*Research article*

FORMULATION AND EVALUATION OF NUTRITIOUS BISCUITS: A COMPARATIVE STUDY OF COMPOSITE FLOUR BLENDS WITH JACKFRUIT SEEDS (*Artocarpus heterophyllus*), KITHUL PALM FLOUR (*Caryota urens*), AND PUMPKIN SEEDS (*Cucurbita maxima*) AGAINST WHEAT FLOUR

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ABSTRACT

Nutritional deficiencies remain a major global health concern particularly in developing countries where monotonous diets fail to provide adequate nutrients. In Sri Lanka, biscuits manufacturing heavy reliance on imported wheat flour (WF) limits dietary diversity and reduces nutritional quality. This study aimed to formulate and evaluate nutritious biscuits using composite flour (CF) consisting of jackfruit seed (*Artocarpus heterophyllus*), kithul palm (*Caryota urens*), and pumpkin seed (*Cucurbita maxima*) flours in a 1:1:1 ratio. Four flour blends were prepared: T1 (100% WF, control), T2 (85% WF + 15% CF), T3 (70% WF + 30% CF), and T4 (55% WF + 45% CF). Techno functional properties of flours, sensory attributes of the biscuits using 48 untrained panelists (9-point hedonic scale), total phenolic content (TPC) and total antioxidant capacity (TAC) of the biscuits were evaluated for all formulations. Proximate composition, shelf-life analyses and physical properties were conducted only for the selected T2 formulation. The T2 flour blend exhibited the highest water absorption, bulk density, emulsion activity and stability, and the lowest gelatinization temperature and gelation concentration. T2 biscuits achieved the highest scores for taste, aroma, and overall acceptability. Proximate analysis of T2 biscuits (per 100 g) showed 3.11 g moisture, 1.56 g ash, 20 g fat, 0.61 g fibre, 7.85 g protein, 66.87 g carbohydrate, and 478.88 kcal energy. The total bacterial count after one week without preservatives (1.9×10^2 CFU/g) remained within safe limits (1×10^4 CFU/g). All composite biscuits showed significantly higher ($p < 0.05$) TPC and TAC compared to wheat flour

biscuit. These findings highlight that substituting 15% of wheat flour by developed composite flour produced nutritionally rich and consumer acceptable biscuits, supporting food security and economic sustainability through local resource utilization.

1. Introduction

Non communicable diseases (NCDs) are chronic conditions resulting as a combination of behavioral, environmental, genetic, and physiological determinants. Most common NCDs such as obesity, diabetes, cancers, and cardiovascular diseases that are prevalent among all age groups are significantly influenced by bad dietary habits. Inadequate intake of fiber, vitamins, and minerals is considered a leading dietary risk factor (World Health Organization, 2024). Notably, NCDs are accounting for approximately 75% of global deaths unrelated to pandemics (World Health Organization, 2024), while the rising incidence of NCDs imposes a substantial economic burden on both developed and developing countries (Okunogbe *et al.*, 2022). Modifying dietary and nutritional behaviors is widely recognized as one of the most effective preventive strategies against these diseases (Budreviciute *et al.*, 2020). In this context, popular foods consumed among all age groups present an opportunity to deliver nutrients often deficient in modern diets (Wang *et al.*, 2023).

Biscuits are classified as fast-moving consumer goods, are widely consumed ready to eat snack across all demographics, all age groups since they are affordable, have appealing sensory qualities, variety, and extended shelf life (Eugenia Ramashia *et al.*, 2024). Traditional biscuit formulations consist of wheat flour, fat, and sugar. However, the nutritional quality of biscuits is limited by the refined wheat flour used as the main raw material. During milling, the bran which contains most of the bioactive compounds, fiber, vitamins, and minerals is partially or completely removed. As a result, refined wheat flour-based products have lower nutritional value compared to composite flour alternatives. Wheat protein is inferior in quality due to low levels of lysine, methionine, and threonine (Cheng *et al.*, 2021).

To address the nutritional limitations of wheat flour, biscuit quality can be enhanced either through fortification with minerals, vitamins, and fiber or by exploring alternative flours (Davidson, 2019). Encouraging the use of composite flour is one of the cost-effective methods to produce nutritionally rich biscuits. Composite flour is a blend of various flours, starches, and other ingredients designed to partially or completely replace wheat flour. This approach offers additional advantages for developing countries, such as reducing reliance on imported wheat, promoting the use of domestic crops, and valorizing agricultural byproducts. Previous research indicates that biscuits made from composite flours can achieve good nutritional, physical, and sensory quality, with characteristics similar to traditional wheat-flour biscuits (Goubgou *et al.*, 2021).

The jackfruit (*Artocarpus heterophyllus*), is originated in the rainforests of Western Ghats of India. Various parts of the jackfruit tree, including fruit pulp, leaves, seeds, skin, and wood provide a range of advantages in industrial processing, trade commodities, medicinal purposes, fuel and construction material (Karunakaran *et al.*, 2025). Jackfruit seed flour, is an underutilized agricultural byproduct suitable for composite flour formulations. It is rich in carbohydrates (73.87 g/100 g), proteins (14 g/100 g), potassium (768.63 mg/100 g), calcium (132 mg/100 g), and iron (8.94 mg/100 g), making it a nutritious food source (Sévérin *et al.*, 2024). The flour's antioxidant properties, derived from polyphenolic compounds help extend product shelf-life. Furthermore, its functional properties, including high bulk density gelation and oil absorption capacity, enhance its suitability as a wheat flour substitute (Paula *et al.*, 2025).

