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Development and Evaluation of Barnyard Millet-Makhana Fortified Biscuits for Nutritional and Functional Enhancement

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Abstract
Nutrient-dense cereals and aquatic seeds can improve the health profile of baked snacks, yet many wheat-based biscuits remain low in protein, minerals and functional lipids. Addressing this limitation, we formulated barnyard millet and makhana fortified biscuits to enhance macro & micronutrients while maintaining consumer acceptability and shelf stability. A control sample of 100 % wheat flour was compared with three formulations V1, V2 and V3 substituting barnyard millet with sorghum and makhana in varying ratios. Physical property analysis showed significant changes in spread and thickness. Diameter increased in fortified samples with V1 and V2 recording F values of 248.16 and 246.58 respectively at $p > 0.05$. Thickness was highest in V1 with F value 305.86 while weight increased significantly in V1 and V3. Proximate composition confirmed strong nutritional improvements. Moisture declined from 10.98 % in control to 8.71 % in V3 while carbohydrates decreased from 52.24 to 35.34 %. Protein content rise from 9.55 to 16.49 % and fat from 9.08 to 24.49 %. Energy values increased from 311.57 kcal to 396.53 kcal. Mineral fortification was evident with calcium rising from 128.87 to 147.98 mg per 100 g and iron from 3.46 to 4.38 mg per 100 g in V3. Ash increased from 2.32 to 2.84 %. One-way ANOVA indicated large variations in all chemical parameters with large F values of 4679.96 and 474.45 for iron and calcium, respectively. Panel testing with five-point scale and 30 panelists resulted in V2 being the most preferred with score of 4.6 for overall acceptability. Shelf-life testing of V2 for 30 days resulted in moisture increasing up to 14.94 % against 5.6 % in the control while free fatty acid values remained constant. These are confirmation of the existence of V2 as a nutritious, consumer-acceptable and stable functional biscuit.

Keywords: Gluten free, Nutritional fortification, Shelf-life stability

Introduction

The global trend towards healthier dietary is inspiring new concepts in the functional food sector, particularly in replacing conventional snacks that generally contain minimal nutrients. An ideal case is the popular biscuit, which normally contains much refined wheat flour and sugars. This combination gives rise to what is popularly referred to as "empty calories" and is attributed to an increased chance of medical issues such as obesity and type 2 diabetes (Goubgou *et al*., 2021) [13]. This non-nutritious content, as well as an increasing number of people with metabolic disorders and celiac disease, indicate a huge demand for food types that are healthy and also popular among the masses (Patil *et al*., 2023) [18]. An encouraging solution is the application of nutrient-rich, gluten-free types of flour from less popular plants. Barnyard millet (*Echinochloa frumentacea*), a hardy ancient cereal, is rich in dietary fiber and valuable minerals such as iron and calcium. It is also rich in glycemic index (GI) that keeps glucose levels stable in the body (Nani & Krishnaswamy, 2021) [17]. Makhana (*Euryale ferox*) is rich in protein, low in fat, with good minerals and low glycemic index (GI) and hence is a desirable option for healthy food (Ekal & Kumbamoorthy, 2024) [7]. It can be able to combine such ingredients and create a product better than ordinary gluten-free biscuits, typically prepared from weak rice or corn flour. Altering the formula of biscuits from barnyard millet and makhana benefits the health of the people in two ways: it enriches nutrition with slow-carb release, rich in fibres and beneficial vitamins and minerals, and it promotes sustainable agriculture with drought-resistant crop with poor soil adaptability

(Goubgou *et al*., 2021; Maharajan *et al*., 2024) [13, 15]. Altering the recipe, however, is not merely an exercise in nutrition; it must also solve major technical and taste problems. Without the gluten, the valuable component of wheat, the product ends up with poor texture and poor mouthfeel. So, it is crucial to balance the quantities of such substitute flours with care and prepare a product as delicious as it is nutritious (Ravi & Rana, 2024) [21]. This necessitates an examination of the behavior of the dough and the characteristics and chemistry of the final product with a careful consideration of the effect of the mixed flours, including spread ratio, physical and chemical characteristics, protein content and dietary fiber (Singh *et al*., 2023) [16]. Though we are aware that makhana and barnyard millet are beneficial, their combined usage in bakery products such as biscuits is not deeply studied. At the moment, there is an increasing demand for millet-based products such as multi-millet biscuits and gluten-free cookies, and people are increasingly consuming them (Porwal *et al*., 2023; Ravi & Rana, 2024) [19, 21]. This study follows through on that trend by devising a new product formulation using barnyard and makhana flour and to evaluate the use of these flours at different concentration levels in the fortified biscuits.

