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Development of a Proteinous Composite Flour for the Bakery Industry

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Abstract

Bakery products are commonly prepared using refined wheat flour, which lacks nutrients such as high-quality proteins and minerals. The incorporation of plant-based ingredients into composite flours has emerged as a promising approach to improve the nutritional quality of bakery products. This study was focused to develop a nutritionally enhanced composite flour by blending exudate soy flour, pumpkin seed powder, and spinach powder with wheat flour and assess its functional suitability in muffin production. Four flour combinations were formulated: T1 (100% wheat as control), T2 (60% wheat + 36% soy + 2% pumpkin + 2% spinach), T3 (65% wheat + 31% soy + 2% pumpkin + 2% spinach), and T4 (70% wheat + 26% soy + 2% pumpkin + 2% spinach). Muffins prepared from these blends were subjected to a series of analyses, including proximate composition (protein, fat, ash, moisture), mineral content (Mg, Zn, Fe, Ca), antioxidant activity (DPPH assay), and sensory evaluation using a 5-point hedonic scale. Results revealed that the T2 formulation demonstrated the highest protein content (26.5%), strong antioxidant activity, and the most favourable sensory acceptance among all variations. These improvements were achieved without compromising taste, texture, or shelf-life stability. The findings confirm that replacing a portion of wheat flour with locally available, nutrient-rich ingredients can significantly improve the nutritional value of bakery products. Therefore, the study supports the practical potential of composite flours in addressing protein deficiency and promoting healthier bakery alternatives.

***Keywords**:* Nutritional enhancement, protein-enriched flour, pumpkin seed powder, spinach powder, sensory evaluation

Introduction

In an increasingly health-conscious world, food is no longer viewed solely as a source of sustenance but as a means to promote wellness, manage lifestyle diseases, and support long-term health. Among processed food categories, bakery products such as bread, muffins, biscuits, and cakes are dietary staples for many populations due to their convenience, palatability, and accessibility. However, most commercial baked goods rely on refined wheat flour (Triticum aestivum), which, while providing carbohydrates and baking structure, is deficient in essential nutrients. Wheat lacks indispensable amino acids like lysine and threonine and is low in protein, dietary fibre, and key micronutrients such as iron (Fe), zinc (Zn), magnesium (Mg), and calcium (Ca) (Dhingra & Jood, 2001; Kamaljit et al., 2010). To overcome these limitations, composite flour technology has emerged as a promising strategy (Kamaljit et al., 2010). The present study explores the development of a novel composite flour using soy flour (Glycine max), pumpkin seed powder (Cucurbita maxima), and spinach powder (Spinacia oleracea), each selected for its unique nutritional and functional benefits. Soybeans contain 30–45% high-quality protein and all indispensable amino acids. Their protein content is approximately two times higher than other pulses, four times that of wheat, six times that of rice, four times that of eggs, and twelve times higher than milk. Additionally, soy contains around 3% lecithin, which supports brain development and improves the texture, yield, and shelf life of baked products by

increasing water absorption and moisture retention (Setchell et al., 2004; Mishra et al., 2021). Pumpkin seeds, traditionally regarded as waste, are rich in essential minerals like Mg, Zn, and Fe, all of which serve as enzymatic cofactors necessary for protein synthesis, oxygen transport, and immune function (Muday et al., 2003; Mills et al., 2019). Moreover, pumpkin seeds offer dermatological and therapeutic properties, supporting collagen production and providing antioxidant protection (Goyeneche, 2015; Gamba et al., 2021). Spinach adds functional and nutritional diversity to the blend, providing bioavailable calcium and iron critical for red blood cell formation, muscular contraction, and enzymatic stability (Ribaya-Mercado & Blumberg, 2004; Hercberg et al., 2018). An important focus of this research is on metabolic synergy, specifically, the role of mineral cofactors in facilitating protein utilisation. Even high-protein foods like soy are ineffective without minerals such as Zn, Mg, Fe, and Ca that act as catalysts in biochemical pathways. This study supports a shift toward locally empowered, nutritionally conscious bakery innovations. By combining antioxidant-rich spinach, mineral-dense pumpkin seeds, and protein- rich soy, the study provides a workable remedy for micronutrient deficiencies and protein-energy malnutrition. It contributes to public health, food sovereignty, and sustainable use of agricultural by-products, aligning with national and global nutrition strategies.