Pumpkin (*Cucurbita maxima*) seed flour is another nutrient dense agriculture byproduct that can be utilized for composite flour

formulation (Kaur and Sharma, 2017). These olive-green kernels are buttery in texture and in flavor. It is a good source of calories, protein, carotenoids, minerals (iron, zinc, magnesium, calcium), fiber, and phytosterols (Syed, 2019). The flour is also a valuable source of unsaturated fatty acids, particularly omega-6 (37.63–51.74%) and omega-9 (28.64–37.45%), which are contributing to cardiovascular, cognitive, and dermal health (Prommaban *et al.*, 2021). Studies have shown that incorporating pumpkin seed flour into wheat-based products significantly boosts protein content and antioxidant activity without negatively affecting sensory appeal (Galenko *et al.*, 2024).

Tropical Asia's native rainforest species, *Caryota urens*, is referred to as the Kithul in Sri Lanka. Additionally, it is among the most prevalent trees in Sri Lanka's highland perennial forest gardens (Malki *et al.*, 2025). A traditional starch source with increasing potential for use in composite flour applications is Kithul flour, which is made from the pith of the *Caryota urens* palm. The inflorescences are reportedly used in traditional medicine to cure rheumatic swellings, migraine headaches, gastric ulcers, and snake bites. Genuine Kitul products are thought to be appropriate for diabetic patients due to their antidiabetic qualities, which may entail the regulation of inflammatory pathways and oxidative damage caused by hyperglycemia (Andrade *et al.*, 2023).

Although these plant-based ingredients have been studied individually for their nutritional and functional properties, limited research has examined their combined use in a single composite flour. This multi-ingredient approach is expected to provide combined benefits, with jackfruit seed flour contributing starch-based functionality, pumpkin seed flour enhancing protein and lipid content, and Kithul flour improving dietary fibre, thereby enhancing both the nutritional value and the techno functional properties of biscuits. Based on this background, the current study was designed to formulate a composite flour blend incorporating jackfruit seed flour, pumpkin

seed flour, and kithul flour to substitute varying proportions of wheat flour in biscuit production. The study involved the preparation of composite flour blends at different substitution levels. The suitable biscuit in this study was determined by the sensory analyses and functional properties. Furthermore, the proximate composition, microbial shelf life, and colour characteristics were analysed for the most consumer accepted biscuit formulation. In addition, TPC and TAC were determined for all the biscuit formulations.

2. Materials and methods

2.1. Materials

2.1.1. Plant material and sample preparation

The Jackfruit seeds, pumpkin seeds, Kithul flour and all-purpose wheat flour and additional ingredients like vegetable oil, sugar, baking soda, baking powder and salt were procured from the local market in Wellawatta, Sri Lanka. Clean mature pumpkin seeds were obtained and washed to eliminate sand and soil. Followed by washing with tap water, seeds were oven dried (Meditry DHG-9053A, China) at 70°C for 30 minutes. Then they were milled and sieved to separate the particles below 500µm (Syam *et al.*, 2023).

Jackfruit seeds were boiled at 100°C for 45 minutes and the arils were removed. Lye peeling was done by soaking in 0.05% NaOH for 3 minutes, then the seeds were washed using distilled water. The spermoderms were removed and were soaked in 0.2% sodium metabisulfite for 30 minutes. Finally, wet milling was done and dried at 70°C for 12 hours, and it was ground and sieved to obtain flour particles below 500µm (Jayampathi *et al.*, 2025). All four types of flour (all-purpose wheat flour, Kithul flour, jackfruit seed flour, and pumpkin seed flour) were stored at 4°C in sealed polyethylene bags for further use.

2.1.2. Chemical and reagents

All chemicals used were of analytical reagent grade, including, Absolute ethanol, Ammonium molybdate, Ascorbic acid, Distilled water, Folin-Ciocalteu reagent, Gallic acid, Sodium carbonate, Sodium hydroxide,

Sodium metabisulfite, Sulfuric acid, Tryptone Soya Agar (TSA) and Yeast extract.

2.2. Methods

A completely randomized design was conducted in this study. Four biscuit formulations (T1-T4) containing different levels of composite flour substitution were prepared and evaluated with control formulation of 100% wheat flour. The optimal formulation was selected based on the sensory evaluation scores, supported by the functional property analyses. Subsequently, the selected formulation was subjected to further analyses including the proximate and microbial shelf-life.

2.2.1. Formulation of composite flour

Three composite flour blends and a control were prepared using jackfruit seed flour, pumpkin seed flour, Kithul flour, and wheat

flour. The composite flour was formulated by blending jackfruit seed flour, pumpkin seed flour, and Kithul flour in equal proportions (1:1:1) to enable a balanced evaluation of their combined effects following the approach reported by Chandra, Singh, and Kumari (2015) (Table 1).

2.2.2. Determination of functional properties of the flours

Oil absorption capacity (OAC), water absorption capacity (WAC), emulsion activity (EA), emulsion stability (ES), bulk density (BD), foaming capacity (FC), water solubility (WS) and swelling power (SP), dispersibility, gelatinization temperature (GT), and least gelation concentration (LGC) were determined in triplicate for the four developed composite flour treatments using the methods described by Chandra, Singh and Kumari (2015).

Table 1. Composition of the composite flour preparations

Treatment	Wheat flour %	Composite flour		
		Jackfruit seed flour %	Kithul flour %	Pumpkin seed flour %
T1	100	0	0	0
T2	85	5	5	5
T3	70	10	10	10
T4	55	15	15	15

2.2.3. Preparation of biscuits

Sugar and butter were mixed using a beater (Philips, China) until the sugar was dissolved. Then, milk powder, wheat flour, composite flour, baking powder, milk, and vanilla essence were added to the creamy mixture and were blended to achieve a homogeneous consistency (Table 2). The obtained dough was piped into circular shapes

(5 cm in diameter) and baked at $170 \pm 2^\circ\text{C}$ for 20 minutes in a preheated bakery oven (Panasonic, Japan). After baking, the biscuits were allowed to cool and were then stored in airtight containers at room temperature for subsequent analysis and sensory evaluation (Figure 1).

