

Article

Development of Reduced-Sugar Gluten-Free Sponge Cakes Using Rice, Amaranth, and Tiger Nut Composite Flours

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Abstract

The absence of a gluten network remains the main technological limitation in developing high-quality gluten-free sponge cakes, leading to poor structure, reduced volume, and impaired texture. This study aims to develop a functional gluten-free sponge cake by partially substituting rice flour with amaranth flour (5 to 15%) and tiger nut flour (25 to 35%), while optimizing the formulation to achieve a 35–45% reduction in added sugar compared to the traditional control cake, in order to improve the antioxidant and technological quality of the product. Several formulations were compared to a positive control (wheat) and a negative control (rice) by combining physicochemical, biochemical, and crumb structure analysis. The parameters evaluated include weight loss, specific volume, moisture, water activity (a_w), texture profile analysis (TPA), crumb cell analysis, color, antioxidant potential and sensory acceptability. This study showed that the incorporation of amaranth and tiger nut flours improved the functional, physicochemical, textural, antioxidant, and sensory properties of gluten-free sponge cakes. Among all formulations, F8, which consisted of 60% rice flour, 10% amaranth flour and 30% tiger nut flour with 60% sugar, exhibited the best overall performance ($p < 0.05$), with a higher specific volume ($5.84 \text{ cm}^3/\text{g}$), higher moisture content (28.76%) and lower water activity (0.58 for the crust and 0.54 for the crumb), the



Academic Editor: Giancarlo Cravotto

Received: 25 May 2026

Revised: 23 June 2026

Accepted: 7 July 2026

Published: 23 July 2026

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highest area fraction (27.73%), stronger antioxidant activity (1.80 mg AAE/g dw), and the highest sensory acceptability (7.23) ($p < 0.05$). These results highlight the potential of combining these flours to develop highly acceptable gluten-free bakery products.

Keywords: celiac disease; pseudocereals; alternative grains; food formulation; antioxidant activity; sensory acceptability

1. Introduction

The rise in the prevalence of celiac disease, an autoimmune disorder triggered by gluten, as well as the growing interest of consumers in healthy diets, has generated a significant rise in global demand for gluten-free products [1,2].

Although the strict elimination diet remains the only effective treatment, current commercial formulations often exhibit nutritional deficiencies, particularly in dietary fiber and bioactive compounds, as well as in certain essential minerals such as iron, zinc, and calcium. Moreover, gluten-free products generally exhibit limited technological and sensory properties as well as short shelf lives. The high starch content and low protein composition of these gluten-free products accelerate staling by enhancing starch retrogradation. To address these shortcomings, the incorporation of ingredients naturally rich in nutrients and bioactive components is now recommended to improve the final quality of these products and ensure lasting adherence to the diet [3,4].

In this context, modern lifestyles promote the emergence of healthy and convenient food options, such as sponge cakes, which have become preferred energy-dense goods and rank among the most appreciated gluten-free bakery products by consumers [3]. Various ingredients are used to address the nutritional, technological, and sensory quality defects observed in gluten-free cakes and to improve their final quality. The incorporation of proteins improves the springiness and softness of the cakes, thereby enhancing their textural properties and overall sensory appreciation [5]. Matos and Sanz [6] developed gluten-free cakes based on rice flour and various protein sources, finding that the addition of soy protein isolates significantly improved the viscoelastic properties of the dough and positively influenced the textural characteristics of the finished products. Similarly, Shevkani and Kaur [7] enriched rice flour cakes with up to 12% protein isolate from cowpea, observing notable differences in the functional properties of this flour, with the extent of these successes depending on the type and amount of proteins incorporated.

However, while innovation in flavors and formulations is progressing rapidly in North America and Europe, the gluten-free-product market still faces major challenges in developing countries. High prices and limited availability highlight the need to develop more accessible gluten-free products with superior sensory properties to meet the needs of these populations [8,9].

The incorporation of pseudocereals and tubers into the human diet contributes to improving nutritional status and reducing the risk of chronic diseases. In this work, pseudocereals such as amaranth and tubers such as tiger nut have received particular attention due to their nutritional value, which includes high levels of proteins, lipids, fiber, essential amino acids, minerals, as well as phenolic compounds recognized for their antioxidant properties, capable of reducing the risk of degenerative diseases. Tiger nut (*Cyperus esculentus* L.), also known as chufa, is an excellent gluten-free material, rich in lipids, fiber, and bioactive compounds. It is rich in essential amino acids such as valine, leucine, and lysine, as well as mineral salts such as potassium, calcium, iron, magnesium, and vitamins A, C, and E [10,11]. It is naturally sweet and rich in carbohydrates, including

glucose and other sugars [11]. On the other hand, amaranth (*Amaranthus* spp.), which is naturally gluten-free, is very rich in high-quality proteins, fiber, minerals, and bioactive compounds, and it is known for its superior protein digestibility [12,13].

Although some gluten-free formulations have been developed to enrich cakes with nutrient-rich materials, the specific combination of rice, amaranth, and tiger nut for the production of gluten-free sponge cakes remains unexplored. The scientific rationale for the choice of these matrices is based on their techno-functional and nutritional complementarity. Structurally, rice flour offers the neutral starchy base necessary for volume, but it frequently lacks the protein network and lipids required to trap air and moisture in gluten-free doughs [9]. The high-quality proteins in amaranth flour can improve the emulsifying properties and the gas retention of the dough, resulting in a better crumb structure. At the same time, tiger nut, with its high monounsaturated fatty acid content, acts as a natural shortening agent that can soften the texture and prevent staling. In a nutritional context a positive synergistic effect can be expected: amaranth compensates for the lysine deficiency inherent in rice and creates a balanced amino acid profile, while the natural sweetness and complex carbohydrate profile of tiger nut allow for a significant reduction in refined sugar without impacting the energetic or sensory profile. For this reason, the present work aimed to develop new enriched gluten-free formulations for the production of sponge cakes based on rice enriched with amaranth and tiger nut, while the natural sweetness of tiger nut allows for a substantial reduction in added sugar compared to the traditional control cake. The developed formulations were systematically evaluated for their technological, sensory and antioxidant properties, offering a highly valuable strategy for the food industry by demonstrating how the strategic combination of pseudocereals and tubers can be used to manufacture low-sugar and gluten-free bakery products that meet consumer demands without compromising industrial processing or sensory quality.

2. Materials and Methods

2.1. Chemical

The chemicals used in this study were provided by Sigma-Aldrich, Steinheim, Germany, and include: 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$), sulfuric acid (H_2SO_4), sodium phosphate (Na_3PO_4), Folin–Ciocalteu reagent, aluminum chloride (AlCl_3), ferric chloride (FeCl_3), potassium ferricyanide $\text{K}_3[\text{Fe}(\text{CN})_6]$, potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$), sodium carbonate (Na_2CO_3), and trichloroacetic acid (TCA). Ascorbic acid, gallic acid, and quercetin were used as standards. The ethanol was supplied by Sigma-Aldrich (St. Louis, MO, USA). All reagents and solvents used were of analytical grade.

2.2. Raw Materials

Wheat flour (Mama, Blida, Algeria), table-sugar powder (Cevital, Bejaia, Algeria), fresh eggs, baking powder (Nouara, Algiers, Algeria), and vanilla (Salwa, Algeria) were purchased from the local market in Constantine city, Algeria. Rice (*Oryza sativa*), Amaranth (*Amaranthus cruentus*) and tiger nut (*Cyperus esculentus*) flours were obtained from the NATURALIM company (Algiers, Algeria).

2.3. Proximate Composition Analysis of Flours

The analysis of protein and fat contents was performed according to ISO methods: ISO 20483:2013 and ISO 11085:2008, respectively [14,15]. The moisture and ash content were determined according to the AACC methods 44-15A and AACC 08-03.01 respectively [16]. The total carbohydrates (TCs) were calculated by difference as $100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash})$ [17].

2.4. Water-Binding and Oil-Binding Capacity of Flours

The water-binding capacity (WBC) and oil-binding capacity (OBC) of the flours were determined following the method of Curti et al. [17], with slight modifications. Briefly, 500 ± 5 mg on a dry basis of each flour sample (W_s) was weighed into 50 mL centrifuge tubes. Then, 25 mL of distilled water or sunflower oil was added to the tubes, which were incubated at 25°C for 30 min with shaking carried out at 0, 10, 20, and 30 min. After incubation, the tubes were centrifuged at $3000 \times g$ for 20 min and then inverted on absorbent paper to drain the excess liquid. Finally, the gel was weighed (W_g). WBC and OBC were calculated as follows:

$$\text{WBC or OBC} \left(\frac{g}{g} \right) = (W_g - W_s) / W_s$$

2.5. Gluten-Free Sponge Cake Formulations

The gluten-free formulations used in this work were based on a mixture of rice, amaranth, and tiger nut flour, with a reduction in sugar content due to the addition of tiger nut. In order to enhance the technological, sensorial, and nutritional qualities of gluten-free sponge cakes, the suggested formula was developed using the established benefits of cereal–pseudocereal supplementation, as amaranth is rich in protein and tiger nut is rich in nutrients and natural sugars [11,18,19]. In these formulations, rice flour was partially substituted with amaranth flour (5–15% w/w) and tiger nut flour (25–35% w/w), together with a reduction in added sugar content. These mixture compositions were chosen based on the literature [20,21] and some preliminary trials based on the taste of the final produced product.

Table 1 summarizes the mixture compositions of the gluten-free sponge cake formulations (F1–F9).

Table 1. Gluten-free sponge cake formulations.

Formulation	Sugar (%)	Rice (% w/w)	Amaranth (% w/w)	Tiger Nut (% w/w)
F1	100	95	5	0
F2	100	90	10	0
F3	100	85	15	0
F4	55	75	0	25
F5	60	70	0	30
F6	65	65	0	35
F7	55	70	5	25
F8	60	60	10	30
F9	65	50	15	35

Sugar level refers to the formulation diagram: 100% in formulations without tiger nut flour and reduced in tiger nut flour formulations.

2.6. Sponge Cake Preparation

The sponge cakes were prepared according to Madadi et al. [22], with minor modifications. Egg whites were separated from the yolks and whipped at high speed using an electric mixer (Moulinex, Écully, France) for 8 min. Sugar was gradually added until stiff peaks formed. Egg yolks and vanilla extract were then incorporated and mixed until the batter became light and creamy.