Materials and Methods

Raw Material Procurement and Biscuit Formulation

Organic barnyard millet, sorghum, makhana and whole wheat flours were procured from organic stores of Guntur. Refined sugar, unsalted butter, baking powder and baking soda were purchased from local market of Guntur. Four biscuit variants were formulated as Control and three variations (V1, V2, V3) with progressive substitution of barnyard millet flour with makhana and sorghum flours (Table 1).

Table 1: Ingredient composition of fortified biscuits

Ingredient	Control	Variation 1	Variation 2	Variation 3
Barnyard millet flour (gm)	50	20	15	10
Sorghum flour (gm)	0	20	15	10
Makhana flour (gm)	0	10	20	30
Wheat flour (gm)	20	20	20	20
Sugar (gm)	13	13	13	13
Butter	15	15	15	15
Baking soda	1	1	1	1
Baking powder	1	1	1	1

Preparation of Fortified Biscuits

Fortified biscuits were prepared by the method adapted from with the slight modifications. Butter and sugar were creamed to a light, fluffy consistency. The composite flours and leavening agents were sieved together (60-mesh) and gradually incorporated into the creamed mixture. The dough was kneaded, rested for 15 minutes at 4°C, sheeted to a 5 mm thickness and cut into uniform shapes. The biscuits were baked at 170°C for 20 minutes in a preheated oven, cooled to ambient temperature and packaged in HDPE pouches for subsequent analysis.

Physical and Chemical Analysis

Physical Parameters

Diameter

The diameter of the biscuits was measured using the AACC (2000). Six biscuits were placed edge to edge, and their total diameter was measured in millimeters using vernier callipers. This procedure was repeated to obtain an average value and the results were reported in centimeters (cm).

Thickness

The thickness of biscuits was measured using the AACC (2000) method. Six biscuits were stacked on top of one another, and the total height was measured in millimetres using vernier callipers. This procedure was repeated to obtain an average value, and the results were reported in centimetres

Spread ratio

The spread ratio was determined by measuring the diameter (D) and thickness (T) of six biscuits from each variant using a vernier callipers. The spread factor (SF) was calculated as

$SF = (D/T) \times CF \times 10$

where CF is the correction factor (0.1).

Proximate Composition

The fortified biscuits were analysed for moisture contents, ash content, crude fat, crude protein, crude fibre and total carbohydrates contents using the standard AOAC (2000) methods. The energy content was estimated using the Atwater general conversion factors as described by Ahmed *et al*., (2022) [2]. Minerals like calcium and iron were analysed by using AOAC (2005) and AOAC (1998) respectively.

Sensory Evaluation

The organoleptic acceptability of the biscuit variants was evaluated by a panel of 30 untrained judges using a 5-point hedonic scale (1 = dislike extremely to 5 = like extremely) as described by Rohith *et al*., (2020) [22]. Attributes evaluated included appearance, flavour, texture, taste and overall acceptability. Samples were presented randomly under controlled lighting conditions.

Shelf-Life Study

The most acceptable variant from sensory analysis was selected for a 30-day shelf-life study. Biscuits were stored in HDPE pouches at ambient conditions. Analyses were conducted at 0, 15 and 30 days for free fatty acids (FFA) and sensory attributes by the methods described by Fatemeh *et al*., (1999) [11].

Statistical Analysis

All analyses were performed in triplicate. Data were subjected to one-way analysis of variance (ANOVA) and mean differences were compared using Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$) using SPSS software (version 16.0).

Results and Discussion

Biscuits fortified with barnyard millet and makhana flour as partial substitutes for wheat flour were tried in the formulation. The results are systematically presented and discussed.

Physical Properties: The physical characteristics, diameter, thickness and weight of the control (100% wheat) and three variations (V1, V2, V3 with increasing barnyard millet and makhana flour) were assessed to understand the impact of fortification on biscuit structure. The results, analyzed via One-Way ANOVA, are summarized in Table 2.