Table 2. Formulation of the ingredients

Ingredient	Weight
Wheat flour/ composite flour	166 g
Butter	100 g
Powdered sugar	100 g
Milk	66 mL
Milk powder	16 g
Baking powder	1.5 teaspoon
Vanilla essence	1 tsp



Figure 1. Biscuits made from developed composite flour types. T1 - biscuit made from 100% wheat flour, T2 - biscuit made from 85% wheat flour + 5% each of other flours, T3 - biscuit made from 70% wheat flour + 10% each of other flours, T4 - 55% wheat flour + 15% each of other flours

2.2.4. Sensory evaluation

The sensory attributes of color, aroma, taste, texture, aftertaste, and overall acceptability were evaluated by a panel of 48 untrained panelists. All participants were informed about the purpose and procedures of the study, and the informed consent was obtained before their participation. Panelists were randomly selected from the student population (age 18-25 years) at the BMS campus in Colombo, Sri Lanka. This might restrict the findings to the general consumer population therefore; future research should consider more diverse age groups. The sensory evaluation procedure was approved by the Ethics Committee of Northumbria University, UK (Protocol code 2024-7357-7464).

Samples were presented in a randomized order, with each assessor receiving one full biscuit under same laboratory environmental conditions. Assessments were conducted using a 9-point hedonic scale (1 = Dislike extremely; 2 = Dislike very much; 3 = Dislike moderately;

4 = Dislike slightly; 5 = Neither like nor dislike; 6 = Like slightly; 7 = Like moderately; 8 = Like very much; 9 = Like extremely).

Based on the highest overall acceptability scores from the sensory analysis, the optimal biscuit formula was selected for further investigation. This selected formula then underwent proximate analysis and total plate count testing (Plustea *et al.*, 2024).

2.2.5. Proximate analysis of composite biscuit

According to the standard analytical methods the moisture content, ash content, crude fiber, crude protein, and crude fat of the developed T2 composite biscuits (85% wheat, 15% composite flour) were analyzed according to standard AOAC analytical methods. All measurements were conducted in triplicate, and the results are reported on a dry weight basis as mean $\pm$ standard deviation (SD) (Twinomuhwezi, Awuchi and Rachael, 2020).

2.2.6. Total plate count

Total plate count of the selected biscuit (T2: 15% composite flour) was evaluated after

a 1-week storage period. Biscuits were stored in airtight containers at ambient room temperature. 10 g sample of the ground biscuit was aseptically transferred into a sterile stomacher bag containing 90 mL of 0.1% peptone water. The bag was homogenized in a stomacher (Seward Stomacher, UK) for 2 minutes to create a 10^{-1} initial homogenate biscuit extract. Then the pour plate technique was employed as per the method described by Kumari *et al.* (2021). Serial dilutions (10^{-1} to 10^{-6}) were prepared in triplicate from the initial homogenate using sterile diluent. From each dilution, 1 mL aliquot was transferred into a sterile petri dish. Approximately 15 mL of molten Tryptone Soya Agar (TSA), supplemented with yeast extract at 45°C, was then poured into each plate. Plates were rotated gently clockwise and anticlockwise on a level surface. The solidified plates were incubated at 30°C for 48 hours. Following incubation, bacterial colonies on plates containing 30-300 colonies were counted. The colony-forming units per gram of biscuit (CFU/g) were calculated using the standard formula (Ramashia *et al.*, 2024):

$$\text{CFU/g} = \frac{\text{Number of colonies counted}}{\text{Dilution factor} \times \text{Volume plated (mL)} \times \text{Sample weight (g)}} \quad (1)$$

2.2.7. Texture profile analysis

The textural properties of the selected biscuit formula (T2: 15% composite flour) were determined using a Texture Analyzer (TX 700 Shimadzu, Japan) to perform a two-cycle compression test. Measurements were conducted in triplicate. A biscuit (4 cm diameter) was placed centrally on the stationary base plate. This was performed using a flat cylindrical probe (P/36) and following parameters were used; 3.0 mm/s pre-test speed, 1.0 mm/s test speed, 2.0 mm/s post-test speed, 70% strain target mode, 0.1N trigger force and 3 seconds pause between cycle. Hardness (N), Cohesiveness (ratio), Gumminess (N), and Chewiness (mJ) were calculated automatically by the instrument's software from the resultant force-time curve.

2.2.8. Colour measurement

The surface color of the selected biscuit formula (T2: 15% composite flour) was measured using a Hunter Lab colorimeter (CR-400, Konica Minolta Inc., Japan). The instrument was calibrated using a standard white calibration plate prior to analysis. Color measurements were taken directly on the top surface of intact biscuits. For each sample, measurements were performed at three distinct, non-overlapping locations. L^* , a^* , and b^* values were recorded in triplicate using different biscuits (Bakare *et al.*, 2020). Then Chroma (C^*) was calculated using the equation: $C^* = \sqrt{(a^{*2} + b^{*2})}$.

2.2.9. Preparation of sample extract

Exactly 1.5 mL of distilled water at 45°C was added with 0.2 g of the ground samples in a centrifuge tube while being swirled for five minutes. Samples were centrifuged (Gemmyco PLC-025, Taiwan) for 10 minutes at 4000 rpm after being rested for 30 minutes at 4°C. The supernatant was transferred to another fresh Falcon tube. The residue was reconstituted with 1 mL water at 40°C and centrifuged again under the same conditions. The resulting supernatant was combined with the first aqueous extract for each sample. These pooled extracts were used for total phenolic content and total antioxidant capacity analysis (Conforti and Patrignani, 2021).