In a separate bowl, the dry ingredients (flour or gluten-free mixture and baking powder) were gradually folded into the egg mixture until a homogeneous, light, and aerated batter was achieved. The resulting batter was poured into a silicone mold and baked in an oven preheated to 175°C for 15 min. After baking, the sponge cakes were

allowed to cool completely and were then stored in insulated bags at room temperature until analysis.

Two control formulations were used in this work: a wheat flour formulation (wheat control named as positive control) and a rice flour formulation (gluten-free control named as negative control). The rice-based formulation also served as the base for gluten-free composite formulations. Both formulations were prepared using the same basic ingredients, namely sugar, egg, baking powder, and vanilla. Table 2 outlines the ingredient proportions used in the production of both wheat-based and gluten-free sponge cakes.

Table 2. Control cake formulations.

Ingredient	Quantity (g)	(%) Based on Flour Weight
Wheat or rice flour	30.36	100
Sugar	30.36	100
Egg	37.95	125
Baking powder	1.26	4.15
Vanilla	0.07	0.23

2.7. Physicochemical Properties of Sponge Cakes

2.7.1. Baking Weight Loss

Baking weight loss (WL) was determined according to Coelho and de las Mercedes Salas-Mellado [23] and calculated in three replicates as the proportional difference between the dough and cake weights according the following equation:

$$WL(\%) = (W_b - W_c) \times 100 / W_b$$

where

W_b —weight of batter before baking (g);

W_c —weight of cake baked and cooled (g).

2.7.2. Specific Volume

The volume ($\text{cm}^3 \cdot \text{g}^{-1}$) was measured in three replicates by the small seed displacement method according to AACC (2000) [24]. The specific volume was then determined by dividing the volume of the sponge cake by its weight.

2.7.3. Moisture Content and Water Activity

The moisture content of the cakes was determined using AOAC standard methods 925.10 [25]. Indeed, moisture content was determined by weight loss after drying 3 g powder in an air oven at 105 °C for 7 h. Water activity (a_w) was measured at 25 °C using a water activity meter (Novasina Aw Sprint TH-500, Axair Ltd., Pfäffikon, Switzerland) [26].

2.7.4. Color Determination

The color of the crumb and crust of the sponge cakes was determined by taking images of the final product according to Eddine Derardja et al. [27], using Adobe Photoshop software (Version 21.2.4, Adobe Systems Inc., USA). To ensure that ambient light does not affect color capture, a closed polystyrene box with a white internal surface, integrated with a 1.2-watt 5-volt white LED light, is used to obtain uniform internal lighting. The images were taken using a Canon EOS-1200 D digital camera (18-megapixel resolution and 3 × 18–55 mm f/3.5–5.6 Zoom Lens, Canon Inc., Tokyo, Japan). The CIELAB color space ($L^* a^* b^*$), as defined by the International Commission on Illumination, was used as it described all the colors visible to the human eye. The lightness value, L^* , defines black at 0 and white

at 100, the a^* value is relative to the opposing colors green–red, with (–) values indicating green and (+) values indicating red. The value of b^* represents the blue–yellow opponents, with (–) values indicating blue and (+) values indicating yellow. The color values of lightness (L), redness–greenness (a), and yellowness–blueness (b) were obtained from the histogram in the menu bar using the LAB color mode in Adobe Photoshop software. The rectangular selection tool in the main menu was used to select the sample area. Next, the L , a , b values of the selected area were transformed into CIELAB (L^* , a^* , b^*) values using the following equations:

$$L^* = \frac{L}{2.5}$$

$$a^* = \frac{240a}{255} - 120$$

$$b^* = \frac{240b}{255} - 120$$

The total color difference, ΔE , was calculated according to the following equation:

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

2.8. Texture Analysis of Sponge Cakes

Texture profile analysis (TPA) was performed using a texture analyzer under the conditions described above (LLOYD instruments, Fareham, England). The parameters recorded were hardness (N), defined as the maximum force required during the first compression cycle; cohesiveness (dimensionless), calculated as the ratio of the area under the second compression curve to that under the first compression curve; springiness (mm), defined as the distance recovered by the sample between the first and second compression cycles; adhesiveness (N), corresponding to the work required to overcome the attractive forces between the sample surface and the probe; and chewiness (N·mm), calculated as the energy required to chew a solid food until it is ready for swallowing. An aluminum cylinder probe of 25 mm diameter compressed the cake to 50% of its height (30 mm) at a speed of 2 mm/s and applied a force of 100 N with a 30 s delay between the first and the second compression. All measurements were taken in a controlled room at 25 °C, 24 h after baking with two independent replicates [28].

2.9. Crumb Cell Analysis

Image analysis was conducted according to Gonzales-Barron and Butler [29] using ImageJ software (1.43u; Java1.7.0–2132 bit, Wayne Rasband, National Institute of Mental Health (NIH), Bethesda, MD, USA). Central slices (10 mm thick) were obtained from the cake samples, and their images were captured using a scanner (HP ScanJet 3530c, HP Inc., USA). The images were saved in TIFF format. Each image was cropped and converted to 8-bit greyscale in order to isolate the crumb region. A binary threshold was applied, where the white represented the crumb and the black represented the crumb pores. The crumb structure was evaluated by measuring cell count, average cell size, and area fraction.

2.10. Assessment of Antioxidant Properties

In accordance with Ayad et al. [30], a 1 g of sponge cake sample and 10 mL of 80% ethanol were sonicated for an hour at 40 °C in an ultrasonic cleaning bath (ultrasons-H, 50/60 Hz, 720 W, Ctra. Nil Km: 585.1, Abrera (Barcelona), Spain). The extracts were extracted, then cooled, and filtered in preparation for further analysis.

2.10.1. Determination of Total Phenolic Content (TPC)

The Folin–Ciocalteu assay is a widely used method for determining the total phenolic content (TPC). The TPC of the samples was determined according the method described by Singleton and Rossi [31] and as reported by Luna Flores et al. [32], with slight modifications. Briefly, 0.50 mL of each sample extract was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10). After 8 min, 2 mL of a 7.5% Na₂CO₃ solution was added and the mixture was incubated in the dark at room temperature for 2 h. The absorbance was measured at 765 nm using a UV/visible spectrophotometer (Thermo Electron Corporation Evolution 100; Madison, WI, USA). The TPC was quantified using a gallic acid calibration curve prepared from standard solutions of standard gallic acid solutions (ranging from 0.78 to 200 µg/mL). The results were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

2.10.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of each formulation was evaluated following the methodology of Djeridane et al. [33]. Briefly, equal volumes of a 2% AlCl₃ solution were mixed with each sample extract and letting the mixture incubate for 15 min. The absorbance was measured at 430 nm. The TFC was determined from extrapolation of the calibration curve, which was prepared from standard quercetin solutions (0.31–40 µg/mL), and expressed as quercetin equivalents per gram of dry weight (mg QE/g dw).

2.10.3. Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) of the tested formulations was determined according to the phosphomolybdate method described by Prieto et al. [34]. Briefly, 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was mixed with 0.3 mL of each tested extract. The absorbance was measured against a blank at 695 nm after incubation for 90 min at 95 °C. The calibration curve was prepared using ascorbic acid as a standard compound (0.78–200 µg/mL). The total antioxidant capacity was expressed as ascorbic acid equivalents per gram of dry weight (mg AAE/g dw).

2.10.4. Scavenger Radical Activity by DPPH

The free radical scavenging activity of the formulations was evaluated according to the method described by Ismail et al. [35] with slight modifications. Serial dilutions were prepared from each sample extract; thereafter, each 2 mL of each dilution was mixed with an equal volume of a 0.2 M DPPH ethanolic solution. After standing in the dark for 30 min, the absorbance was taken at 517 nm against ethanol. The control was prepared without any sample under the same conditions.

The percentage of inhibition (I%) was determined using the following equation:

$$I\% = \frac{(\text{Abs. of Control} - \text{Abs. of sample})}{\text{Abs. of Control}} \times 100$$

Ascorbic acid was used as the standard antioxidant.

2.10.5. ABTS Radical Scavenger Activity

ABTS radical scavenging activity was evaluated according to the method described by Re et al. [36]. The ABTS•+ radical solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark for 12–16 h. The resulting ABTS•+ solution was then diluted to an absorbance of approximately 0.70 ± 0.02 at 734 nm [37,38]. Then, 2 mL of ABTS•+ solution was mixed with 1 mL of each diluted

sample extract, incubated for 10 min in the dark, and the absorbance was measured at 734 nm. The control solution was prepared without any sample under the same conditions.

The percentage of inhibition (I%) was calculated using the following equation:

$$I\% = \frac{(\text{Abs. of control} - \text{Abs of sample})}{\text{Abs. of control}} \times 100$$

The results were expressed as IC50 (mg dw/mL).

2.10.6. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) of the formulations was determined according to Oyaizu et al. [39]; 0.5 mL of each sample extract were mixed with 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide (1%, *w/v*), incubated at 50 °C for 20 min, and then followed by the addition of 2.5 mL of trichloroacetic acid (10%). After centrifugation (3000 rpm for 10 min), 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 250 µl of freshly prepared FeCl₃ (0.1%, *w/v*), and absorbance was recorded at 700 nm. The reducing power was expressed as A0.5 value (mg dw/mL), with ascorbic acid used as the reference standard.

2.11. Sensory Analysis

A total of 26 trained panelists composed of twenty women and six men, aged 20 to 26, used the hedonic scale method to perform sensory evaluation of the samples. They were familiar with gluten-free products. A random three-digit code was assigned to each sample, which was then presented to the panel randomly. Each sample was evaluated by the panelists using a 9-point hedonic scale. Score ranged from 1 (dislike extremely) to 9 (like extremely) [40]. The characteristics evaluated for the cakes were taste, flavor, texture, appearance, and overall acceptability. Panelists had access to water to rinse their mouths between samples in order to minimize any lingering effects. The sensory analysis was completed 24 h after baking. The room where the sensory session took place was well-lit and spacious. Before the test, the participants were informed of the study's aim and granted their consent in accordance with the ethical procedures of the INATAA Institute, located at Constantine 1 Frères Mentouri University.

