ability to retain more carbon dioxide during mixing. Additionally, the protein's potential for cross-linking might have improved the batter's viscoelastic properties, thereby enhancing gas retention during baking (Yano, 2019).

**Table 7. Physical properties of Gluten-free Cupcake different formulas**

| Physical properties \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Weight g | 39.83±0.47$^a$ | 39.67±0.11$^a$ | 39.17±0.28$^a$ | 39.33±0.11$^a$ | 38.5±0.18$^a$ | 37.15±0.17$^a$ |
| Volume $cm^3$ | 100.7±0.88$^a$ | 93.6±0.57$^b$ | 88.20±1.15$^c$ | 86.00±1.15$^c$ | 82.23±1.61$^d$ | 81.23±1.25$^d$ |
| Specific volume $g/cm^3$ | 2.53±0.09$^a$ | 2.36±0.04$^b$ | 2.25±0.07$^c$ | 2.19±0.08$^d$ | 2.16±0.05$^e$ | 2.18±0.04$^d$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$)

## Color Measurements of Gluten-Free Cupcakes

Color is one of the most critical sensory attributes that directly influences consumer acceptance of food products. In bakery items, visual appeal plays a key role in attracting consumers (Krupa-Kozak et al., 2019). The color of cupcakes made from white rice flour and their formulations incorporating brown rice and red beans at various substitution levels was analyzed using the CIELAB color space system (L*a*b*). In this system, L* represents lightness (ranging from 0 = black to 100 = white), a* indicates the red-green axis (positive values = red, negative = green), and b* reflects the yellow-blue axis (positive values = yellow, negative = blue). The corresponding data are summarized in Table 8. The results demonstrated that the L*, a*, and b* values of cupcake formulations substituted with brown rice and red beans were significantly different from those of the control (100% white rice flour). The control sample exhibited significantly higher L* and b* values, indicating greater lightness and yellowness, respectively, compared to the other formulations. In contrast, the increased presence of brown rice and red beans led to reductions in lightness and yellowness and a noticeable increase in redness (a* value), which can be attributed to the inherent deep brown and red pigments of these ingredients. The observed darker coloration in cupcakes containing brown rice and red beans may also be linked to a higher degree of Maillard browning reactions, likely due to the higher protein content of these flours (Paz et al., 2020). In comparison, the control sample made solely from white rice flour appeared significantly lighter in color.

**Table 8. Color of gluten-free Cupcake different formulas**

| Color parameters \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| L* Lightness | 60.24±0.96$^a$ | 50.58±0.46$^b$ | 41.87±0.36$^c$ | 52.02±0.45$^b$ | 51.50±0.51$^b$ | 49.42±0.46$^c$ |
| a* Redness | 12.57±0.21$^c$ | 13.88±0.21$^b$ | 14.09±0.14$^a$ | 12.85±0.13$^c$ | 13.85±0.12$^b$ | 13.72±0.17$^b$ |
| b* Yellowness | 29.36±0.25$^a$ | 24.41±0.24$^b$ | 21.25±0.20$^c$ | 27.71±0.22$^{ab}$ | 24.76±0.32$^b$ | 25.56±0.28$^{ab}$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$)