2.2.10. Total Phenolic Content (TPC)

A stock solution containing 1 mg/mL of gallic acid was prepared in order to create the TPC standard and a range of working standard values, from 10 to 100 mg/mL were prepared for all four treatment biscuit extracts (Najjar *et al.*, 2022). Each biscuit sample had three extracts made, and each extract was made in triplicate. An aliquot of 300 µL of sample, each standard solution or blank (distilled water) were mixed with 1.2 mL of diluted Folin-Ciocalteu reagent. The test tubes were then left in the dark for five minutes at room temperature (RT). Each test tube was then filled with 1.5 mL of 7.5% Na_2CO_3 , and the tubes were left in the dark for an hour at RT. A UV-visible spectrophotometer (JENWAY 6305, United Kingdom) was used to measure

the absorbance at 765 nm, and the standard curve was used to calculate the unknown amounts. TPC was expressed as grams of gallic acid equivalents per gram of the sample (g GAE/g) (Ferradji *et al.*, 2024).

2.2.11. Testing for Total Antioxidant Capacity (TAC)

The standard for TAC was made by preparing a 1 mg/mL ascorbic stock solution. A series of working standard concentrations, 25 to 200 mg/mL, was prepared by dilution for all four treatment biscuit extracts (Batista *et al.*, 2021). Three extracts per sample were prepared, and for each extract, a triplicate was performed. Phosphomolybdenum reagent was prepared with 2.3% (w/v) Na_3PO_4 , 0.392% (w/v) ammonium molybdate, and 8 mL of concentrated H_2SO_4 . In each test tube 2.5 mL of the prepared reagent was added, along with 250 μL of the sample, the blank (distilled water) and the working standard solutions. For 90 minutes, the tubes were incubated at 95°C in the dark. A UV-visible spectrophotometer (JENWAY 6305, United Kingdom) was used to measure the absorbance at 695 nm, and the standard curve was used to calculate the unknown amounts. TAC was expressed as grams of ascorbic acid equivalents per gram of the sample (g AAE/g) (Korese *et al.*, 2021).

2.2.12. Data analysis

For statistical analysis, IBM SPSS Statistics Version 29 Windows (v29.0.2.0) was used. The parametric data were assessed using analysis of variance (ANOVA), and testing was conducted using one-way ANOVA. Mean significant differences of triplicate data were separated using Tukey's Honestly Significant Difference (HSD), where a *p*-value of less than 0.05 was considered statistically significant. Microsoft Excel (version 2506) was used to generate graphs, and values are presented as mean $\pm$ SD.

3. Results and discussions

3.1. The functional properties of different composite flour formulations

The functional characteristics of flour play a crucial role in determining the physicochemical behavior of dough during processing, the structural and sensory qualities of the final baked product. Replacing wheat flour with composite flours (T2, T3, and T4) resulted in significant changes in these functional properties as shown in Table 3. Attributes such as Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Emulsion Stability (ES), Bulk Density (BD), Swelling Power (SP), Foaming Capacity (FC), and Water Solubility (WS) directly influence the texture, structure, and crispness of biscuits.

The developed T2 flour blend (85% wheat, 15% composite flour) showed a particularly advantageous hydration property balance in this study. Although it was statistically similar to wheat flour (T1), its Water Absorption Capacity (WAC) was significantly greater than that of T3 and T4 blends. This indicates that hydrophilic elements, such as the globulin proteins from pumpkin seeds and the soluble dietary fibers from Kithul flour, provide extra water binding sites at a 15% substitution level without significantly altering the natural starch-gluten matrix (Giannoutsos *et al.*, 2025). Significant reduction in WAC at higher substitution levels (T3 at 30% and T4 at 45%) suggests that once a threshold limit is exceeded, the increased concentration of insoluble fiber and non-gluten proteins impedes water absorption and starch swelling, while simultaneously weakening the gluten matrix responsible for retaining moisture (Li *et al.*, 2023). In contrast, the stable WAC observed in T2 likely supported better dough structure and handling, resulting in biscuits with less crumbly, more desirable texture according to sensory analysis. A significant increase in the Oil Absorption Capacity (OAC) was observed in all composite flour blends, with the highest value recorded in the T3 formulation. This increase is likely due to the dense and porous structure of jackfruit seed fiber, which physically traps oil, and the high levels of

hydrophobic amino acids (leucine and alanine) in pumpkin seed protein, which provide strong non-polar sites for binding fat molecules (Hasmadi *et al.*, 2020). As the proportion of composite flours increased the oil absorption capacity (OAC) increased. This could be due to differences in non-polar side chains in the proteins, which are responsible for binding oil through their hydrophobic nature. In a study by Aremu *et al.* (2007), on composite flours showed OAC values ranging from about 130% to 156%. They observed that higher OAC was linked to increased levels of legume and tuber flour. This was explained by the presence of more non-polar (hydrophobic) amino acids in proteins, which improve the ability of flour to bind oil. Based on this, the moderate OAC value of T2 (160.00), moderate oil absorption is often suitable for many baked items, because

very high OAC can make products too greasy and negatively affect texture. In addition, the T2 blend showed a significantly higher Emulsion Stability (ES), more than twice that of the control sample. This suggests that the balance of soluble fibers and amphiphilic proteins in T2 creates a viscoelastic, mechanically robust interfacial film at the oil-water interface. This film helps stop oil droplets from merging during the intense mixing of biscuit dough (Liu *et al.*, 2016; Qiu, Zhao & McClements, 2015). As a result, the fat is more evenly distributed throughout the dough. Also, T2 had the highest Bulk Density (BD) among all treatments, while T3 and T4 showed the lowest values. This suggests that the flours in T3 and T4 are more porous and less compact compared to refined wheat flour (T1) and T2.

Table 3. The mean functional scores for the four flour compositions.