2.12. Statistical Analysis

All analytical measurements were performed in triplicates. The results presented in this work are expressed as mean ± standard deviation. The means were compared by one-way analysis of variance (ANOVA) followed by a post hoc Fisher's least significant difference (LSD) post hoc test using STATISTICA software (Version 7, Stat soft, Inc., USA). The significance of differences between the samples was determined at a *p*-value < 0.05. The Tukey post hoc test (HSD) was used to distinguish the different groups for the sensory analysis at a significance level of 0.05. We used the statistical software R (www.R-project.org) version 4.5.1 [41] to carry out both hierarchical clustering analysis (HCA) and principal component analysis (PCA) to analyze the distribution of the wheat-based and gluten-free sponge cakes under investigation and to determine correlations between the physico-chemical, textural, crumb cell, antioxidant and sensory properties. HCA was carried out using the R package {pheatmap} version 1.0.13, whereas the PCA was conducted with the package {factoextra} version 2.0.0.

3. Results and Discussion

3.1. Proximate Composition and Functional Properties of Flours

The proximate compositions (including moisture, ash, protein, fat, and carbohydrate contents) and functional properties (water-binding capacity and oil-binding capacity) of the wheat (control), rice, amaranth, and tiger nut flours are presented in Table 3. Significant differences ($p < 0.001$) were observed among all flours for their compositional and functional characteristics.

Table 3. Proximate composition (wet basis) and functional properties of the studied flours.

	Moisture Content (%)	Ash Content (%)	Protein Content (%)	Fat Content (%)	Carbohydrates (%)	WBC (g/g)	OBC (g/g)
Wheat flour	14.5 ± 0.42 ^d	0.450 ± 0.00 ^a	9.05 ± 0.01 ^b	2.33 ± 0.01 ^b	73.67	0.940 ± 0.038 ^a	0.52 ± 0.06 ^b
Rice flour	11.50 ± 0.14 ^b	0.630 ± 0.080 ^b	10.70 ± 0.03 ^c	0.84 ± 0.01 ^a	76.33	1.560 ± 0.018 ^d	0.23 ± 0.10 ^a
Amaranth flour	12.60 ± 0.28 ^c	1.140 ± 0.014 ^c	15.80 ± 0.02 ^d	8.05 ± 0.02 ^c	62.41	1.160 ± 0.020 ^b	0.55 ± 0.12 ^b
Tiger nut flour	8.40 ± 0.00 ^a	1.390 ± 0.040 ^d	5.07 ± 0.01 ^a	24.42 ± 0.02 ^d	60.72	1.259 ± 0.005 ^c	0.51 ± 0.02 ^b
<i>p</i> -value	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	/	0.000 ^{***}	0.000154 ^{***}

WBC: Water-binding capacity; OBC: oil-binding capacity. *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

Moisture content ranged from 8.40% to 14.50%, with tiger nut flour exhibiting the lowest value and wheat flour the highest. The reduced moisture content of tiger nut flour may contribute to improved storage stability and lower susceptibility to microbial deterioration. Ash content also differed significantly among flours, with tiger nut flour showing the highest value (1.39%), followed by amaranth (1.14%), indicating a greater mineral content compared with wheat (0.45%) and rice (0.63%). Protein content varied markedly depending on flour type. Amaranth flour exhibited the highest protein content (15.80%), confirming its nutritional value as a protein-rich pseudocereal, whereas tiger nut flour showed the lowest level (5.07%). Fat content followed a different pattern, with tiger nut flour containing substantially higher lipid levels (24.42%) than wheat (2.33%), rice (0.84%), and amaranth (8.05%) flours. In contrast, rice and wheat flours exhibited the highest carbohydrate contents (>73%), while amaranth and tiger nut flours presented lower values (approximately 60–62%). The compositional profile of tiger nut flour agrees with previous findings reported by Semdé et al. [42]. Differences observed for rice and amaranth flours compared with values reported by Banumathy et al. [43] and Suychinov et al. [44] may be attributed to variations in genotype, environmental conditions, agronomic practices, and analytical methodologies, which are known to influence starch, protein, lipid, and mineral composition [45,46].

The functional properties of the flours also differed significantly ($p < 0.001$). Water-binding capacity (WBC) ranged from 0.94 to 1.56 g/g, with wheat and rice flours showing the lowest and highest values, respectively. The elevated WBC observed in rice flour suggests a greater ability to absorb and retain water, which may positively influence batter hydration, moisture retention, and the softness of the final baked products. Amaranth flour also exhibited higher WBC than wheat flour, consistent with previous reports for gluten-free systems [47]. Differences in WBC among flours are generally associated with variations in starch structure, protein composition, dietary fiber content, particle size, and the availability of hydrophilic sites capable of forming hydrogen bonds with water [48,49]. Our results are consistent with those reported in the literature [47,50].

Oil-binding capacity (OBC) showed significant differences among samples, ranging from 0.23 to 0.55 g/g. Rice flour exhibited the lowest OBC, indicating limited lipid retention capacity, whereas wheat, amaranth, and tiger nut flours showed comparable and significantly higher values. Higher OBC values are generally associated with protein hy-

drophobicity, the presence of non-polar amino acid residues, and lipid-interactive surfaces, all of which contribute to flavor retention and improved mouthfeel in bakery applications [48,49]. Overall, the observed differences in WBC and OBC compared with previous studies [51,52] are likely related to differences in flour composition, botanical origin, and experimental conditions used during functional property determination, including hydration time, flour-to-solvent ratio, and centrifugation parameters.

3.2. Physicochemical Properties of Sponge Cake

The physicochemical properties of the wheat and gluten-free sponge cakes, including the specific volume, weight loss, moisture content, and water activity, are presented in Table 4. Significant differences were observed among formulations for all parameters evaluated ($p < 0.001$), indicating that flour substitution substantially influenced batter behavior and moisture distribution during baking.

Table 4. Physicochemical properties of the sponge cake formulations.

	Weight Loss (%)	Specific Volume (cm ³ /g)	Moisture Content (%)	Water Activity	
				Crust	Crumb
PC	13.58 ± 0.89 ^d	4.80 ± 0.10 ^h	17.85 ± 0.10 ^a	0.53 ± 0.00 ^a	0.60 ± 0.00 ^{ab}
NC	9.78 ± 0.71 ^a	3.13 ± 0.01 ^{bcd}	19.76 ± 0.10 ^b	0.62 ± 0.00 ^b	0.64 ± 0.01 ^{bc}
F1	10.80 ± 0.18 ^b	3.15 ± 0.03 ^{cd}	22.65 ± 1.13 ^c	0.69 ± 0.01 ^b	0.75 ± 0.02 ^e
F2	14.36 ± 0.46 ^e	3.08 ± 0.02 ^{abc}	23.47 ± 0.96 ^{cd}	0.68 ± 0.01 ^b	0.69 ± 0.01 ^{cd}
F3	13.26 ± 0.22 ^d	3.05 ± 0.08 ^{ab}	24.91 ± 0.25 ^e	0.66 ± 0.01 ^b	0.72 ± 0.05 ^{de}
F4	13.17 ± 0.23 ^d	3.38 ± 0.06 ^g	28.64 ± 0.37 ^g	0.59 ± 0.02 ^b	0.54 ± 0.03 ^a
F5	11.33 ± 0.52 ^{bc}	3.02 ± 0.03 ^a	26.94 ± 1.44 ^f	0.72 ± 0.05 ^b	0.80 ± 0.02 ^e
F6	11.65 ± 0.13 ^c	3.17 ± 0.04 ^{de}	27.06 ± 0.0 ^f	0.67 ± 0.01 ^b	0.65 ± 0.02 ^{bc}
F7	13.27 ± 0.16 ^d	3.23 ± 0.01 ^{ef}	24.71 ± 1.30 ^{de}	0.79 ± 0.05 ^c	0.76 ± 0.03 ^e
F8	11.43 ± 0.27 ^{bc}	5.84 ± 0.04 ⁱ	28.76 ± 0.47 ^g	0.58 ± 0.02 ^b	0.54 ± 0.01 ^a
F9	9.32 ± 0.29 ^a	3.30 ± 0.03 ^{fg}	27.56 ± 0.15 ^{fg}	0.76 ± 0.02 ^c	0.65 ± 0.02 ^{bc}
<i>p</i> -value	0.000 ^{***}	0.000 ^{***}	0.000 ^{***}	<0.001 ^{***}	<0.001 ^{***}

PC: Positive control; NC: negative control. *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

Specific volume (SV), an important indicator of aeration and gas retention capacity in sponge cakes, varied significantly among formulations ($p < 0.001$). The positive control exhibited a high specific volume (4.80 cm³/g), reflecting the ability of the gluten network to stabilize incorporated air and support expansion during baking. Interestingly, formulation F8 (60% rice flour, 10% amaranth flour, and 30% tiger nut flour) showed the highest specific volume (5.84 cm³/g), surpassing even the wheat-based control. In contrast, formulation F5 (70% rice flour and 30% tiger nut flour) exhibited the lowest value (3.02 cm³/g).

The reduced specific volume observed in F5 may be attributed to the absence of amaranth proteins, which are known to possess favorable foaming and interfacial properties that enhance air bubble stabilization and improve gas retention during baking. Conversely, formulations containing amaranth alone (F1–F3) also exhibited relatively low specific volumes, suggesting that amaranth supplementation by itself was insufficient to promote expansion. This behavior may result from excessive batter viscosity induced by higher protein and fiber contents, which can restrict bubble growth and limit cake expansion during thermal processing [20]. These results suggest that the combined incorporation of amaranth and tiger nut generated a synergistic effect, producing a more favorable balance between batter viscosity, gas retention, and structural stability.

Weight loss during baking also differed significantly among formulations ($p < 0.001$). The highest weight loss was observed in F2 (14.36%), whereas F9 exhibited the lowest value (9.32%). Such variations are primarily associated with differences in water evaporation and moisture retention during baking. Correspondingly, formulation F8 exhibited the highest moisture content (28.76%), while the wheat control showed the lowest value (17.85%). The higher moisture levels observed in the composite formulations may be explained by the enhanced water-binding capacity of amaranth proteins and tiger nut fiber, which likely improved water retention and reduced moisture loss during baking [53]. According to Ayeni et al. [21], fiber-rich foods have been reported to absorb moisture due to the presence of hydrophilic molecules.