Materials and Methods

Refined wheat flour (*Triticum aestivum*), dehydrated soy meat (*Glycine max*), raw pumpkin seeds (*Cucurbita maxima*), and fresh spinach leaves (*Spinacia oleracea*) were obtained from certified local markets and vendors. Dehydrated soy meat was used as a practical alternative to soy protein isolate. Fresh spinach leaves were selected for their high micronutrient and antioxidant content. Pumpkin seeds, commonly discarded in domestic settings, were chosen for their mineral richness and functional properties. Wheat flour served as the control and base ingredient in all formulations. Each raw material underwent specific preparation procedures before inclusion in the flour blends.

Preparation of Key Ingredients

To remove bitterness, pumpkin seeds were thoroughly rinsed to remove debris, then boiled in water for 5 min to reduce bitterness caused by polyphenolic compounds. Immediately after boiling, the seeds were immersed in an ice water bath for 5 min to halt enzymatic activity and preserve nutrient quality. Tissue paper was used to remove surface moisture. After that, the seeds were oven-dried at 110°C until their weight remained constant. Once fully dehydrated, the seeds were ground using a domestic blender and sieved through a 30-mesh standard sieve to achieve uniform granularity. The resulting flour was stored in airtight polyethylene (PE) containers in a cool, dark place to prevent oxidative spoilage. Fresh spinach leaves were sorted, blanched in hot water (95°C) for 5 min, and rapidly cooled in an ice water bath to retain color and nutrients. After drying surface moisture, the leaves were oven-dried at 65°C for 4 hours, then ground and sieved into a fine powder. Dehydrated soy chunks (Lanka Soy) were oven-dried at 120°C for 2 min to remove residual moisture. The cooled chunks were ground and sieved through a 30-mesh screen to produce fine soy flour rich in protein.

Formulation of Composite Flour

Table 1: *Preparation of composite flour blend*

Treatment	Pumpkin Seed Flour (g)	Spinach Powder (g)	Wheat Flour (g)	Soy Flour (g)
T1 (369)	0	0	100	0
T2 (301)	2	2	60	36
T3 (312)	2	2	65	31
T4 (345)	2	2	70	26

To develop nutritionally enhanced muffins, four flour blends were formulated using refined wheat flour (WF), soy flour (SF), pumpkin seed powder (PSF), and spinach powder (SP). While pumpkin seed and spinach powders were held constant at 2% each (2 g per 100 g flour mix), wheat and soy flour ratios were adjusted to study the effect of protein enrichment and sensory quality. Each blend was maintained at a total of 100 g to standardize comparison across treatments. Table 1 depicts the proportions of composite flour in the three experimental variations such as: T1 (Control): 100:0:0:0 (WF: SF: PSF: SP), T2: 60:36:2:2 (WF: SF: PSF: SP), T3**:** 65:31:2:2 (WF: SF: PSF: SP), T4: 70:26:2:2 (WF: SF: PSF: SP).

Figure 1*: Composite flour formulations and their raw ingredients. (a) Packaged flour blends (T1– T4) prepared for muffin production. (b) Individual raw ingredients including wheat flour, soy flour, pumpkin seed flour, and spinach powder before blending.*

As shown in Figure 1, the raw ingredients and prepared blends demonstrate the uniformity of particle size and homogeneity after processing.

