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# Formulation and shelf life evaluation of nutrient rich *Urochloa ramosa* muffins enriched with *Madhuca longifolia* extract

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**This study investigates the development and shelf life of nutrient-enriched muffins suspended with Mahua flower extract (MFE) and brown top millet (BTM) flour. BTM is a nutrient-dense grain, while mahua flowers are rich in bioactive compounds. Muffins were prepared with varying BTM (5–7%; w/w), MFE (4–6%; v/w) levels and baking temperature (170–180 °C) and their physicochemical properties, total phenolic content (TPC), antioxidant activity (AA), and sensory qualities were analyzed. Process optimization was done by CCRD (Central Composite Rotatable Design) using design expert software tools. Results showed significant ($p < 0.05$) increases in proximate composition and bioactive compounds in MFE-enriched muffins. Muffins with 6% BTM flour and 5% MFE, baked at 175 °C showed maximum scores for sensorial properties and overall acceptability. Similarly, TPC and TFC were significantly ($p < 0.05$) higher in optimized muffins compared to the control. The muffins maintained their quality over 28 days storage, demonstrating the potential of mahua and BTM in enhancing nutritional value and extending shelf life in baked goods. UPLC (Ultra Performance Liquid Chromatography) Analysis of optimized muffin showed p-benzoic acid, caffeic acid, mericitin and catechin as potential phenolics having antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic properties. SEM EDX (Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy) showed optimized muffin having better hygroscopicity and high amount of potassium, sulphur and phosphorus. Future work should aim to bridge the gap between laboratory-scale formulation and industrial application, including detailed assessments of consumer perception, product positioning, and market readiness.**

**Keywords** Mahua flower extract, Brown top millet, Muffin, PCA, UPLC, TFC, TPC

A worldwide known wheat-based snack, muffins are widely accepted and recognized as a convenient food in today's eating habits and food choices due to their ready-to-eat form[1].

Muffins that have an appealing taste and consumer properties are very popular today[2]. Muffins are cupcake-like baked products with a small mass. They combine the crumbliness and porosity of cakes and the puffy and light structure of sponge cakes. They may include a variety of fillings (marmalade, jam, fillers) and various additives (seeds, nuts, dried berries and fruits, chocolate chips, etc.)[3]. However, they have high energy and sugar indicators and low nutritional value[4,5].

Studies have shown that the phytochemicals content and antioxidant activity of muffins are improved when fortified with fruit and vegetable by-product such as pineapple peel, banana peel and pumkin seed flour[6], pomegranate peel powder[7], apple peel[8].

Millets are small-seeded annual grasses belonging to Poaceae family. They are cultivated as grain crops, primarily on marginal lands in dry areas in temperate, subtropical and tropical regions. These crops have many advantages in terms of ability to thrive and give good yield even under adverse conditions[9]. Amongst various small millets, the importance of brown top millet (BTM) (*Urochloa ramosa; Panicum ramosum L.; Brachiaria ramosa L. Stapf*) has been recognized recently as its ability to thrive and give good yields in resource-poor and

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fragile ecological conditions and thus can ensure economic and nutrition security as well as sustainability in production of small farm holders[10].

Each millet contains certain nutritional profiles in its grains and provides valuable nutritional resources to humans[11]. Among the nutritionally important millets, the brown-top millet has gained momentum in recent years. It is multipurpose millet, grown for human consumption, used as a raw material in industries, and fodder for animals and birds[12].

*Madhuca longifolia* is the botanical name of Mahua tree which belongs to family-Sapotaceae[13]. A medium to large-sized deciduous tree characterized by spreading branches and a broad, rounded crown. The leaves are elliptic to obovate in shape and appear in clusters at the tips of branches. Small, cream-colored flowers are borne in clusters at the branch ends[13].

Mahua flower has significant amounts of calcium, phosphorus, and vitamins A and C. Mahua seeds are loaded with not just energy giving carbohydrates, but also essential fatty acids like linoleic and arachidonic. Mahua is said to help increase milk production and secretion in lactating mothers[14]. Mahua flowers contain vitamin-C which is responsible for their antioxidant activity[15].

The development of functional bakery products necessitates precise optimization of formulation and processing parameters to achieve desirable nutritional and sensory qualities. Response Surface Methodology (RSM) serves as a robust statistical and mathematical tool for modeling and analyzing problems where multiple variables influence one or more response variables. By employing a structured experimental design, RSM facilitates the identification of optimal conditions while minimizing the number of experimental trials required. Within RSM, the Central Composite Rotatable Design (CCRD) is particularly advantageous, as it allows for the estimation of quadratic response surfaces and provides rotatability, ensuring uniform precision of predicted responses across the design space. In this study, RSM coupled with CCRD was utilized to optimize the formulation of nutrient-enriched muffins by varying three independent variables: mahua flower extract (4–6%), brown top millet flour (5–7%), and baking temperature (170–180 °C). This approach enabled the systematic evaluation of individual and interactive effects of the variables on muffin quality attributes, thereby facilitating the development of a product with enhanced nutritional profile and consumer acceptability[16–19].

This research explores the use of mahua flower extracts (MFE) and brown top millet (BTM) in muffin preparation to develop a nutritious, sustainable, and unique alternative to traditional baked goods. Both ingredients offer significant health benefits- mahua flowers are rich in antioxidants and medicinal properties, while brown top millet is a gluten-free, high-fiber grain. By combining these underutilized resources, the study aims to address nutritional gaps, promote public health, and contribute to sustainable food practices, all while enhancing biodiversity in food systems.