Functional properties	Wheat flour	Composite flours		
	T1	T2	T3	T4
OAC %	137.33±21.33 ^a	160.00±10.00 ^{ac}	246.67±3.33 ^b	200.00±0.00 ^{bc}
WAC %	133.30±6.67 ^a	146.70 ^a ±3.33 ^a	56.67±3.33 ^b	67.35±17.35 ^b
EA %	46.03 ±1.59	49.35 ±5.96	43.95 ±0.36	44.06 ±0.68
ES %	38.00 ±1.15 ^a	91.96±4.39 ^b	79.3 ±0.67 ^c	74.8±0.47 ^c
BD (g/cm ³)	0.70±0.01 ^a	0.74±0.00 ^b	0.53±0.00 ^c	0.52±0.00 ^c
FC %	11.11 ±2.22 ^a	7.41 ±0.00 ^{ab}	7.41 ±0.00 ^{ab}	3.85 ±0.00 ^b
WS %	14.37±1.42 ^a	15.43 ± 2.36 ^{ab}	24.30 ±3.24 ^b	18.17± 0.98 ^{ab}
SP %	572.55±5.90 ^a	428.76±37.93 ^b	473.70±31.71 ^{ab}	411.56±32.96 ^b
D %	52.00±0.00 ^a	48.00±0.00 ^b	44.00 ±0.00 ^c	41.00±0.00 ^d
GT (°C)	60.00 ±0.00 ^a	68.00 ±0.00 ^a	75.00 ±0.00 ^a	84.00 ±0.00 ^a
LGC (g/ml)	10.00 ±0.00	12.00 ±0.00	14.00 ±0.00	18.00 ±0.00

Values are expressed as mean ± standard deviation (SD) of triplicate determinations. Means within the same row followed by different superscript letters are significantly different ($p < 0.05$). T1-100% wheat flour, T2 - 85% wheat flour + 5% each of other flours, T3 - 70% wheat flour + 10% each of other flours, T4 - 55% wheat flour + 15% each of other flours, OAC- Oil absorption capacity, WAC- Water absorption capacity, EA- Emulsion activity, ES- emulsion stability, BD- Bulk density, FC- Foaming capacity, WS- Water solubility, SP- Swelling power, D- Dispersibility, GT- Gelatinization temperature, LGC- Least gelation concentration.

A higher bulk density, which is often associated with better packing and finer particles, is desirable in food processing because it allows for more consistent and predictable dough yield (Jima, Abera, & Kuyu, 2025). The developed composite flours (T2 and T4) have lower SP indicating that their starch granules retain less water during heating compared to refined wheat flour. This is advantageous for biscuits, as it helps achieve a crisp texture with reduced gumminess (Devi, Sindhu, & Khatkar, 2018). The simultaneous rise in water solubility (WS), especially in T3, suggests higher amounts of other soluble components or soluble amylose. This may lead to greater spread of the dough during baking and could result in a firmer texture once cooled if the fat content is not properly balanced (Xiong *et al.*, 2023). Foaming Capacity (FC) decreased with increasing composite blends, T4 showing the lowest value (3.85%). According to Dusabumuremyi *et al.* (2025), a lower FC is beneficial for biscuit quality, as it indicates during mixing the composite flours are less likely to form big, unstable air bubbles. This allows the fat and chemical leavening agents to produce a uniform, fine, and tender crumb structure. Emulsion Stability (ES) was significantly higher in the composite flours compared to wheat flour. Although biscuits are not emulsion-based, increased ES illustrates the presence of surface-active compounds (as proteins from non-wheat ingredients). These components can help create a more stable dough, contribute to a more uniform texture, improve fat distribution, and potentially extend shelf life by decreasing the spread of moisture (Chandra, Singh, and Kumari, 2014).

Dispersibility and Emulsion Activity (EA) are crucial for dough handling and mixing. Dispersibility gradually decreased from T1 to T4, indicating that the composite flour does not easily form a uniform suspension in water. As a result, T3 and T4 require longer mixing times to develop a homogeneous dough. Among the developed composite flours, T2 remains the most advantageous in this instance. On the other hand, EA showed no significant differences between the treatments, suggesting that variations in dough handling are unlikely to be originated by this property (Nawaz *et al.*, 2021). Gelatinization Temperature (GT) and Least Gelation Concentration (LGC) affect the thermal behavior of flours during baking. GT in this study, increased considerably from T1 (60°C) to T4 (84°C), meaning that starch in the composite flours requires more heat to gelatinize and form the biscuit structure. This can delay the setting of the biscuit in the oven, allowing the dough to spread more and produce thinner biscuits, particularly in T3 and T4. Additionally, LGC increased from T1 (10%) to T4 (18%), which is favorable for biscuit quality, as greater LGC illustrates weaker gel-forming ability. As gelation leads to hardness, the elevated LGC from T2-T4 suggests that biscuits made from these composites are likely to be less hard and more tender compared to the 100% wheat control (Kristanti & Setiaboma, 2022). Among all treatments, T2 exhibited the best overall functional properties. It recorded the highest WAC, EA, ES, and BD, indicating superior water-binding ability, emulsification performance, and packing characteristics compared to the other treatments.