Preparation and Treatment of Muffins

Muffins were prepared using a modified AACC (2000) standard method. Four batches were created: one control (T1) using 100% wheat flour, and three composite treatments (T2, T3, T4) incorporating varying ratios of soy flour, pumpkin seed flour, and spinach powder as detailed in Table 1. For each batch, dry ingredients, including composite flour, sugar, and baking powder, were first mixed thoroughly. In a separate bowl, wet ingredients such as eggs, milk, and melted butter were combined. The wet mixture was gently folded into the dry ingredients to form a smooth batter without over-mixing, ensuring optimal texture. The batter was divided into muffin cups and baked in a preheated oven at 178°C for 20–25 min. Muffins were then cooled for 1–2 hours at room temperature on a wire rack before proceeding to further analysis. Sensory evaluation was conducted using a semi-trained panel of 40 individuals. Each muffin sample (T1–T4) was sliced evenly and coded with random numbers to avoid bias (369,301,312,345). Panelists were instructed to evaluate each samples crust and crumb color, aroma, texture, mouthfeel, taste, and overall acceptability. A structured nine-point hedonic scale was used, where: 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. All the panelists rinsed their palates between samples using water and unsalted crackers.

Results

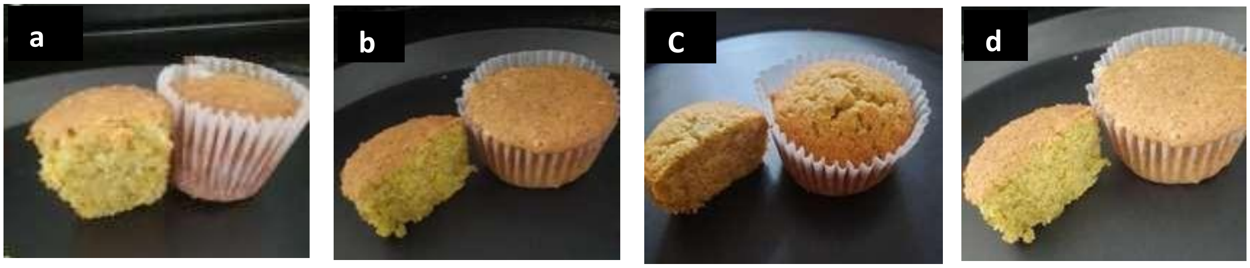

Figure 2: *Muffins prepared using different flour formulations. (a) Muffin made from T1 (control, 100% spinach powder). (c) Muffin made from T3 (65% wheat flour + 31% soy flour + 2% pumpkin seed flour + 2% spinach powder). (d) Muffin made from T4 (70% wheat flour + 26% soy flour + 2% pumpkin seed flour + 2% spinach powder)*

Each muffin was then cut into equal portions for evaluation. The products were examined for their external and internal features, including crust color, crumb color, texture, and overall appearance. All batches showed uniform size and shape, with visible differences in crumb structure and coloration between the control and composite flour treatments. As shown in Figure 2, muffins prepared with the composite flour (T2) had a darker crumb colour and more uniform texture compared to the control. These documented physical properties provided the basis for subsequent sensory evaluation. Sensory evaluation results are shown in Figure 3, where T2 consistently outperformed other treatments across attributes such as taste, aroma, and overall acceptability