Table 2: One-Way ANOVA for Physical Properties of Biscuits

Parameter	F-value	p-value	Significant ($p < 0.05$)
Diameter_Control	2.6063	0.1817	No
Diameter_V1	248.1606	0.0001	Yes
Diameter_V2	246.58	0.0001	Yes
Diameter_V3	96.9665	0.0006	Yes
Thickness_Control	49.0282	0.0022	Yes
Thickness_V1	305.8615	0.0001	Yes
Thickness_V2	3.025	0.157	No
Thickness_V3	16.1916	0.0158	Yes
Weight_Control	22.0934	0.0093	Yes
Weight_V1	106.2274	0.0005	Yes
Weight_V2	0.4444	0.5415	No
Weight_V3	9.9031	0.0346	Yes

Variations V1, V2 and V3 showed statistically significant differences ($p < 0.05$) in diameter, indicating that fortification altered the spread ratio during baking. This is visually supported by Figure 1, which shows a pronounced increase in post-baking diameter for V1 and V2. The high F-values suggest strong variation, likely due to differences in water absorption and gluten dilution affecting dough viscosity and spread. These results were consistent with previous studies where incorporation of non-wheat flours reduced dough elasticity and altered spread behavior (Singh *et al.*, 2020) [18]. For thickness, V1 and V3 exhibited significant changes, while V2 did not differ significantly from the control. This indicates that the specific flour blend in V2 maintained a structural height comparable to wheat flour, potentially due to a balanced protein-starch matrix. The control sample also showed a significant difference, underscoring the sensitivity of biscuit height to minor recipe alterations. The post-baking thickness increase was most substantial in V1 in Figure 1, correlating with its highest F-value (305.86). Weight was significantly affected in the control, V1, and V3, but not in V2. This aligns with the thickness data, suggesting V2's composition resulted in a product with physical properties (mass and height) most similar to the conventional biscuit, a crucial factor for consumer acceptance and production standardization.

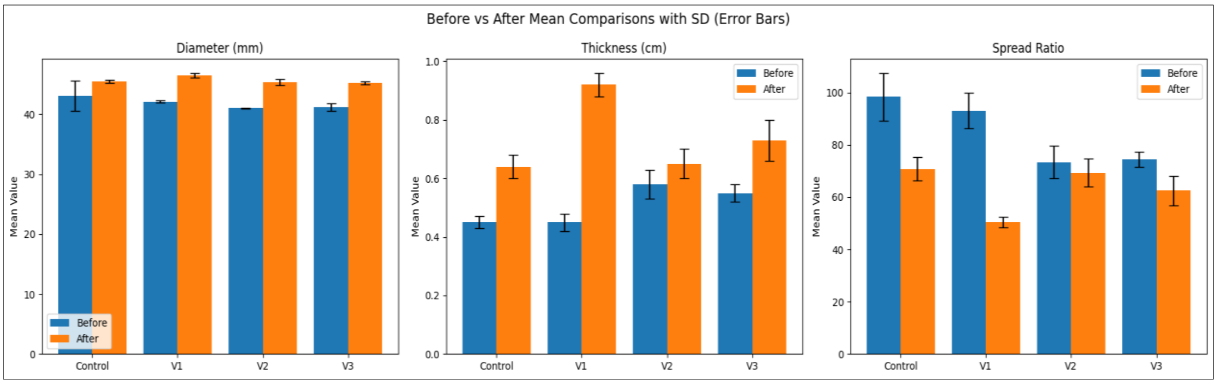

Fig 1: Effect of Baking on Physical Properties of Biscuits (Diameter, Thickness, Spread Ratio) - Comparison of Mean Values Before and After Baking with Standard Deviation (SD) Error Bars

Chemical Composition and Nutritional Enhancement

The proximate composition revealed a substantial nutritional improvement with fortification in Table 3. A progressive decline in moisture content from control (10.98%) to V3 (8.71%) was observed, which can enhance shelf stability. Carbohydrate content decreased inversely with the level of substitution (52.24% in control to 35.34% in V3).