Water activity (a_w) of both crust and crumb also showed significant differences among formulations. Crust water activity ranged from 0.53 to 0.79, corresponding to the positive control and F7, respectively, whereas crumb water activity varied from 0.54 to 0.80, with the lowest values observed in F4 and F8 and the highest in F5. In most formulations, crust water activity remained lower than crumb water activity, which is consistent with moisture migration occurring during baking. The crumb generally retains more moisture, whereas the crust undergoes intensive dehydration, generating a moisture gradient between the two regions [54]. After baking, this gradient promotes water redistribution from the crumb to the crust, resulting in gradual crumb dehydration and crust softening during cooling and storage.

However, an atypical behavior was observed in formulations containing tiger nut flour and reduced sugar levels (F4, F6, F7, F8, and F9), where crust water activity approached or exceeded crumb values. This phenomenon may be explained by the role of sucrose in water binding and mobility. Previous studies have demonstrated an inverse relationship between sucrose concentration and water activity, whereby reducing the sucrose content decreases the number of available hydrogen-bonding interactions and increases the proportion of unbound water within the matrix [55,56]. Consequently, sugar reduction may alter moisture redistribution and modify the conventional crust–crumb water activity gradient in sponge cakes. Overall, the results demonstrate that the incorporation of amaranth and tiger nut flours significantly affected moisture management, gas retention, and structural development, with the combined formulations, particularly F8, exhibiting the most favorable physicochemical performance.

3.3. Color Properties

The colorimetric characteristics of the crust and crumb (L^* , a^* , b^*) differed significantly among the sponge cake formulations ($p < 0.001$), demonstrating that flour substitution strongly affected the visual appearance of the final products (Table 5). These differences are primarily associated with variations in browning intensity, pigment composition, and the interactions among proteins, sugars, lipids, and water during baking.

For crust color, the wheat-based positive control exhibited the lowest L^* value (62.72), indicating a darker surface than the gluten-free formulations. This darker coloration is attributable to more extensive non-enzymatic browning reactions, particularly Maillard reactions promoted by the availability of proteins and reducing sugars during baking. In contrast, the rice-based negative control showed higher L^* values and a lighter crust, which may be attributed to its lower protein content and reduced browning potential. The incorporation of amaranth flour significantly altered crust color. Formulations containing higher levels of amaranth (F1–F3) generally exhibited increased luminosity (L^*) and reduced redness (a^*) and yellowness (b^*), with F3 showing the highest L^* value (76.69). This behavior suggests a reduction in browning intensity and may be associated with changes in batter composition, moisture retention, and heat transfer during baking. In addition,

the compositional characteristics of amaranth, including its protein profile and naturally occurring bioactive compounds, may influence thermal reactions and consequently modify crust color development.

Table 5. Crust and crumb color of the sponge cake formulations.

	Crust Color				Crumb Color			
	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE
PC	62.72 ± 0.13 ^a	11.68 ± 0.004 ^f	43.44 ± 0.22 ^g	/	90.51 ± 0.16 ^h	−1.77 ± 0.26 ^a	30.20 ± 0.45 ^c	/
NC	70.23 ± 0.21 ^f	9.41 ± 0.090 ^d	42.32 ± 0.47 ^f	7925 ± 0.15	91.82 ± 0.13 ⁱ	−0.83 ± 0.12 ^{bc}	24.31 ± 0.30 ^a	6.106 ± 0.16
F1	70.17 ± 0.22 ^f	9.36 ± 0.01 ^d	39.27 ± 0.06 ^{bcd}	8847 ± 0.17	89.75 ± 0.06 ^g	−1.11 ± 0.17 ^b	32.53 ± 0.56 ^{de}	2538 ± 0.20
F2	68.74 ± 0.27 ^e	9.59 ± 0.100 ^d	38.94 ± 1.12 ^{bc}	7801 ± 0.18	88.26 ± 0.02 ^f	−0.14 ± 0.20 ^d	31.39 ± 0.81 ^{cd}	3022 ± 0.50
F3	76.69 ± 0.08 ^g	7.36 ± 0.050 ^a	36.98 ± 0.29 ^a	15,986 ± 0.10	87.98 ± 0.24 ^f	0.00 ± 0.10 ^{de}	28.36 ± 0.37 ^b	3594 ± 0.22
F4	63.18 ± 0.21 ^b	9.90 ± 0.120 ^e	38.49 ± 0.02 ^b	5280 ± 0.13	79.69 ± 0.08 ^e	−0.67 ± 0.43 ^c	34.40 ± 2.87 ^{fg}	11,658 ± 1.20
F5	68.95 ± 0.63 ^e	8.86 ± 0.480 ^c	42.55 ± 0.67 ^f	6896 ± 0.55	77.73 ± 0.04 ^c	−0.63 ± 0.06 ^c	34.99 ± 0.20 ^g	13,695 ± 0.10
F6	70.34 ± 0.01 ^f	7.58 ± 0.200 ^a	39.84 ± 0.47 ^d	9372 ± 0.25	76.93 ± 0.04 ^b	0.27 ± 0.31 ^e	31.43 ± 0.32 ^{cd}	13,787 ± 0.25
F7	67.42 ± 0.2 ^d	9.63 ± 0.000 ^{de}	43.36 ± 0.00 ^g	5128 ± 0.01	78.83 ± 0.12 ^d	0.80 ± 0.06 ^f	30.36 ± 0.37 ^c	11,960 ± 0.19
F8	66.60 ± 0.11 ^c	8.22 ± 0.040 ^b	39.33 ± 0.41 ^{cd}	6627 ± 0.30	78.67 ± 0.03 ^d	−0.68 ± 0.11 ^c	27.85 ± 0.15 ^b	12,120 ± 0.90
F9	68.92 ± 0.18 ^e	8.29 ± 0.120 ^b	40.86 ± 0.03 ^e	7522 ± 0.11	76.17 ± 0.03 ^a	0.25 ± 0.39 ^e	33.09 ± 0.39 ^{ef}	14,767 ± 0.23
<i>p</i> -value	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***

PC: Positive control; NC: negative control. *L**, *a**, and *b** represent the CIELAB color coordinates, *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

Formulations enriched with tiger nut flour (F4–F6) showed intermediate color characteristics. Compared with the rice-only formulations, tiger nut incorporation tended to promote darker and more yellow crusts, particularly at higher incorporation levels. This behavior may be attributed to the naturally occurring sugars, amino compounds, and lipid fraction of tiger nut flour, which can intensify caramelization and Maillard reactions during baking [57]. Similar trends, including decreased *L** and increased *a** and *b** values following tiger nut incorporation (10, 20, and 40 g/100 g) to wheat flour, have previously been reported in bakery products [58]. Regarding crumb color, the negative control exhibited the highest *L** values, indicating a lighter internal structure. In contrast, formulations containing amaranth and tiger nut showed progressively lower luminosity values, producing darker crumbs. The lowest crumb brightness was observed in F5, F6, and F9, suggesting increased color development and greater structural compactness. Crumb redness (*a**) remained generally low across all formulations, although samples containing both amaranth and tiger nut (F6, F7, and F9) exhibited slightly higher values, indicating a modest shift toward reddish tones. Crumb yellowness (*b**) increased in enriched formulations, particularly in F4, F5, and F9, reflecting greater development of yellow pigments. Overall, the results demonstrate that flour substitution significantly influenced crust and crumb color through modifications in thermal browning reactions and matrix composition [59]. The differences observed in the color parameters may also be attributed

to the natural pigments present in amaranth and tiger nut such as carotenoids, chlorophyll and lignin [20]. The total color difference (ΔE) values of the crust ranged from 5.12 to 15.98, corresponding respectively to F7 and F3, indicating that all formulations exhibited visually perceptible color differences compared with the positive control. The high ΔE value of F3 was mainly associated with a substantial increase in lightness and reductions in redness (a^*) and yellowness (b^*). The total color difference (ΔE) values of the crumb revealed significant visual differences among sponge cake formulations compared with the negative control (NC). Formulations F1 and F2 exhibited the lowest ΔE values (2.54 and 3.02 respectively), indicating slight-to-moderate color changes. In contrast, formulations F4 and F9 showed markedly higher ΔE values (11.66 and 14.77 respectively), reflecting substantial color modifications that are readily perceptible to the human eye. The highest ΔE value was observed in F9 (14.77) suggesting that the incorporation of a higher levels of amaranth and tiger nut flour considerably altered the overall appearance of the cakes. The combined incorporation of amaranth and tiger nut produced visually distinct sponge cake with enhanced color intensity, which may positively contribute to consumer perception and product acceptability.

3.4. Crumb Structure

The crumb structure of the sponge cakes was significantly affected by flour composition ($p < 0.001$), as reflected by variations in cell count, average cell size, and area fraction (Table 6, Figure 1). These parameters provide valuable information on gas retention, bubble stability, and crumb development during baking.

Table 6. Crumb structure of the sponge cake formulations.

	Cell Count	Average Cell Size (mm ²)	Area Fraction (%)
PC	377 ± 3.60 ⁱ	0.465 ± 0.030 ^{ab}	19.33 ± 1.34 ^a
NC	177 ± 11.53 ^b	1.300 ± 0.120 ^h	25.16 ± 0.80 ^d
F1	289.33 ± 8.32 ^f	0.678 ± 0.020 ^{de}	21.50 ± 0.17 ^{bc}
F2	134.66 ± 6.65 ^a	1.390 ± 0.080 ^h	20.50 ± 0.4 ^{ab}
F3	195.33 ± 9.81 ^c	1.030 ± 0.080 ^{fg}	22.13 ± 1.09 ^c
F4	246.66 ± 9.01 ^e	0.938 ± 0.020 ^f	25.73 ± 0.35 ^d
F5	347 ± 21.07 ^h	0.552 ± 0.040 ^{bc}	21.20 ± 0.55 ^{bc}
F6	494.66 ± 2.88 ^j	0.398 ± 0.008 ^a	21.93 ± 0.58 ^c
F7	323.33 ± 13.65 ^g	0.593 ± 0.010 ^{cd}	21.13 ± 0.58 ^{bc}
F8	225.33 ± 12.65 ^d	1.130 ± 0.050 ^g	27.73 ± 0.66 ^e
F9	307 ± 12.66 ^{fg}	0.742 ± 0.010 ^e	25.33 ± 0.85 ^d
<i>p</i> -value	0.000 ***	0.000 ***	0.000 ***

PC: Positive control; NC: negative control. *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

The positive control (wheat-based sponge cake) exhibited the highest cell count (377 cells), the smallest average cell size (0.465 mm²), and a relatively low surface fraction (19.33%), indicating the formation of a fine, homogeneous, and well-distributed porous structure. This behavior is characteristic of gluten-containing systems, where the viscoelastic gluten network efficiently stabilizes air bubbles during mixing and limits bubble coalescence during baking. In contrast, the negative control formulated exclusively with rice flour showed a substantially lower cell count (177 cells), the largest average cell size (1.30 mm²), and a higher surface fraction (25.16%), reflecting a coarse and heterogeneous crumb structure. The absence of gluten likely reduced gas retention capacity and promoted bubble coalescence, resulting in fewer but larger pores. Similar structural characteristics have previously been reported for rice-based gluten-free cakes and breads [60]. The incor-

poration of amaranth flour modified crumb architecture depending on the substitution level. A low level of incorporation (5%) improved crumb structure, as evidenced by an increased cells count (289 cells), and reduced average pore size (0.678 mm^2), suggesting improved batter viscosity and foam stabilization associated with the higher protein content of amaranth. However, increasing amaranth levels (10–15%) led to larger pores and lower structural uniformity, indicating that excessive protein and fiber incorporation may increase batter viscosity and restrict uniform gas expansion. Similar effects of pseudocereal enrichment have been reported in previous studies, where moderate inclusion improved structure whereas excessive substitution negatively affected crumb homogeneity [50]. Tiger nut flour incorporation generally contributed to improving crumb organization by increasing cell density and reducing average pore size. This behavior may be explained by the combined effects of dietary fiber, elevated water-binding capacity, and lipid content, which may improve batter stability and reduce air bubble collapse during baking.