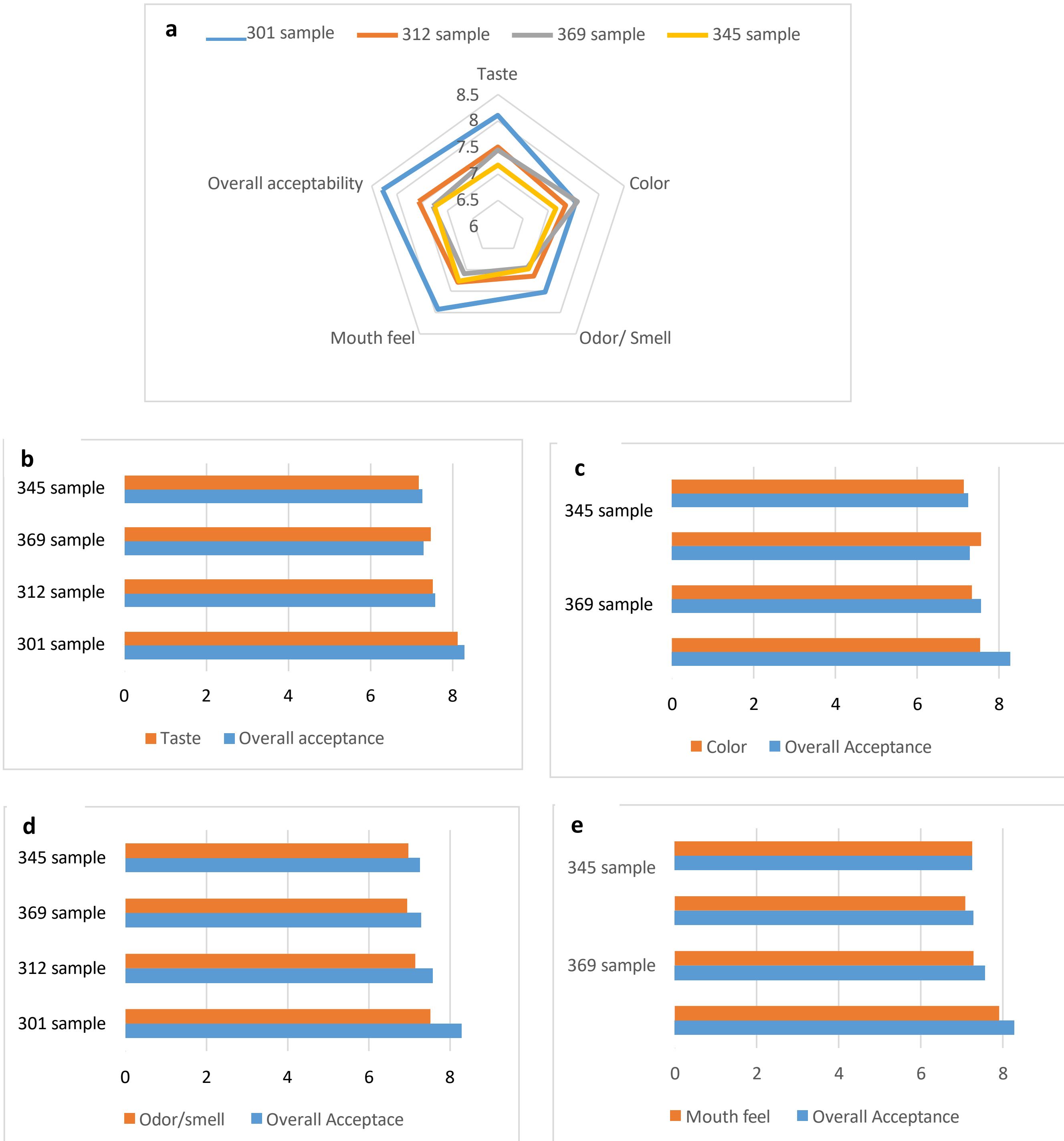

Figure 3: *Sensory evaluation results (a) Sensory evaluation scores across attributes. (b) Taste correlation with overall acceptability. (c) Color contribution to overall acceptability. (d) Aroma association with overall acceptability. (e) Mouthfeel influence on overall acceptability.*

Leavening Index Analysis

The Control dough showed a gradual increase from 1.01 ± 0.01 at 10 min to a peak of 1.06 ± 0.02 at 50 min, demonstrating typical wheat flour leavening behaviour. The T2 sample exhibited a more stable performance, maintaining a moderate but consistent leavening index around 1.02 ± 0.01 throughout the fermentation. This stability suggests the composite flour (60% wheat, 36% soy, 2% pumpkin seed, 2% spinach) provided enhanced gas retention (CO_2), likely due to the binding properties of soy protein and pumpkin seed fiber. In contrast, T2 with higher yeast concentration achieved a faster and stronger rise, starting at 1.04 ± 0.01 at 10 min and reaching 1.05 ± 0.01 by 50–60 min, indicating an improved fermentation response.