Table 3: Mean and Standard Deviation of Chemical Composition Parameters

	Mean	Standard Deviation		Mean	Standard Deviation
Moisture_Control	10.976667	0.650641	Ash_Control	2.32	0.655515
Moisture_V1	10.263333	0.650641	Ash_V1	2.533333	0.650641
Moisture_V2	9.393333	0.602771	Ash_V2	2.593333	0.602771
Moisture_V3	8.713333	0.550757	Ash_V3	2.843333	0.550757
CHO_Control	52.236667	0.666058	Energy_Control	311.566667	0.650641
CHO_V1	48.663333	0.650641	Energy_V1	340.023333	0.650641
CHO_V2	41.913333	0.602771	Energy_V2	368.133333	0.602771
CHO_V3	35.343333	0.550757	Energy_V3	396.533333	0.550757
Proteins_Control	9.546667	0.650641	Calcium_Control	128.866667	0.650641
Proteins_V1	13.993333	4.225321	Calcium_V1	94.616667	69.860027
Proteins_V2	14.083333	0.602771	Calcium_V2	141.533333	0.602771
Proteins_V3	16.493333	0.550757	Calcium_V3	147.983333	0.550757
Fat_Control	9.08	0.492443	Iron_Control	3.456667	0.650641
Fat_V1	14.333333	0.650641	Iron_V1	3.803333	0.650641
Fat_V2	19.323333	0.602771	Iron_V2	4.003333	0.602771

Fat_V3	24.493333	0.550757	Iron_V3	4.383333	0.550757
Fiber_Control	1.406667	0.650641			
Fiber_V1	1.406667	0.650641			
Fiber_V2	1.183333	0.602771			
Fiber_V3	1.183333	0.550757			

Conversely, protein and fat content increased significantly, from 9.55% to 16.49% and 9.08% to 24.49%, respectively, highlighting the high-quality macronutrient profile of makhana flour. This directly resulted in a marked increase in energy content, from 311.57 kcal in the control to 396.53 kcal in V3. Ash content, an indicator of total minerals, also increased. Notably, calcium and iron content showed an upward trend, with V3 containing 147.98 mg and 4.38 mg, respectively. These findings align with previous studies by Kumar *et al*. (2019) and Thilagavathi *et al*. (2021) [14, 25], who demonstrated that composite flours enhance protein, fiber, and mineral contents in bakery products.

The consistency of these trends across replications is visually confirmed in the line plots in Figure 2. One-Way ANOVA confirmed that all chemical parameters differed significantly ($p < 0.05$) among the formulations in Table 4, with exceptionally high F-values for moisture (F=1435.96), fiber (F=311.65), and energy (F=163.64). This statistical validation confirms that fortification significantly and positively altered the nutritional matrix of the biscuits.

Table 4: One-Way ANOVA for Chemical Composition Parameters

Component	F-value	P-value	F-critical	Significant ($P < 0.05$)
Moisture	1435.9578	0	4.0662	Yes
CHO	16.0385	0.00096	4.0662	Yes
Proteins	4.9162	0.03188	4.0662	Yes
Fat	22.5049	0.0003	4.0662	Yes
Fiber	311.6467	0	4.0662	Yes
Ash	8.4102	0.00743	4.0662	Yes
Energy	163.6368	0	4.0662	Yes
Calcium	32.0892	0.00008	4.0662	Yes
Iron	12.2877	0.0023	4.0662	Yes