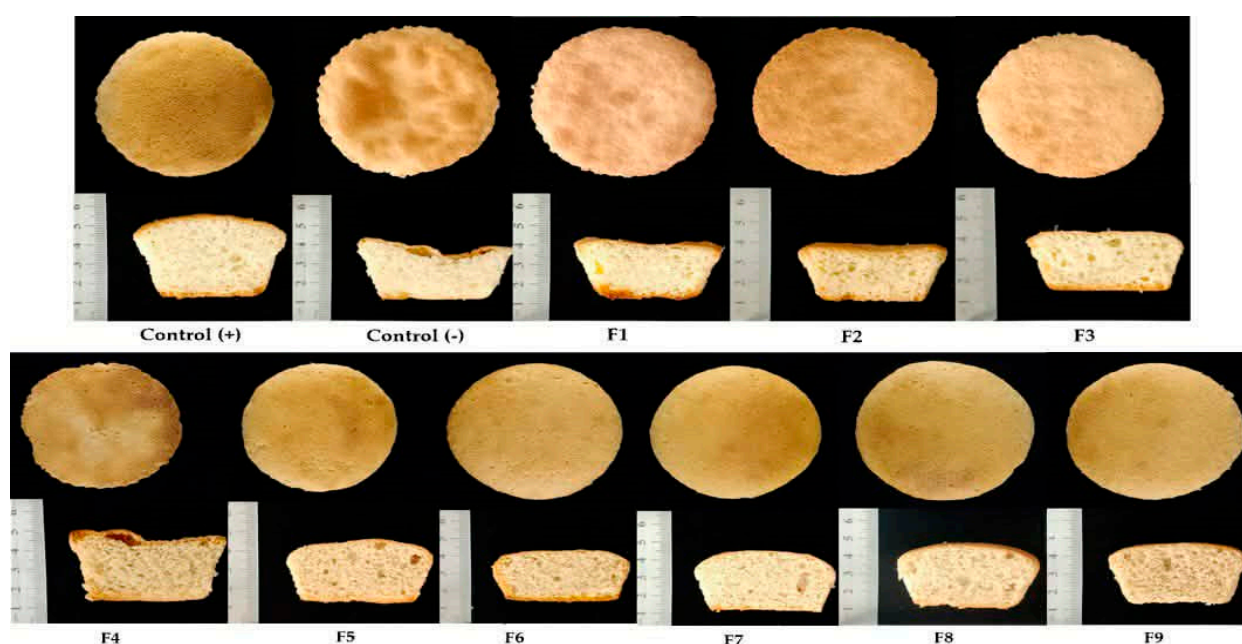


Figure 1. Whole cross-section and crust images of the sponge cake formulations (F1–F9) including positive control (C+) and negative control (C–).

For the combined formulations, crumb development depended on the ratio of amaranth and tiger nut flours. Formulation F7 exhibited a relatively high cell count (323 cells) and a small average cell size (0.593 mm^2), indicating efficient gas retention and a stable crumb network. In contrast, F8 showed fewer cells (225 cells) and larger average cell size (1.13 mm^2) together with the highest surface fraction (27.73%), suggesting greater pore expansion and partial bubble coalescence. Interestingly, F8 also exhibited the highest specific volume, indicating that although some pore enlargement occurred, the overall expansion of the crumb structure remained favorable. This observation suggests that increased volume in gluten-free systems does not necessarily correspond to structural deterioration but rather reflects a balance between gas expansion and matrix stabilization. Overall, the observed differences in crumb structure appear to result from interactions among proteins, starch, lipids, and water-binding components, which govern batter rheology, gas cell stability, and film formation during baking. Variations in protein functionality and starch behavior influence the rigidity of gas cell walls and ultimately determine pore expansion and the final crumb architecture [50].

3.5. Textural Properties

The textural properties of the wheat and gluten-free sponge cakes, including hardness, cohesiveness, springiness, adhesiveness, and chewiness, are presented in Table 7. Significant differences were observed among formulations for all textural parameters ($p < 0.001$), indicating that the incorporation of amaranth and tiger nut flours substantially influenced the mechanical properties of the crumb.

Table 7. Textural properties of the sponge cake formulations.

	Hardness (N)	Cohesiveness (—)	Springiness (mm)	Adhesiveness (N)	Chewiness (N·mm)
PC	4.059 ± 0.31 ^d	0.4200 ± 0.0540 ^f	11.68 ± 3.54 ^d	1.70 ± 0.08 ^d	23.67 ± 0.53 ^e
NC	1.54 ± 0.012 ^a	0.2300 ± 0.1300 ^a	6.54 ± 0.78 ^a	0.69 ± 0.26 ^a	4.42 ± 1.21 ^a
F1	6.09 ± 0.16 ^f	0.3500 ± 0.0050 ^{de}	7.81 ± 1.53 ^{ab}	2.14 ± 0.09 ^e	16.82 ± 3.99 ^d
F2	3.65 ± 1.52 ^{cd}	0.4100 ± 0.0600 ^{ef}	10.82 ± 3.75 ^{bcd}	1.45 ± 0.37 ^{cd}	15.05 ± 1.39 ^d
F3	4.65 ± 2.11 ^{de}	0.3500 ± 0.0200 ^{de}	9.12 ± 3.33 ^{abcd}	1.69 ± 0.89 ^d	16.91 ± 13.76 ^d
F4	5.44 ± 1.40 ^{ef}	0.3100 ± 0.0200 ^{bcd}	9.23 ± 1.21 ^{abcd}	1.70 ± 0.29 ^d	15.91 ± 4.78 ^d
F5	4.19 ± 0.42 ^d	0.2800 ± 0.0090 ^{abc}	6.89 ± 0.15 ^a	1.21 ± 0.08 ^{bc}	8.35 ± 0.40 ^{abc}
F6	4.48 ± 0.24 ^{de}	0.3200 ± 0.0060 ^{cd}	7.69 ± 0.12 ^{ab}	1.47 ± 0.10 ^{cd}	11.31 ± 0.64 ^{cd}
F7	2.88 ± 0.60 ^c	0.3100 ± 0.0300 ^{bcd}	11.17 ± 5.42 ^{cd}	0.91 ± 0.29 ^{ab}	10.98 ± 8.25 ^{bcd}
F8	2.80 ± 0.10 ^c	0.2600 ± 0.0100 ^{ab}	7.41 ± 0.21 ^a	0.73 ± 0.08 ^a	5.41 ± 0.45 ^{abc}
F9	2.59 ± 0.70 ^{bc}	0.2400 ± 0.0004 ^a	8.14 ± 2.13 ^{abc}	0.63 ± 0.17 ^a	4.96 ± 0.04 ^{ab}
<i>p</i> -value	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***

PC: Positive control; NC: negative control. *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

The negative control formulated with 100% rice flour exhibited the lowest values for hardness (1.54 N), cohesiveness (0.23), springiness (6.54), adhesiveness (0.69), and chewiness (4.42), indicating a weak and fragile crumb structure. This behavior is commonly observed in gluten-free bakery products and is mainly attributed to the absence of a continuous viscoelastic gluten network required to stabilize gas cells and maintain structural integrity [61]. Conversely, the wheat-based positive control showed significantly higher cohesiveness (0.42), springiness (11.68), adhesiveness (1.70), and chewiness (23.67), confirming the essential role of gluten in promoting elasticity, gas retention, and structural resilience during baking [62].

The incorporation of amaranth flour markedly altered cake texture. Formulation F1 (5% amaranth flour) exhibited the highest hardness (6.09 N), whereas formulations containing higher amaranth levels (F2 and F3) showed intermediate values. The increase in hardness may be attributed to the high protein and dietary fiber contents of amaranth flour, which likely increased batter viscosity and produced a denser crumb structure. Similar observations have been reported in gluten-free products enriched with pseudocereal flours, where increasing amaranth incorporation resulted in firmer structures because of restricted gas expansion and greater matrix compactness [20,61]. Nevertheless, moderate amaranth incorporation (F2 and F3) improved cohesiveness and springiness compared with the rice-based control, suggesting that amaranth proteins contributed to reinforcing the internal crumb structure organization and enhancing elastic recovery.

Tiger nut flour also significantly influenced texture. Formulations containing tiger nut flour alone (F4–F6) generally exhibited higher hardness and chewiness than the negative control. This effect may be attributed to the combined influence of dietary fiber, starch characteristics, and the high water- and oil-binding capacities of tiger nut flour, which promote matrix strengthening and reduce crumb collapse during baking [63]. At the same

time, the naturally occurring lipid fraction of tiger nut may have contributed to maintaining moderate cohesiveness and improving crumb softness.

Interestingly, formulations combining amaranth and tiger nut flours (F7–F9) produced more balanced textural properties than formulations containing either flour alone. In particular, F8 and F9 exhibited hardness values approaching those of the rice based control together with lower chewiness and adhesiveness, suggesting the formation of a softer crumb structure. This behavior may result from complementary interactions between amaranth proteins and tiger nut lipids which may improve moisture retention, reduce matrix rigidity, and promote more uniform gas cell expansion. Among the combined formulations, F7 exhibited the highest springiness value (11.17), approaching that of the wheat based control (11.68), indicating improved elastic recovery and better preservation of crumb structure after compression.