Protein, Moisture, Ash, and Fat Content

Table 2: *Calculated result of Functional properties in standard and composite flour*

Characteristic	Control	Composite Flour
Protein (N x 5.7), on dry basis, per cent by mass, min	10.1	26.5
Moisture, per cent by mass, max	13.45 ± 0.13	11.76 ± 0.12
Total ash, on dry basis, per cent by mass, max	1.13 ± 0.01	2.64 ± 0.03
Total Fat content	1.2 ± 0.01	2.64 ± 0.02

Note. Mean values bearing the same superscript do not differ significantly ($p < .01$). Control = 100% wheat flour; Composite flour = 60% wheat, 36% soy, 2% pumpkin seed, 2% spinach.

Nutritional composition is presented in Table 2. Protein-enriched composite flour was developed soy flour, pumpkin seed powder, and spinach powder. The results clearly demonstrate that the formulation was not only successful but exceeded the initial protein target of 15%, achieving 26.5% protein content. According to the test results, the composite flour exhibited a crude protein content of 26.5% by mass, with an expanded uncertainty of ±1.4% (k=2). The moisture content (AOAC 934.01) of the composite flour (11.76%) falls within the optimal range for storage and microbial stability, slightly lower than that of standard wheat flour. An ash content (AOAC 942.05) of 2.64% indicates substantial mineral enrichment, primarily contributed by spinach and pumpkin seed components. This reflects the presence of essential inorganic elements such as iron, calcium, magnesium, and zinc, which are not only vital for human health but also serve as cofactors in protein metabolism further enhancing the nutritional quality of the flour. The fat content (AOAC 989.05) (2.64%) was moderately elevated due to the inclusion of soy and pumpkin seed, known sources of unsaturated fatty acids.

Mineral Content Analysis (AAS Method)

Table 3: *Atomic Absorption Spectrometry (AAS) results*

Sample	Concentration (mg/L)			
	Ca	Mg	Zn	Fe
Composite Flour	6.65	28.6	0.30	0.25

Mineral concentrations determined by atomic absorption spectrometry are summarised in Tabl 3. Muffins prepared with composite flour contribute meaningfully to daily micronutrient intake, particularly zinc, highlighting their functional food potential.

Shelf-Life Analysis – Total Plate Count (TPC)

The Total Plate Count (TPC) results revealed that muffins made with the composite flour, especially T2, remained microbiologically stable for up to 21 days, staying below the acceptable threshold of 10^{-5} CFU/g. This indicates effective shelf stability under ambient conditions. The improved microbial resistance is likely due to the moderate moisture content (11.76%) and the presence of bioactive compounds in spinach and pumpkin seed flours, which are known to have mild antimicrobial effects.

DPPH Radical Scavenging Activity

The composite flour formulation demonstrated notable antioxidant capacity, reaching 42.92% inhibition at 4000 µg/mL, which was higher than wheat flour but lower than individual ingredients like soy flour (69.89%) and spinach powder (66.18%).

Discussion

The results indicate that the incorporation of soy, pumpkin seed, and spinach powders into wheat flour significantly influenced both the sensory and functional properties of muffins. Among the treatments, T2 (60% wheat, 36% soy, 2% pumpkin seed, 2% spinach) consistently exhibited superior performance in terms of sensory attributes, nutritional content, and functional behavior (Kamaljit et al., 2010; Manivannan, Kim, & Lee, 2019). The high sensory scores for T2 suggest that soy contributed to a moist and tender crumb through its emulsifying and water-holding properties, while pumpkin seed and spinach flours enhanced texture, structure, and mouthfeel. The natural pigments from spinach and pumpkin seed improved the visual appeal without the need for artificial colorants, aligning with consumer preferences for clean-label products. Aroma and taste improvements are likely attributed to volatile compounds in soy and pumpkin, which impart subtle nutty and vegetal notes, contributing to overall acceptability.