## Instrumental Analysis of Gluten-Free Cupcakes

Texture Profile Analysis (TPA) was conducted on gluten-free cupcakes made with 100% white rice flour (control) and formulations substituted with brown rice and red bean flour at 10% and 20% levels. The results are presented in Table 9. The inclusion of white rice flour influenced the structural characteristics of the cupcake batter by diluting the overall protein content and reducing the firmness of the gluten-free cupcakes formulated with red bean flour. This can be explained by the lower amylose content in rice flour (approximately 25%) compared to red bean flour (34–41%) (Zhou et al., 2002), resulting in reduced starch retrogradation and a softer crumb texture. The dilution of amylose concentration through rice flour addition contributes to decreased hardness in the final baked product. Increased batter viscosity, as supported by prior studies, facilitates gas entrapment during mixing, leading to a more aerated crumb structure, greater cake volume, thinner cell walls, and lower overall firmness (Wilderjans et al., 2008). Hardness, as measured by a texture analyzer, reflects the force required to compress the cupcake. According to Purnomo (2014), it represents the force applied by the teeth until the food breaks. The control cupcake (100% white rice) showed a hardness value of 27.00N. In comparison, cupcakes with 10% and 20% brown rice substitution recorded values of 25.38N and 29.58N, respectively. Cupcakes substituted with red beans at 10% and 20% had higher hardness values of 29.33N and 31.00N. Interestingly, a formulation containing 10% substitution of both brown rice and red beans yielded the lowest hardness value at 21.97N. The increase in hardness with red bean flour addition could be attributed to its higher fiber content, which retains water and reduces evaporation, thereby increasing structural rigidity (Solaka and Zain Al Abidin, 1995). Additionally, Hapsari and Niken (2018) noted that a high starch content can lead to a denser and more complex texture. The elevated protein content of red bean flour may also enhance its water-binding capacity, further influencing firmness. Springiness, which describes the ability of the cupcake to recover its shape after compression, remained relatively unchanged across formulations and was even slightly higher in some cases. This increase could be due to protein aggregation, resulting in greater elasticity (Shevkani and Singh, 2014). However, resilience and cohesiveness slightly declined in the substituted formulations compared to the control. Resilience dropped from 0.21 $mm^3$ in the control to 0.19$mm^3$, and cohesiveness decreased from 0.40 $mm^3$ to 0.38$mm^3$. These reductions may be due to the lower protein and higher fiber content in the substituted flours (Bhol and Bosco, 2014). Cohesiveness is the measure of how well the sample withstands a second deformation relative to the first, reflecting internal bonding (Chevanan et al., 2006). Chewiness, defined as the energy required to chew the sample until it is ready for swallowing (Bertolino et al., 2011), was highest in cupcakes containing red bean flour. Conversely, white rice and brown rice cupcakes had the lowest gumminess and chewiness values, which correlate closely with hardness. A proportional relationship between chewiness and hardness was confirmed, as reported by Ozawa et al. (2009).

## Peroxide Value in Gluten-Free Cupcake Formulas

The peroxide value (PV) is a key indicator for assessing the primary oxidation of lipids, as it measures the concentration of hydroperoxides, which are early byproducts of lipid peroxidation (Huang et al., 2021). As shown in Table 10, enrichment of cupcake formulations with brown rice and red bean flour influenced the oxidative stability of the products. Throughout the storage period, the PV of all cupcake samples increased progressively, indicating gradual lipid oxidation over time. Notably, the control sample made with 100% white rice flour exhibited the highest peroxide value after four weeks of storage, rising from 1.35mg KOH/g oil at time zero to 13.28mg KOH/g oil. In contrast, cupcakes containing 10% and 20% red bean flour demonstrated the lowest peroxide values at the end of the storage period, followed by the cupcakes enriched with brown rice. This improved oxidative stability is likely due to the high phenolic and flavonoid contents present in red beans, which act as natural antioxidants. These compounds help inhibit the formation of lipid peroxides by interrupting the radical chain reactions that occur during lipid oxidation. Specifically, antioxidants can either scavenge free radicals, preventing hydroperoxide formation during the propagation phase, or inhibit hydroperoxide decomposition into secondary oxidation products such as aldehydes (Seppanen et al., 2010).