Overall, the results demonstrate that textural behavior was strongly influenced by the interactions among flour composition, moisture retention, and crumb microstructure. The combined incorporation of amaranth and tiger nut generated more favorable textural characteristics than the use of either flour, supporting their potential to improve the quality of gluten-free sponge cakes.

3.6. Antioxidant Properties

Four methods were used to evaluate the antioxidant activity (AA) of the samples: total antioxidant capacity (TAC) [34]; DPPH radical scavenging activity [64]; ABTS radical scavenging assay [36]; and ferric reducing antioxidant power (FRAP) [39]. Ferric reducing power (FRAP) was recorded as an A0.5 value corresponding to the absorbance of 0.5 that was computed from the graph plotting absorbance against sample concentration; total antioxidant activity (TAC) was measured in milligrams of ascorbic acid equivalent per gram dry weight (mg AAE/g dw); and DPPH and ABTS scavenging activities were expressed as EC50 value in mg dw/mL (the concentration providing 50% inhibition).

The antioxidant properties of the wheat and gluten-free sponge cakes including total phenolic content, total flavonoid content, and antioxidant activities are presented in Table 8.

Table 8. Total phenolic content, total flavonoid content, and antioxidant activities of the sponge cake formulations.

	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	TAC (mg AAE/g dw)	ABTS: IC50 (mg dw/mL)	DPPH: IC50 (mg dw/mL)	FRAP (mg dw/mL)
PC	1.44 ± 0.22 ^a	0.300 ± 0.002 ⁱ	1.070 ± 0.020 ^{abc}	4.08 ± 0.24 ^c	32.00 ± 0.07 ^f	16.85 ± 0.23 ^e
NC	4.17 ± 0.45 ^e	0.250 ± 0.003 ^h	1.250 ± 0.100 ^{de}	3.50 ± 0.17 ^b	24.72 ± 0.10 ^d	10.82 ± 0.63 ^a
F1	1.53 ± 0.18 ^{ab}	0.150 ± 0.002 ^d	1.300 ± 0.050 ^{eg}	4.61 ± 0.21 ^d	49.16 ± 1.44 ⁱ	16.97 ± 0.29 ^e
F2	1.75 ± 0.25 ^{abc}	0.110 ± 0.001 ^b	1.360 ± 0.030 ^{eg}	3.48 ± 0.05 ^b	20.36 ± 0.67 ^c	21.15 ± 0.43 ^h
F3	1.49 ± 0.13 ^a	0.160 ± 0.001 ^e	1.110 ± 0.100 ^{bcd}	4.13 ± 0.23 ^c	44.13 ± 0.08 ^g	17.91 ± 0.50 ^f
F4	1.47 ± 0.20 ^a	0.120 ± 0.004 ^c	1.430 ± 0.100 ^g	9.81 ± 0.06 ^f	28.99 ± 0.60 ^e	19.54 ± 0.69 ^g
F5	1.93 ± 0.01 ^{cd}	0.160 ± 0.003 ^e	1.210 ± 0.100 ^{cde}	5.35 ± 0.10 ^e	17.94 ± 0.12 ^b	12.55 ± 0.34 ^c
F6	1.46 ± 0.06 ^a	0.170 ± 0.006 ^f	0.960 ± 0.080 ^a	11.90 ± 0.05 ^h	91.55 ± 0.54 ^j	15.68 ± 0.66 ^d
F7	1.80 ± 0.24 ^{abcd}	0.180 ± 0.004 ^g	1.010 ± 0.070 ^{ab}	10.63 ± 0.20 ^g	46.62 ± 1.82 ^h	15.48 ± 0.33 ^d
F8	1.87 ± 0.20 ^{bcd}	0.110 ± 0.001 ^b	1.800 ± 0.150 ^h	2.61 ± 0.27 ^a	10.53 ± 0.09 ^a	11.70 ± 0.27 ^b
F9	2.12 ± 0.01 ^d	0.060 ± 0.007 ^a	1.220 ± 0.004 ^{cde}	3.27 ± 0.17 ^b	47.19 ± 0.61 ^h	13.04 ± 0.47 ^c
<i>p</i> -Value	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***

PC: Positive control, NC: negative control, TPC: total phenolic content, TFC: total flavonoid content, TAC: total antioxidant capacity, ABTS: 2,2'-azinobis(3-éthylbenzothiazolin-6-sulfonate), DPPH: 2,2-diphényl-1-picrylhydrazyl, FRAP: ferric reducing power. *** indicate significant differences at $p < 0.001$. Different superscript letters within the same column indicate statistically significant differences among means ($p < 0.05$) using Fisher's LSD post hoc test.

The samples' total phenolic (TPC), expressed as gallic acid equivalent (GAE mg/g dw), was determined to be between 1.44 ± 0.22 mg GAE and 2.12 ± 0.01 mg GAE per g of dry weight. The total flavonoid content (TFC), expressed as quercetin equivalent (QE), varied between 0.06 ± 0.007 and 0.30 ± 0.002 mg QE per g of dry weight. Comparative analysis of the formulations (F1–F9) and negative control as well as revealed significant differences in TPC and TFC. All formulations exhibited lower TPC and TFC values than the controls. The negative control exhibited the highest TPC with 4.17 ± 0.22 mg GAE/g dw.; this higher value may be attributed to the presence of non-phenolic compounds that also reacted with the Folin–Ciocalteu reagent. The reactivity of the Folin–Ciocalteu phenol reagent with nitrogen compounds reduces the usefulness of the reagent for the detection and determination of phenolic compounds [65]. Moreover, phenolic compounds can form complexes with proteins in the matrix, and the heating process can also modify phenolic antioxidants during cooking to varying degrees [66]. It is well documented that rice is a rich source of phenolic compounds, nitrogen compounds like hydroxycinnamic acid amides, and carbohydrates [67]. Similarly, the positive control exhibited the highest TFC value of 0.30 mg QE/g d.w. Regarding the antioxidant activity data, both the controls and the formulations (F1–F9) exhibited considerable antioxidant activity, with F8 having the highest total antioxidant capacity value at 1.80 ± 0.15 mg AAE/g. Consistent with these results, the formulation exhibiting the highest antioxidant activity also showed the lowest IC50 values, with 2.61 mg/mL for ABTS and 10.53 mg/mL for DPPH compared with the other formulations. Regarding ferric reducing antioxidant power (FRAP), although the negative control exhibited the highest reducing power (A 0.5 as 10.82 mg/mL), F8 reached its A 0.5 value at a lower concentration (11.70 mg/mL) than the other gluten-free formulations indicating a higher reducing power. This suggests that the combination of 60% sugar, 60% rice, 10% amaranth, and 30% tiger nut enhanced the reducing capacity of the sponge cake, possibly due to the presence of antioxidant compounds including reductones and minerals which are capable of reducing Fe^{3+} to Fe^{2+} . Polyphenols diverse and widely distributed class of phytochemicals plant-derived. These compounds which are widely found in rice (*Oryza sativa*), wheat (*Triticum aestivum*), tiger nut (*Cyperus esculentus* L.), and amaranth (*Amaranthus cruentus*), are increasingly recognized for their important role in human health. Among the irbiological functions, poly-phenols are potent antioxidants that can neutralize reactive oxygen species (ROS) and reduce oxidative stress, which is a major factor in the development of chronic diseases like cancer, heart disease, and neurodegenerative conditions like Parkinson's and Alzheimer's diseases [68–71].

3.7. Sensory Properties

The sensory profiles of the wheat and gluten-free sponge cakes are presented in Figure 2. Significant differences were observed among formulations for all evaluated sensory attributes, including taste, flavor, texture, appearance, and overall acceptability ($p < 0.001$), demonstrating that flour substitution substantially influenced consumer perception.