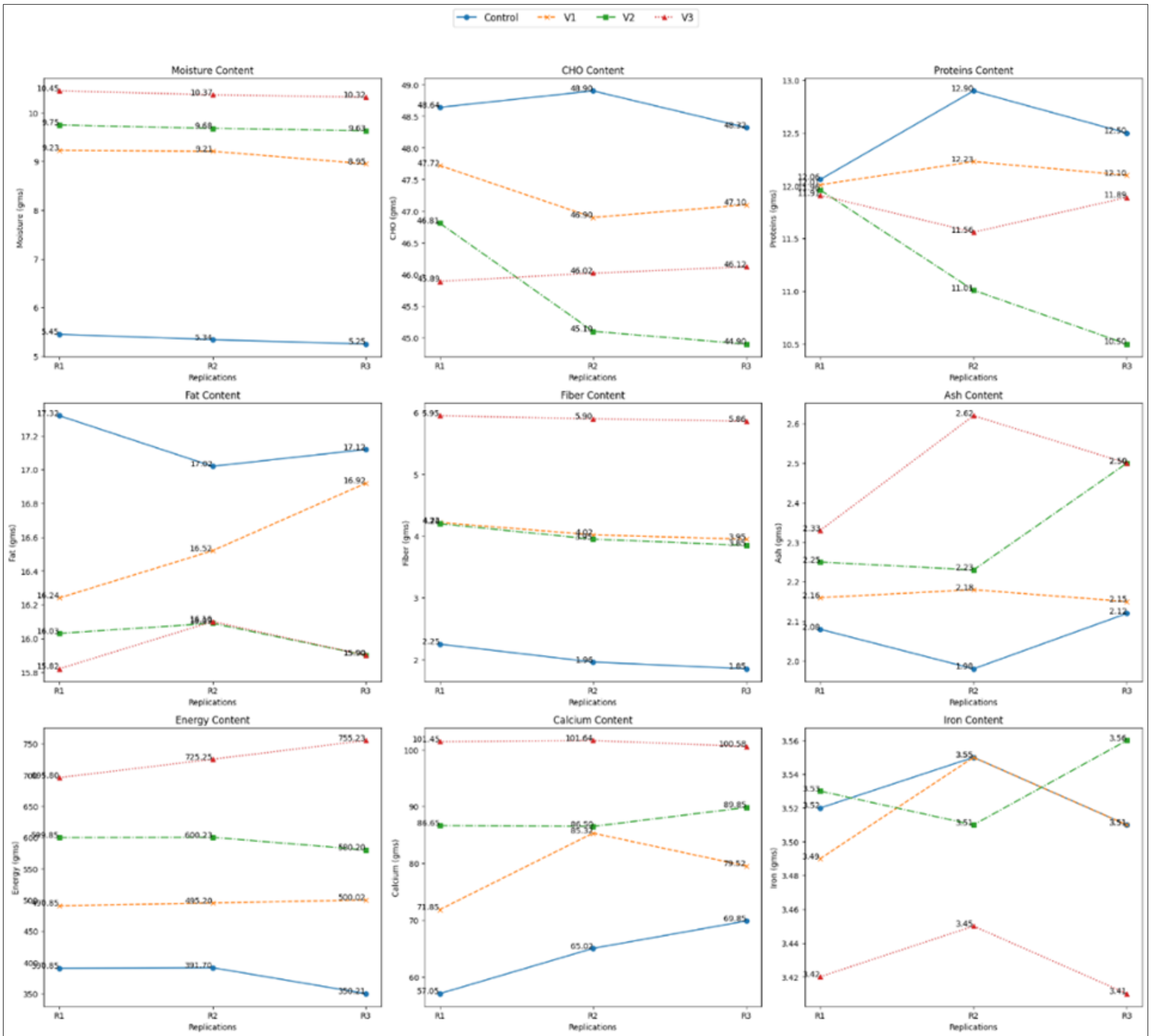

Fig 2: Line Plot of Chemical Composition Parameters Across Replications (R1-R3) for Control and Variations (V1, V2, V3)

Organoleptic Acceptance

Sensory evaluation in Figure 3 revealed that Variation 2 (V2) was the most preferred formulation. It scored highest in appearance & colour (4.5/5), taste (4.4/5), texture (4.5/5), crunchiness (4.5/5) and overall acceptability (4.6/5). This suggests that the specific flour ratio in V2 achieved an optimal balance, enhancing nutritional value without compromising sensory properties. The control sample performed moderately, while V3, despite its superior nutritional profile, received lower scores, likely due to a more pronounced earthy taste and denser texture from the highest level of fortification. Similar findings were observed by Devi *et al*. (2019) [6], who noted that 20-30% substitution with non-wheat flours like legume flour and millet flour maintained desirable sensory characteristics in biscuits.

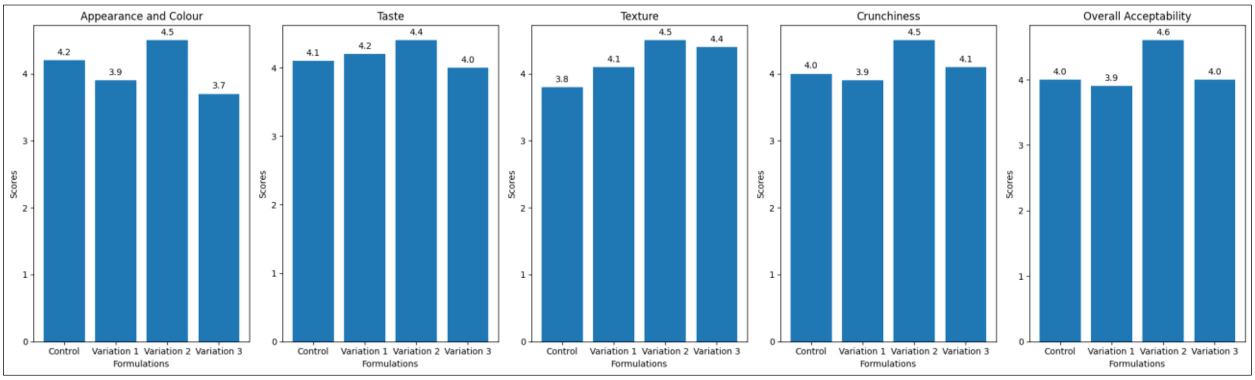

Fig 3: Bar Charts Representing Sensory Scores of Control and Variations Based on 5-Point Hedonic Scale