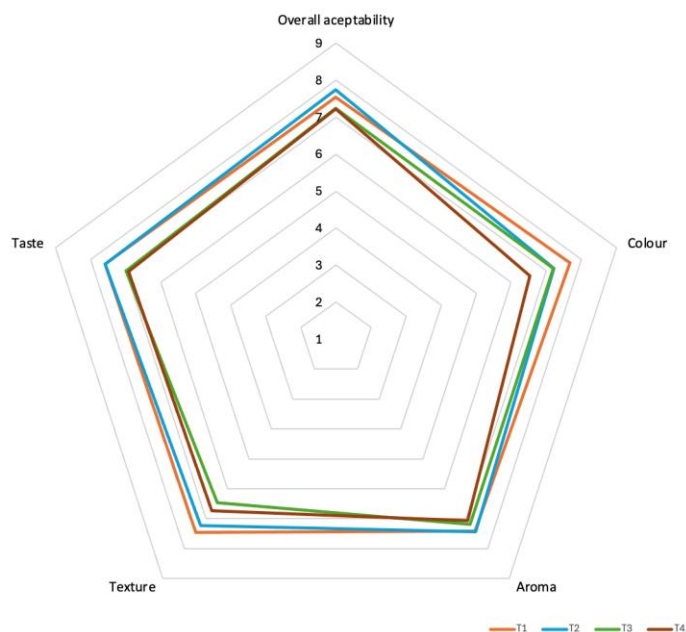


Figure 2. Radar chart of mean scores obtained for the sensory attributes of developed biscuits from composite flour (T1-100% wheat flour, T2 - 85% wheat flour + 5% each of other flours, T3 - 70% wheat flour + 10% each of other flours, T4 - 55% wheat flour + 15% each of other flours). Each attribute is rated on a scale from 1 to 9 (Scale: 9- Like extremely, 1- Dislike extremely). Values are presented as mean $\pm$ SD. The sensory data were statistically analysed using one-way ANOVA followed by Tukey's HSD test, with statistical significance considered at $p < 0.05$.

Table 4. Mean sensory scores of developed biscuits from composite flour.

Sensory attributes	T1	T2	T3	T4
Overall acceptability	7.54 $\pm$ 1.41 ^a	7.75 $\pm$ 1.14 ^a	7.25 $\pm$ 1.55 ^a	7.23 $\pm$ 1.33 ^a
Colour	7.67 $\pm$ 1.43 ^a	7.21 $\pm$ 1.41 ^{ab}	7.19 $\pm$ 1.33 ^{ab}	6.52 $\pm$ 1.60 ^b
Aroma	7.42 $\pm$ 1.35 ^a	7.44 $\pm$ 1.22 ^a	7.19 $\pm$ 1.51 ^a	7.06 $\pm$ 1.47 ^a
Texture	7.46 $\pm$ 1.90 ^a	7.23 $\pm$ 1.49 ^{ab}	6.46 $\pm$ 1.86 ^b	6.73 $\pm$ 1.85 ^{ab}
Taste	7.58 $\pm$ 1.53 ^a	7.58 $\pm$ 1.15 ^a	6.98 $\pm$ 1.51 ^a	6.92 $\pm$ 1.49 ^a

Values are expressed as mean $\pm$ SD (n=48). Means within the same row followed by different superscript letters are significantly different ($p < 0.05$). Sensory attributes were rated on a 9-point hedonic scale (Scale: 9- Like extremely, 1- Dislike extremely). T1-100% wheat flour, T2 - 85% wheat flour + 5% each of other flours, T3 - 70% wheat flour + 10% each of other flours, T4 - 55% wheat flour + 15% each of other flours.

3.2. Sensory Evaluation of Biscuits Formulated with Composite Flours

The sensory evaluation data (Figure 2, Table 4) reveals that moderate substitution with composite flours yielded biscuits that were identical from the wheat flour control in terms of consumer liking. For texture, the biscuit made from T3 (30% substitution) received a score (6.46) that was significantly lower ($p < 0.05$) than the T1 control (7.46). The texture scores for T2 and T4 were not statistically

different from T1. This result is likely due to the reduced WAC (56.67%) and SP (47.70%) of the T3 composite flour. However, T2 composite flour, with a WAC (146.70%) and SP (428.76%) closer to the control, avoided this textural defect. Similarly, for colour, only biscuits made from T4 flour (45% substitution) were rated significantly lower than those made from refined wheat flour (T1). This is likely due to altered Maillard browning at high levels of non-wheat components (Antonyrasan,

Pushparaja and Vasantharuba, 2025). However, T2 and T3, at lower substitution levels, avoided this significant colour rejection. For aroma and taste, no significant differences were found among any of the formulations. This indicates that the composite flours did not introduce off-flavors or detract from the pleasant taste profile at any substitution level. Overall, although the treatments showed relatively minor differences among the tested parameters, T2 obtained the highest overall acceptability score, therefore it was identified as most suitable formulation based on combined evaluation of sensory and functional analysis.

3.3. Proximate analysis

The proximate composition of the biscuit made from T2 (85% wheat, 15% composite flour) is mentioned in Table 5. The analysis reveals the product contain favorable macronutrient profile modulated by the inclusion of Kithul, Jackfruit seed, and Pumpkin seed flours. The moisture content of the biscuit was determined to be 3.11g in 100g

of biscuit (on a dry matter basis). This value is critically below the 5% threshold widely recommended for biscuits to ensure microbial stability, prevent rancidity, and maintain texture during storage (El-Sayed *et al.*, 2023). The crude fat content was determined to be 20g in 100g of biscuit. This substantial level is the combined result of two sources: butter added as a standard ingredient in the biscuit formula, and the intrinsic lipids contributed by the lipid-rich Jackfruit seed and Pumpkin seed flours. While the added fat increases energy density, the fat from the composite flours is important because it may contain unsaturated fatty acids such as oleic and linoleic acids which are predominant in these seeds (Prommaban *et al.*, 2021). Therefore, compared to a traditional biscuit made with wheat flour and the same amount of added shortening, the T2 biscuit delivers a higher total fat content with a potentially improved fatty acid composition, shifting the lipid profile towards more health beneficial unsaturated fats.

Table 5. Proximate analysis of biscuits developed from T2 composite flour (on dry matter basis).