**Table 9. Texture properties of Gluten-free Cupcake formulas**

| Texture properties \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Firmness g | 2970±5.28$^{a}$ | 2254±2.24$^{a}$ | 2036±2.76$^{a}$ | 1709±3.12$^{b}$ | 1619±3.38$^{b}$ | 1968±4.29$^{b}$ |
| Hardness N | 27.00±0.21$^{b}$ | 25.38±0.19$^{b}$ | 29.58±0.34$^{a}$ | 29.33±0.48$^{a}$ | 31.00±0.51$^{a}$ | 21.97±0.42$^{c}$ |
| Resilience | 0.21±0.04$^{a}$ | 0.20±0.04$^{a}$ | 0.19±0.03$^{b}$ | 0.19±0.03$^{b}$ | 0.19±0.03$^{b}$ | 0.19±0.02$^{b}$ |
| Cohesiveness (mm3) | 0.40±0.05$^{a}$ | 0.39±0.03$^{b}$ | 0.40±0.02$^{a}$ | 0.38±0.04$^{c}$ | 0.40±0.04$^{a}$ | 0.38±0.03$^{c}$ |
| Springiness (mm) | 7.58±0.13$^{a}$ | 7.08±0.05$^{a}$ | 7.80±0.04$^{a}$ | 7.99±0.05$^{a}$ | 7.37±0.06$^{a}$ | 7.36±0.04$^{a}$ |
| Chewiness (mj) | 120.50±1.17$^{b}$ | 103.10±0.91$^{c}$ | 135.4±0.82$^{ab}$ | 145.70±0.97$^{a}$ | 148.7±1.01$^{a}$ | 96.30±0.91$^{c}$ |
| Gumminess (N) | 15.90±0.17$^{c}$ | 14.66±0.14$^{d}$ | 17.35±0.17$^{c}$ | 18.24±0.25$^{ab}$ | 17.29±0.21$^{b}$ | 13.08±0.35$^{a}$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$).

**Table 10. Peroxide value in Gluten-free Cupcake formulas during storage period**

| Peroxide value \ Formulas | WRF 100:00 | BRF 90:10 | BRF 80:20 | RBF 90:10 | RBF 80:20 | Mix 80:10:10 |
|---|---|---|---|---|---|---|
| Zero time | 1.35±0.04$^{a}$ | 1.35±0.04$^{a}$ | 1.35±0.04$^{a}$ | 1.35±0.04$^{a}$ | 1.35±0.04$^{a}$ | 1.35±0.04$^{a}$ |
| 7 Days | 3.52±0.05$^{a}$ | 2.98±0.03$^{b}$ | 2.54±0.02$^{b}$ | 2.57±0.04$^{b}$ | 2.15±0.03$^{b}$ | 2.94±0.02$^{b}$ |
| 14 Days | 7.28±0.13$^{a}$ | 4.39±0.07$^{b}$ | 3.98±0.06$^{c}$ | 4.09±0.05$^{b}$ | 3.57±0.02$^{c}$ | 4.58±0.03$^{b}$ |
| 21 Days | 10.51±0.28$^{a}$ | 5.98±0.14$^{b}$ | 5.45±0.16$^{b}$ | 5.13±0.09$^{b}$ | 4.52±0.07$^{c}$ | 5.69±0.06$^{b}$ |
| 28 Days | 13.28±0.34$^{a}$ | 7.57±0.17$^{b}$ | 7.19±0.16$^{b}$ | 6.84±0.09$^{c}$ | 6.17±0.08$^{c}$ | 7.38±0.09$^{b}$ |

Data are mean ± standard deviation (n = 3). Values with different superscript letters within a row are significantly different ($p \leq 0.05$).

## 4. Conclusion

Rice, in its various forms, is a staple food in many countries, with both white and brown rice being widely consumed. In this study, gluten-free cupcakes were developed using white rice flour as the base, with partial substitution by brown rice and red bean flours at different levels. The incorporation of 10% and 20% of these ingredients produced cupcakes with improved crumb texture and enhanced color characteristics brown from the brown rice and reddish hues from the red beans. Nutritionally, the enriched formulations demonstrated higher protein, fiber, and antioxidant contents, attributed to the superior nutritional profiles of red beans and brown rice. In terms of storage stability, cupcakes formulated with red bean and brown rice showed lower peroxide values over time, indicating better resistance to lipid oxidation. These findings suggest that substituting part of the white rice flour with red bean or brown rice flour in gluten-free cupcake recipes can significantly improve both the nutritional and sensory qualities, while also extending shelf life.

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