Shelf-Life Study of the Most Accepted Biscuit (V2)

Based on sensory results, V2 was selected for a 30-day shelf-life study against the control, monitoring moisture and free fatty acids (FFA) as indicators of staling and rancidity in Table 5.

Table 5: F-Test Results for Moisture and Free Fatty Acid Content at 0-, 15- and 30-Days Storage

	0 DAY STORAGE			
	MOISTURE		**FFA**	
	Control	**Variation 2**	**Control**	**Variation 2**
Mean	5.303333333	9.716666667	0.2466666667	0.25666
Variance	0.01923333333	0.02503333333	0.004933333333	0.009033333333
Observations	3	3	3	3
Df	2	2	2	2
F	0.7683089214		0.5461254613	
P(F<=f) one-tail	0.4344879518		0.353221957	
F Critical one-tail	0.05263157895		0.05263157895	
	15 Days Storage			
	Moisture		**FFA**	
	Control	**Variation 2**	**Control**	**Variation 2**
Mean	5.386666667	9.956666667	0.3466666667	0.3466666667
Variance	0.02643333333	0.004633333333	0.004233333333	0.02333333333
Observations	3	3	3	3
Df	2	2	2	2
F	5.705035971		0.1814285714	
P(F<=f) one-tail	0.1491416309		0.15356711	
F Critical one-tail	19		0.05263157895	
	30 Days Storage			
	Moisture		**FFA**	
	Control	**Variation 2**	**Control**	**Variation 2**
Mean	5.6	14.94	0.4433333333	0.4566666667
Variance	0.0499	91.7452	0.01493333333	0.023333333
Observations	3	3	3	3
Df	2	2	2	2
F	0.0005438976644		0.64	
P(F<=f) one-tail	0.0005436020005		0.3902439024	
F Critical one-tail	0.05263157895		0.05263157895	

While V2 initially had higher moisture, the difference was not statistically significant ($p > 0.05$) until day 30, when its moisture content rose sharply to 14.94% compared to the control's 5.6% ($p < 0.05$). This indicates a higher hygroscopicity of the fortified flour blend, which could affect crispness over extended storage. Conversely, FFA levels increased gradually in both samples but showed no statistically significant difference ($p > 0.05$) at any interval. This indicates that V2 was not more susceptible to hydrolytic rancidity than the control wheat biscuit over the one-month time interval. These results are consistent with Gajera *et al*. (2020) [5], who reported that millet-based biscuits maintain acceptable oxidative quality when stored under ambient conditions. Results indicate that barnyard millet and makhana flour are functional constituents with potential for enriching biscuits. V2 was the most efficacious formulation that was successful in improving protein, fat, and mineral enrichment along with physical characteristics and sensory scores comparable to the control product. Overall acceptability was the best of all samples. Even though there was higher moisture uptake in the case of V2 over a span of 30 days, stability against rancidity was comparable with the control. This suggests that with appropriate moisture-proof packaging, there is potential for retaining quality for at least one month in the case of V2 biscuits. Thus, V2 is a potential, nutrient-rich functional food with huge consumer acceptance.

Conclusion

Fortification with barnyard millet and makhana significantly enhanced the nutritional and qualitative value of biscuits. Protein increased from 9.55 percent in the control biscuits to 16.49 percent in V3 and fat content increased to 24.49 percent, increasing the energy to 396.53 kcal/100 g. Minerals were enhanced with calcium up to 147.98 mg and iron up to 4.38 mg, indicating that the composite flours are rich in their nutrient profile. The taste panel ranked V2 as the best choice with an overall score of 4.6 while maintaining a desirable texture and flavor. Shelf-life studies indicated that although the fortified biscuits absorbed higher moisture at 14.94 percent after 30 days, the biscuits did not become rancid and the free fatty acids remained around 0.46. These outcomes confirm that barnyard millet and makhana are potential materials for manufacturing gluten-free biscuits with extensive nutrition, attractiveness, and storable for a short period.

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