Biscuits made from T2	
Moisture (g/ 100g)	3.11 ± 0.82
Crude Protein (g/ 100g)	7.85 ± 0.45
Crude Fat (g/ 100g)	20.00 ± 1.13
Ash (g/ 100g)	1.56 ± 0.62
Crude Fibre (g/ 100g)	0.61 ± 0.11
Carbohydrate (g/ 100g)	66.87 ± 14.01
Energy content (kcal/100 g)	478.88 ± 18.25

Composition of T2 - 85% wheat flour, 5% Jackfruit seed flour, 5% Kithul flour, 5% Pumpkin seed flour. Values are presented as mean ± SD. T2 was the selected formulation for further characterization; therefore, comparison with T1, T3, and T4 was not performed for these parameters.

The developed T2 biscuit had a crude protein content of 7.85%, which is lower than the exclusive wheat biscuit reported in other studies (11.70%) (Ramashia *et al.*, 2024). This low protein level may be due to the small amount of protein rich ingredients, and the composition of the formulation, which may naturally contain less protein than commercial biscuit. In comparison, some studies on biscuits made with composite flours often report higher protein levels (12–18%) because they include substantial amounts of protein-rich flours, such

as soybean or legume flours, which enhance both amino acid composition and overall protein content. These findings suggest that T2 biscuit provides some protein, and by increasing the proportion of protein-rich composite flours the content of protein could be increased to surpass commercial and experimental standards (Victor-Aduloju, 2024).

In terms of crude fiber, the T2 biscuit value of 0.61% is slightly higher than the 0.27% crude fiber found in the wheat biscuit reported by Ramashia *et al.* (2024). The

relatively low crude fiber observed can be due to the limited substitution levels of the composite flour, where wheat flour had the higher composition. This dilution effect may have reduced the overall fiber contribution from jackfruit seeds and pumpkin seed flours. However, composite flour biscuits in the literature that include high-fiber ingredients such as pulses, hulls, or mushroom typically exhibit greater crude fibre levels than both wheat-only and T2. For instance, biscuits produced from wheat supplemented with pulses such as African yam bean and tigernut showed crude fibre in the range of 3.96–7.43%, reflecting substantial increases due to fibre-rich legume contributions. These studies collectively indicate that although T2 crude fiber content modestly exceeds the commercial wheat biscuit, yet it remains lower than other composite formulations enriched with fibrous non-wheat flours, which tend to significantly increase total dietary fiber in the final product (Bello, Olufwamukomi and Enujiugha, 2017).

The carbohydrate content of the developed biscuit (66.87%) was slightly higher than the 65.46% reported for a commercial wheat biscuit in Ramashia *et al.* (2024), reflecting the high proportion of starch-rich ingredients typical of cereal-based bakery products. In studies on composite flour biscuits, carbohydrate values often vary depending on the nature and level of substitution; for example, biscuits from blends of wheat, soy okara and tigernut residue flours showed carbohydrate contents in the range of 56.9–59.2%, which were lower than both the commercial wheat and the current formulation, likely due to the higher protein and fat contributions of the non-wheat ingredients reducing the relative carbohydrate proportion. Overall, the carbohydrate content of the current biscuit is slightly higher than wheat biscuit but tends to exceed a few other composite biscuit formulations where substitution with non-starchy ingredients lowers carbohydrate proportion (Agu, Ihionu and Mba, 2023).

3.4. General microbial quality assessment

The aerobic bacterial count in the biscuits made from T2 flour increases from $0.3 \pm 0.2 \times 10^2$ CFU/g at the date of manufactured to $1.9 \pm 1 \times 10^2$ CFU/g at 7 days, indicating growth over time under ambient storage conditions ($27 \pm 2^\circ\text{C}$). The total aerobic bacterial count remained below 1×10^4 CFU/g, which is commonly accepted limits for biscuits in terms of general microbial quality (Koh *et al.*, 2023). However, since pathogen specific tests for *Salmonella*, *E. coli*, and *S. aureus* were not conducted, microbiological safety cannot be confirmed.

3.5. Texture analysis

The texture profile analysis (Table 6) explains the sensory panel's favorable perception of the T2 biscuit. The combination of moderate hardness, low chewiness and gumminess, and low cohesiveness is characteristic of a tender and crisp biscuit, which aligns with quality attributes reported for acceptable biscuit products. The hardness value of 46.7 N falls within the range 20 N and 80 N commonly reported for biscuits and composite-flour biscuits, where hardness is strongly influenced by formulation and moisture content (Chauhan *et al.*, 2016). Such moderate hardness indicates a product that is crisp yet easy to bite. This textural behavior can be attributed to the functional properties of the T2 flour blend, where balanced water absorption capacity and swelling power promote adequate starch gelatinization and protein hydration during baking, resulting in a continuous but non-dense matrix (Masri *et al.*, 2026). Similar observations were reported by Oyeyinka *et al.* (2018), who found that biscuits produced from composite flours exhibited improved crispness and desirable textural properties compared to control wheat biscuits. The low values for chewiness (8.55 N) and gumminess (8.57 N) confirm the product's non-chewy texture. Moreover, low cohesiveness (0.18) is an indicator of the biscuit crumbles easily rather than deforming plastically. The dilution of wheat gluten by the 15% substitution with Kithul, Jackfruit seed, and pumpkin seed flours

disrupts the formation of a strong, elastic gluten network. While this is detrimental in bread, it is highly desirable in biscuits, where a short, tender texture is preferred. This aligns

with the work of Gbenga *et al.* (2018), who noted that partial substitution with non-gluten flours significantly reduced dough elasticity and final product cohesiveness.