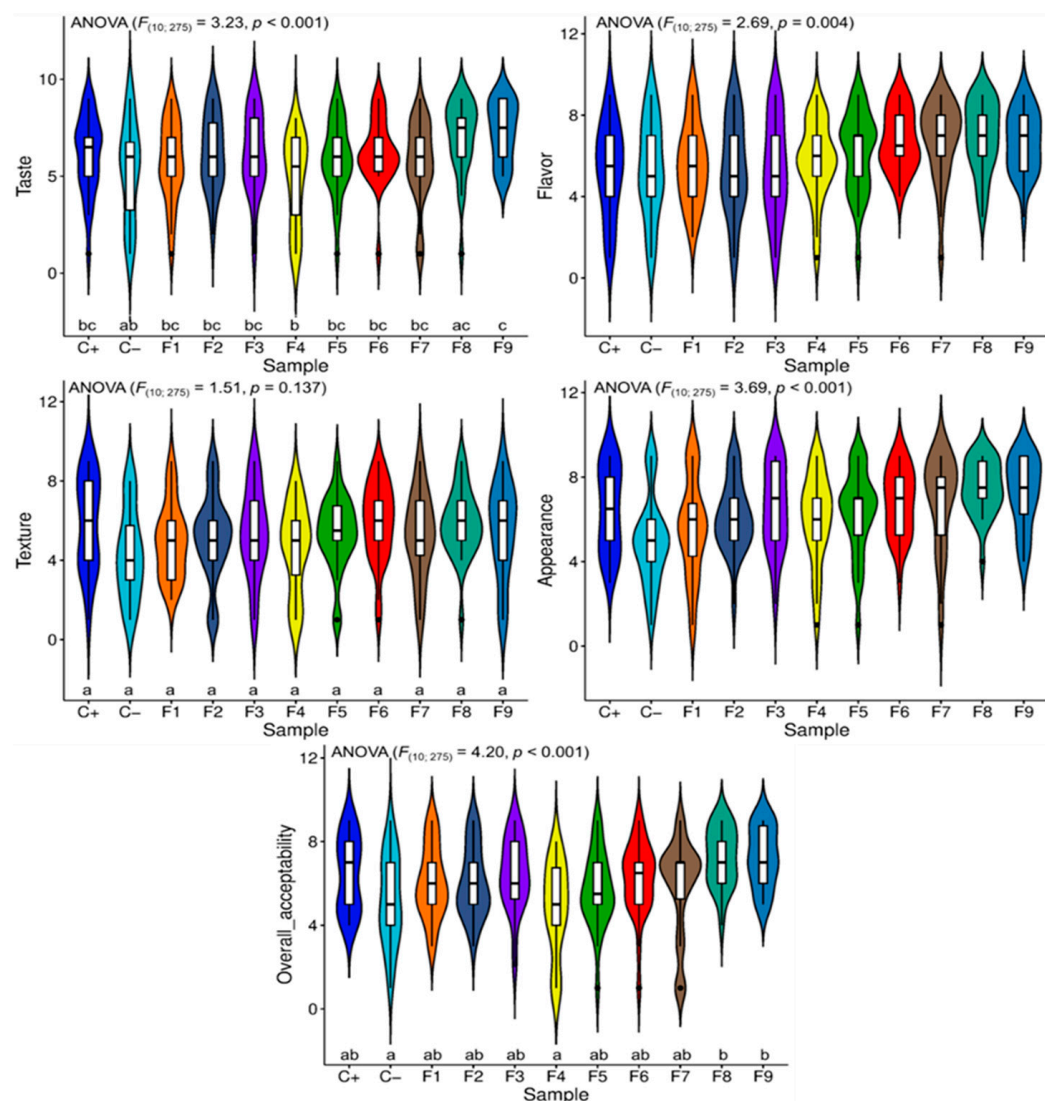


Figure 2. Violin plots with overlaid boxplots showing the variation of sensory analysis scores obtained from 26 panelists among the sponge cake formulations (F1–F9), including positive (C+) and negative (C–) controls. Different letters indicate significant differences according to Tukey's HSD post hoc test ($p < 0.05$).

The positive control formulated with wheat flour received high scores for most sensory parameters, particularly texture and overall acceptability, confirming the technological advantages of gluten in producing bakery products with desirable structure, springiness, and mouthfeel. In contrast, the negative control formulated exclusively with rice flour exhibited significantly lower sensory scores, especially for texture (4.30) and appearance (4.96). These results are consistent with its lower specific volume and less homogeneous crumb structure observed in previous sections, reflecting the limited ability of rice flour alone to reproduce the structural functionality of gluten. Formulations containing amaranth flour alone (F1–F3) generally showed improved sensory performance compared with the rice control. Taste, texture, and appearance scores increased progressively with amaranth incorporation, particularly in F3, suggesting that moderate enrichment enhanced overall product perception. These improvements may be related to the higher protein content of amaranth and its influence on moisture retention, crumb development, and visual appearance. Similar observations were reported by Hamzhepour and Dastgerdi [20], who found improved sensory acceptance in cakes containing moderate levels of amaranth flour. However, flavor scores remained relatively moderate at higher amaranth levels,

suggesting that excessive incorporation may introduce characteristic cereal-like or earthy notes that could reduce consumer preference. Formulations enriched with tiger nut flour (F4–F6) showed variable sensory responses. While F4 received comparatively lower scores, increasing tiger nut incorporation improved overall sensory quality. These effects may be attributed to the naturally occurring lipids and sugars present in tiger nut flour, which likely contributed to enhanced flavor perception, improved softness, and a smoother mouthfeel. Similar improvements in sensory performance following tiger nut incorporation have been reported in bakery products [58]. The formulations combining amaranth and tiger nut (F7–F9) generally achieved the highest sensory acceptance among all gluten-free formulations. In particular, F8 and F9 obtained the highest scores for taste, appearance, and overall acceptability, in some cases exceeding those of the wheat control. These findings support the presence of complementary effects between the two ingredients, where the structural contribution of amaranth and the moisture-retaining and flavor-enhancing properties of tiger nut jointly improved product quality. The superior appearance scores of these formulations may be associated with improved crust coloration and more desirable crumb characteristics, whereas enhanced taste and flavor perception may result from the interaction of aromatic compounds, balanced moisture retention, and improved textural properties. Overall, the sensory results indicate that combining amaranth and tiger nut flours represents an effective strategy to improve consumer acceptance of gluten-free sponge cakes while maintaining desirable technological characteristics.

3.8. Principal Component Analysis and Hierarchical Clustering Analysis

Principal component analysis (PCA) was performed to investigate the relationships among the physicochemical, structural, textural, antioxidant, colorimetric, and sensory properties of the sponge cake formulations. The PCA biplot (Figure 3, Table 9), including ellipses representing the 68% confidence interval, was mainly defined by the first two principal components (PC1 and PC2), which together explained 42.0% of the total variance (22.9% for PC1 and 19.1% for PC2). Although moderate, this proportion of explained variance provided a satisfactory overview of the multivariate relationships within the dataset.

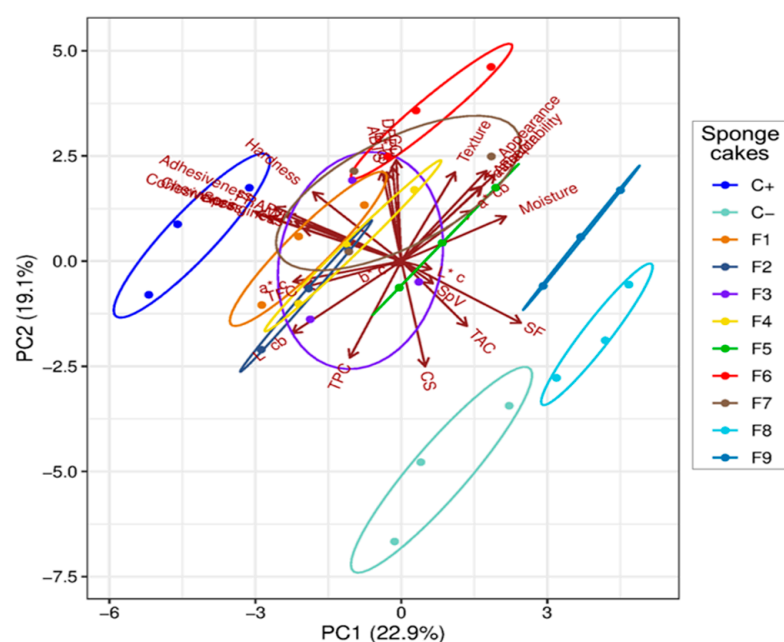


Figure 3. PCA biplot of physicochemical, color, textural, antioxidant, sensory properties of sponge cake formulations (F1–F9) including positive (C+) and negative (C−) controls. The circle represents the correlation circle, whereas the colored ellipses indicate 68% confidence intervals for formulations.

Table 9. Pearson correlation coefficients between the studied variables and the first five PCA dimensions derived from the standardized dataset.

Variables	Dim. 1 Var. = 22.85%		Dim. 2 Var. = 19.15%		Dim. 3 Var. = 15.67%		Dim. 4 Var. = 12.51%		Dim. 5 Var. = 9.61%	
	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value	<i>r</i>	<i>p</i> -Value
a* crust	0.609	<0.001	×	×	0.460	0.007	×	×	0.438	0.011
a* crumb	−0.388	0.026	×	×	−0.500	0.003	×	×	×	×
ABTS	×	×	0.583	<0.001	−0.456	0.008	×	×	×	×
Acceptability	−0.443	0.010	0.482	0.004	0.703	<0.001	×	×	×	×
Adhesiveness	0.710	<0.001	0.346	0.049	×	×	0.377	0.030	×	×
Appearance	−0.478	0.005	0.592	<0.001	0.607	<0.001	×	×	×	×
b* crust	×	×	×	×	×	×	−0.850	<0.001	×	×
b* crumb	×	×	0.578	<0.001	×	×	×	×	0.359	0.040
CC	×	×	0.659	<0.001	×	×	−0.610	<0.001	×	×
Chewiness	0.824	<0.001	×	×	×	×	×	×	×	×
Cohesiveness	0.750	<0.001	×	×	×	×	×	×	×	×
CS	×	×	−0.686	<0.001	×	×	0.567	0.001	×	×
DPPH	×	×	0.609	<0.001	−0.425	0.014	×	×	−0.381	0.029
Flavor	−0.525	0.002	0.567	0.001	0.557	0.001	×	×	×	×
FRAP	0.653	<0.001	×	×	×	×	0.554	0.001	×	×
Hardness	0.497	0.003	0.445	0.009	×	×	0.415	0.016	×	×
L* crust	×	×	×	×	×	×	×	×	−0.856	<0.001
L* crumb	0.612	<0.001	−0.471	0.006	0.421	0.015	×	×	−0.404	0.020
Moisture	−0.588	<0.001	×	×	−0.512	0.002	0.350	0.046	×	×
SF	−0.675	<0.001	−0.403	0.020	×	×	×	×	×	×
Springiness	0.555	0.001	×	×	×	×	×	×	×	×
SpV	×	×	×	×	0.446	0.009	×	×	0.620	<0.001
TAC	−0.368	0.035	−0.426	0.013	×	×	0.547	0.001	0.478	0.005
Taste	−0.452	0.008	0.490	0.004	0.697	<0.001	×	×	×	×
Texture	×	×	0.582	<0.001	0.710	<0.001	×	×	×	×
TFC	0.543	0.001	×	×	0.409	0.018	−0.592	<0.001	×	×
TPC	×	×	−0.631	<0.001	×	×	×	×	−0.388	0.026
WL	0.600	<0.001	×	×	×	×	0.359	0.040	×	×

Only statistics (*r*: Correlation coefficient and *p*-value) of significant variables ($p < 0.05$) are shown. Var: Explained variance of each principal component dimension. × indicates that no significant correlation was observed between the variable and the corresponding PCA dimension ($p > 0.05$).

PC1 was mainly associated with positive loadings for moisture content, sensory texture, appearance, overall acceptability, specific volume (SV), total antioxidant capacity (TAC), surface fraction, average cell size, crust luminosity (L*), and crumb redness (a*). Conversely, instrumental textural parameters, total phenolic content (TPC), total flavonoid content (TFC), FRAP activity, crumb luminosity (L*), and crust color coordinates (a* and b*) were negatively associated with this axis. These relationships indicate that formulations located on the positive side of PC1 tended to exhibit improved sensory performance, higher moisture retention, larger pore development, and greater perceived product quality, whereas those positioned on the negative side were associated with firmer and more compact structures.