Leavening index analysis demonstrated that composite flour blends, particularly T2, exhibited more stable gas retention compared to the control. This stability suggests that the protein and fiber components from soy and pumpkin seed enhanced CO_2 retention during fermentation, allowing for consistent dough rise.

The variant with higher yeast concentration showed accelerated leavening, highlighting the potential for controlled manipulation of fermentation rates in bakery production.
The proximate composition of the composite flour is presented in Table 2. Protein content was significantly higher (26.5%) compared to control wheat flour (10.1%), while moisture content was slightly reduced. This result confirms the effectiveness of soy and pumpkin seed in enhancing protein density, consistent with previous studies that reported significant increases in protein quality when legumes and oilseeds were incorporated into cereal-based products (Dhingra & Jood, 2001; Setchell, Brown, & Lydeking-Olsen, 2004). The slightly lower moisture content in composite flour contributed to improved microbial stability, while the higher ash content reflected mineral enrichment derived from spinach and pumpkin seeds. These outcomes indicate that the developed formulation not only meets but exceeds the expected improvements in nutritional composition.

Mineral analysis further demonstrated that muffins made with composite flour contribute meaningfully to dietary intake of essential micronutrients, particularly zinc. This is noteworthy given that zinc deficiency remains a global nutritional challenge, especially in low- and middle- income populations. According to the World Health Organization (2020), the recommended daily intake of zinc for adults is 8–11 mg, and the contribution of a single muffin towards this requirement underscores the functional food potential of the formulation. In addition to zinc, enhanced levels of calcium, magnesium, and iron highlight the value of using spinach and pumpkin seeds as cost-effective fortifying agents.

Shelf-life analysis confirmed that muffins prepared with composite flour maintained microbial stability for up to 21 days under ambient conditions. This extended stability is attributable to the reduced moisture content and the presence of bioactive compounds with antimicrobial properties. Comparable findings have been reported by Goyeneche (2015), who identified natural antimicrobial effects of pumpkin seed extracts. The observed stability supports the potential for composite muffins to serve as a safe and nutritious product in commercial bakery markets without reliance on artificial preservatives.

The antioxidant capacity of the composite flour, while lower than that of individual raw ingredients, nevertheless conferred a functional advantage compared to wheat flour alone. This outcome is consistent with reports that polyphenols and isoflavones in soy and carotenoids in spinach contribute to oxidative stress reduction (Hercberg et al., 2018). From a public health perspective, such properties are valuable in addressing lifestyle-related health conditions associated with oxidative stress.
Overall, the discussion confirms that the T2 formulation represents a nutritionally enriched, functionally viable, and sensory-acceptable composite flour blend. The successful integration of protein- and mineral-rich plant ingredients demonstrates the potential of composite flour to address both protein-energy malnutrition and micronutrient deficiencies. These results reinforce the view that composite flour technology can transform conventional bakery products into functional foods that support improved dietary quality and public health outcomes.

Conclusion

This research was carried out to develop a protein-enriched composite flour using locally available ingredients, extruded soy protein, pumpkin seed powder, and spinach powder as partial substitutes for wheat flour in muffin preparation. Among the tested formulations, 60% wheat, 36% soy, 2% pumpkin seed, and 2% spinach were identified as the most successful based on its nutritional composition, leavening properties, antioxidant activity, and highest sensory preference. The inclusion of soy significantly enhanced protein content, while pumpkin seed and spinach contributed essential minerals and antioxidant properties. This research demonstrates how nutrient-dense and often underused ingredients can be incorporated into widely consumed foods to improve public health outcomes. The developed composite flour offers a natural, accessible solution to enhance protein and micronutrient intake, particularly beneficial in regions where nutritional deficiencies remain a concern. Hence, this innovation not only supports healthier eating habits but also encourages the sustainable use of local agricultural resources.

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