Table 6. Texture properties of the biscuits made from T2 composite flour

Texture attributes	Biscuits made from T2
Hardness (N)	46.7 ± 3.49
Chewiness (N)	8.55 ± 2.22
Gumminess (N)	8.57 ± 2.64
Cohesiveness	0.18 ± 0.04

Composition of T2 - 85% wheat flour, 5% Jackfruit seed flour, 5% Kithul flour, 5% Pumpkin seed flour. Values are presented as mean ± SD. T2 was the selected formulation for further characterization; therefore, comparison with T1, T3, and T4 was not performed for these parameters.

3.6. Colour analysis

The colorimetric analysis of the T2 biscuit (Table 7) reveals a product with a moderately dark, reddish-yellow hue, which aligns with expectations for baked goods containing Jackfruit seed, Kithul, and Pumpkin seed flours. The measured L* value of 53.4 ± 0.7 indicates a product of medium lightness. This is lower than values reported for biscuits made from 100% refined wheat flour, which typically exhibit L* values ranging from 60 to 75 (Bortlíková, Kolarič & Šimko, 2019; Jan *et al.*, 2018). This reduction in lightness is a consequence of incorporating non-wheat ingredients. For instance, studies enriching biscuits with jackfruit seed flour (Noor *et al.*,

2021) and pumpkin seed meal (Mbaeyi-Nwaoha & Umeh, 2016) have reported similar decreases in L* values, attributable to intrinsic pigments like carotenoids and higher fiber content that reduce light reflectance. Moreover, the a* value of 8.70 ± 0.3 exhibits a pronounced red tone. This is notably higher than the a* values of 2.0 to 6.0 commonly reported for wheat biscuits (Bortlíková *et al.*, 2019). The increase in redness can be attributed due to an altered Maillard reaction during baking. Similarly, the b* value of 23.5 ± 0.9 reflects a strong yellow component. While this falls within the typical range for baked goods (20-30) (Pathare, Opara and Al-Said, 2013).

Table 7. Colorimetric properties of the biscuits made from T2 composite flour

Colour parameter	Biscuits made from T2	Description
L*	53.4 ± 0.7	0 - Black, 100 - White
a*	8.70 ± 0.3	+a* - Red, -a* - Green
b*	23.5 ± 0.9	+b* = Yellow, -b* = Blue
Chroma (C*)	25.1 ± 1.0	Color Saturation

Composition of T2 - 85% wheat flour, 5% Jackfruit seed flour, 5% Kithul flour, 5% Pumpkin seed flour. Values are presented as mean ± SD. T2 was the selected formulation for further characterization; therefore, comparison with T1, T3, and T4 was not performed for these parameters.

3.4. Antioxidant properties of biscuits made from composite flours

The analysis of TPC and TAC (Table 8) shows that incorporating Jackfruit seed, Kithul, and Pumpkin seed flours significantly enhances

the bioactive profile of biscuits. A significant ($p < 0.05$), and progressive increase in both TPC and TAC was observed with increasing levels of composite flour substitution. The TPC rose from 0.95 g GAE/100g in T1 to 2.25 g

GAE/100g in T4, representing a 137% increase at the highest substitution level. Similarly, TAC increased from 2.97 g AAE/100g in T1 to 4.14 g AAE/100g in T4, a 39% increase. This confirms that Jackfruit seed, Kithul, and Pumpkin seed flours are rich sources of phenolic compounds and other antioxidants, which were successfully transferred to the final baked product. These findings align with the

literature on the high antioxidant potential of these underutilized ingredients. For instance, jackfruit seeds are known for their gallic acid and ellagic acid content, while pumpkin seeds contain phenolic acids and flavonoids, all of which contribute to the measured TPC and TAC (Peng *et al.*, 2021, Rodríguez *et al.*, 2021).

Table 8. Comparison of Total Phenolic Content (TPC) and Total Antioxidant Capacity (TAC) in biscuits made from different composite flours.

Type of flour use to make biscuits	TPC (g GAE/100g)	TAC (g AAE/100g)
T1	0.95 ± 0.01 ^a	2.97 ± 0.01 ^a
T2	1.42 ± 0.11 ^b	3.38 ± 0.03 ^{ac}
T3	1.95 ± 0.05 ^c	3.74 ± 0.04 ^{bc}
T4	2.25 ± 0.06 ^c	4.14 ± 0.00 ^b

Values are expressed as mean ± SD. Means within the same column followed by different superscript letters are significantly different ($p < 0.05$). T1-100% wheat flour, T2 - 85% wheat flour + 5% each of other flours, T3 - 70% wheat flour + 10% each of other flours, T4 - 55% wheat flour + 15% each of other flours.

4. Conclusion

This study successfully developed, characterized, and optimized nutritionally enhanced biscuit through the strategic substitution of wheat flour with a composite of Jackfruit seed, Kithul, and Pumpkin seed flours. All four formulations were initially evaluated and compared based on their functional and sensory properties. The investigation revealed that the functional properties of the flours; specially increased oil absorption, emulsion stability, and altered hydration dynamics directly affect the physicochemical and sensory quality of T2. Sensorially, T2 (15% total substitution) was statistically equivalent to the 100% wheat biscuit across all key attributes, avoiding the significant textural and color defects observed at higher substitution levels (T3, T4). Based on these results, T2 was identified as the most acceptable formulation and selected for further detailed characterization including proximate composition and microbial analysis. This

approach is commonly used in product development where comprehensive analyses are done on the optimized formulation. The incorporation of the novel ingredients significantly improved the TPC, TAC. This formulation successfully transforms a conventional biscuit into a product with an improved nutritional profile. It provides a model for enhancing the nutritional quality of staple foods without compromising the sensory characteristics essential for consumer acceptance and market success. The absence of full compositional analysis and extended microbial shelf-life assessment for all formulations represents a limitation of the present study. Future research should address this limitation by conducting these analyses across all formulations, thereby enabling a more comprehensive comparison of their nutritional and storage characteristics.

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