PC2 was mainly characterized by positive associations with instrumental texture parameters, sensory properties, and crumb color coordinates (a* and b*), whereas negative loadings were observed for surface fraction, average cell size, antioxidant indicators (TPC/TFC, TAC), crust and crumb luminosity (L*), crust chromatic coordinates (a* and b*), and specific volume. This separation suggests that texture, color development, and antioxidant properties contributed independently to formulation discrimination.

Hierarchical clustering analysis (HCA) further classified sponge cake formulations into five distinct groups according to their overall quality characteristics (Figure 4).

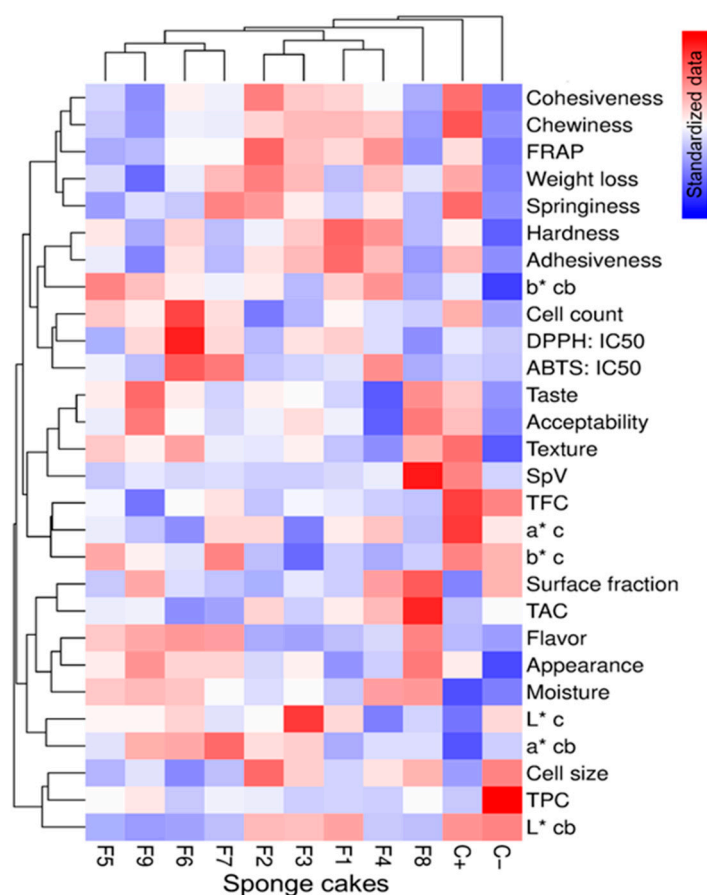


Figure 4. Heat map of the hierarchical clustering analysis of the gluten-free sponge cake formulations (F1–F9), including the positive (C+) and negative (C−) controls. The color scale represents standardized values, ranging from low values (blue) to high values (red).

The first cluster included only the negative control (C−), which was clearly separated from all other formulations and characterized by lower sensory acceptance, reduced textural performance, and inferior technological quality, reflecting the limited structural functionality of rice flour alone. Nevertheless, this formulation was associated with higher crumb luminosity. The second cluster consisted exclusively of the positive control (C+), which was strongly associated with hardness, cohesiveness, adhesiveness, chewiness, springiness and specific volume, confirming the central role of gluten in generating an elastic and cohesive sponge cake structure. The third cluster grouped F1–F4, which exhibited intermediate technological performance characterized by moderate structural and sensory quality, relatively low antioxidant levels (TFC and TPC), and limited specific volume development while chewiness remained high. The fourth cluster included F5, F6, F7, and F9, characterized by improved moisture retention and favorable sensory attributes, particularly flavor and appearance. These formulations also exhibited a finer crumb structure with a smaller average cell size and greater structural homogeneity.

Interestingly, F8 formed an independent cluster, clearly separated from all other gluten-free formulations. This formulation was associated with superior sensory performance (taste, flavor, texture, appearance, and overall acceptability), elevated moisture content, high specific volume, and enhanced antioxidant activity. The distinct positioning of F8 supports previous observations and suggests that this formulation achieved the most favorable balance between structural development, technological functionality, and consumer acceptance.

The correlation matrix (Figure 5) revealed strong and significant relationships among the evaluated variables. Overall acceptability showed very strong positive correlations with taste ($r = 0.99$), texture ($r = 0.95$), appearance ($r = 0.95$), and flavor ($r = 0.90$), confirming that consumer preference was primarily driven by these attributes.

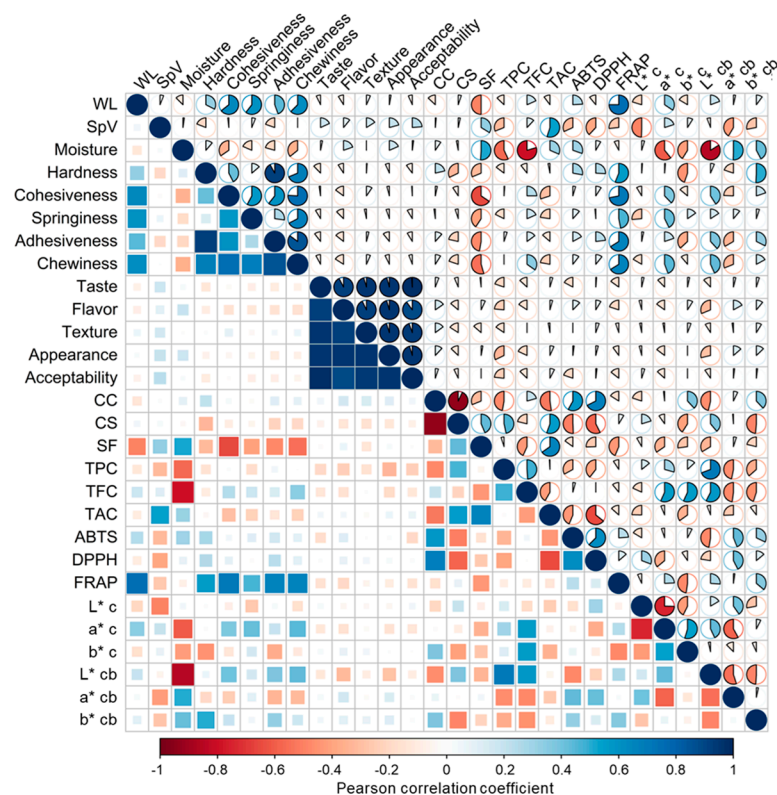


Figure 5. Correlation matrix of physicochemical, color, textural, antioxidant, sensory properties of the sponge cake formulations (F1–F9) including positive (C+) and negative (C–) controls.

Strong associations were also observed among instrumental texture parameters, particularly between hardness and adhesiveness ($r = 0.92$), chewiness and adhesiveness ($r = 0.85$), chewiness and cohesiveness ($r = 0.76$), and chewiness and hardness ($r = 0.69$), indicating a high degree of interdependence among mechanical characteristics of the crumb. Moisture content exhibited negative correlations with TFC ($r = -0.80$), TPC ($r = -0.57$), crust redness (a^* , $r = -0.61$), crust yellowness (b^* , $r = -0.41$), and crumb lightness (L^* , $r = -0.84$)*, whereas positive correlations were observed with surface fraction ($r = 0.57$), crumb redness ($r = 0.50$), and crumb yellowness ($r = 0.42$). These findings suggest that moisture retention influences both structural development and color formation.

Weight loss was positively correlated with cohesiveness ($r = 0.64$), springiness ($r = 0.60$), adhesiveness ($r = 0.44$), chewiness ($r = 0.62$), and FRAP activity ($r = 0.75$), while showing a negative association with surface fraction ($r = -0.50$). Color variables also exhibited strong interdependence, particularly between crust L^* and crust a^* ($r = -0.75$) and between crust L^* and crust b^* ($r = -0.43$), indicating that darker crusts were associated with greater redness and yellowness, consistent with increased thermal browning during baking. Overall, multivariate analysis confirmed that flour substitution significantly altered the quality attributes of sponge cakes and identified F8 as the formulation providing the most balanced combination of physicochemical, structural, antioxidant, and sensory characteristics.

4. Conclusions

The present study evaluated the effects of amaranth and tiger nut flours on the functional, physicochemical, structural, textural, antioxidant, and sensory properties of gluten-free sponge cakes formulated with rice flour. The incorporation of these alternative flours significantly influenced the quality characteristics of the cakes compared with the rice-based control.

Amaranth flour, characterized by a higher protein content, was associated with modifications in crumb structure and texture, whereas tiger nut flour, characterized by higher lipid and mineral contents, was associated with changes in moisture content, color characteristics, and sensory attributes. The results also indicated that the quality of the sponge cakes depended on both the proportion and combination of the flours used.

Among the gluten-free formulations, F8 (60% rice flour, 10% amaranth flour, and 30% tiger nut flour) exhibited the highest specific volume (5.84 cm³/g), moisture content (28.76%), and total antioxidant capacity (1.80 mg AAE/g), together with improved textural properties and the highest scores for taste, appearance, and overall acceptability, in some cases exceeding those of the wheat-based control. PCA and hierarchical clustering analyses further distinguished F8 from the other gluten-free formulations and highlighted its association with favorable physicochemical, antioxidant, and sensory attributes. Importantly, these results were obtained in formulations containing 35–45% less sugar than the conventional sponge cake formulation, suggesting that substantial sugar reduction may be achieved while maintaining acceptable technological and sensory quality.

Overall, the findings suggest that the combined use of amaranth and tiger nut flours may represent a promising approach for improving several technological, nutritional, and sensory attributes of gluten-free sponge cakes. However, some limitations of the present study should be acknowledged. Batter rheology, storage stability, staling behavior, digestibility, and large-scale consumer acceptance were not investigated. Further studies addressing these aspects would provide additional insights into the factors underlying the observed effects and help to further evaluate the potential of these formulations for gluten-free bakery applications.

Author Contributions: Conceptualization, H.M. and H.B.; methodology, H.M., H.A. (Hadil Adoum); and M.M. (Manel Merimeche); investigation, H.M., H.A. (Hadil Adoum) and M.M. (Manel Merimeche), software, N.G. and H.C.; validation, H.M.; data curation, R.A. and N.G.; formal analysis, R.A.; resources, H.M. and M.M. (Meriem Meradji); writing—original draft preparation, H.M.; writing—review and editing, H.B., I.F., M.W., R.R. and H.A. (Hamadi Attia); visualization, R.R. and M.W.; supervision, I.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in the work are included in the article; requests for further information and details can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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