## Materials and methods

### Raw materials

The brown top millet (BTM) used in this study was procured from B & B Organics India Limited, Coimbatore, Tamil Nadu. The grains were of uniform size (1.5–2 mm diameter), harvested during the late Kharif season (October–November 2023), and verified for purity before use. The millet was stored in airtight, food-grade polyethylene bags at room temperature (23 ± 1 °C) and processed within 24 h of receipt to ensure freshness. Mahua (*Madhuca longifolia*) flowers were sourced from the Sundarpur market in Varanasi, collected during their natural blooming season (March–April), and manually sorted to remove extraneous materials before drying and extraction. Other ingredients, including Amul white butter, brown sugar, baking powder, baking soda, and vanilla essence, were obtained from local markets in Varanasi. Cupcake liners were purchased from Narayanpur market, Mirzapur, and fresh cow milk was collected from the certified dairy unit at Banaras Hindu University (BHU).

### Prepration of mahua flower extract (MFE)

To prepare mahua flower extract, the dried mahua flowers were grinded into a powder form. Measured out 4 g of this powder and mixed it with 10 mL of distilled water, weighing was done by weighing machine (Mettle Toledp, Switzerland) and heated the mixture in a water bath at 45 °C for 30 min to facilitate the extraction of soluble components. After heating, allowed the mixture to cool to room temperature and then refrigerated it overnight at 7–8 °C for 24 h. The following day, reheated the mixture in the water bath at 45 °C. For optimal extraction, manually crushed and ground the flower powder once it was reheated. Finally, strained the mixture through muslin cloth to separate the liquid extract from the solid residue. Collected the filtered extract in a clean, airtight container and refrigerated it if not used immediately to ensure its quality and stability[1].

### Nutrient-enrich muffin Preparation

To prepare nutrient-rich muffins, the muffins were prepared as described by Man et al. (2014) the process began with the accurate measurement of ingredients (mention below in Table 1). For every 100 g of muffins, the following quantities were required: 30 g of refined wheat flour (RWF), 4.3–7.6 g of brown top millet flour,

| Ingredients | Refine wheat flour (RWF) | Brown top millet flour (BTM) | Mahua flower extract (MFE) | Brown sugar | Butter | Baking powder | Baking soda | Milk | Salt |
|---|---|---|---|---|---|---|---|---|---|
| Amount(100 g) | 30 g | 4.3–7.6 g | 3.38–6.8 g | 18–22 g | 15 g | 1.29 g | 1.29 g | 25 mL | 0.13 g |

**Table 1**. Ingredients of Nutrient-Rich muffins.

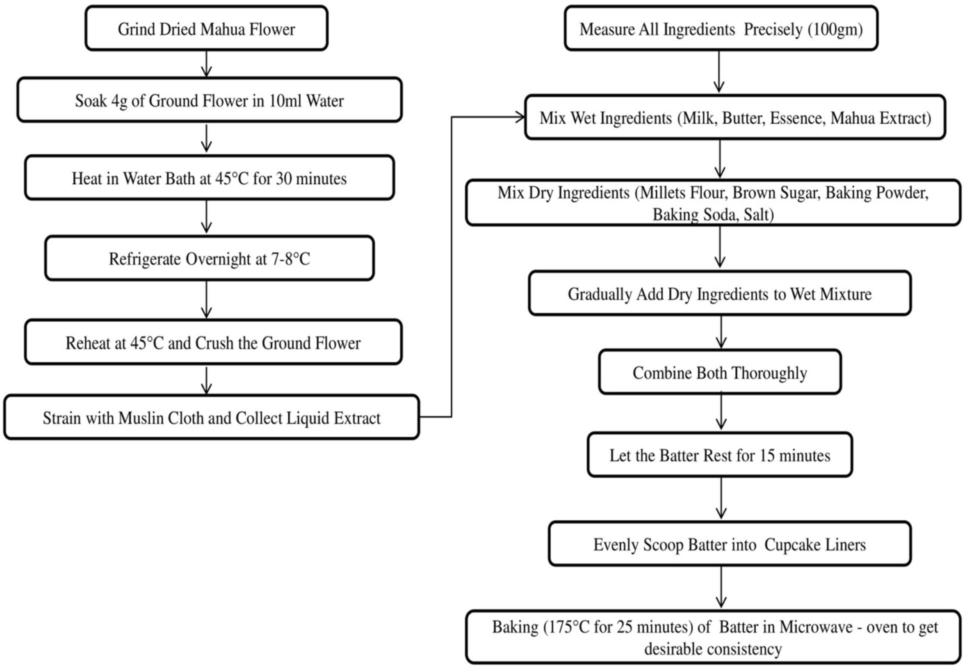


**Fig. 1**. Flowchart illustrating the manufacturing process of brown top millet muffins.

| Factor | Name | Units | Type | Minimum | Maximum |
|---|---|---|---|---|---|
| A | Brown Top Millet | % | Numeric | 5 | 7 |
| B | Mahua flower extract | % | Numeric | 4 | 6 |
| C | Temperature | ˚C | Numeric | 170 | 180 |

**Table 2**. Independent variables used for optimization.

3.38–6.8 g of mahua flower extract (MFE), 18–22 g of brown sugar, 15 g of butter, 1.29 g each of baking powder and baking soda, 25 mL of milk, and 0.13 g of salt.

In a large bowl, the wet ingredients were mixed: 25 mL of milk, 15 g of butter, a few drops of vanilla essence (optional), and the prepared mahua flower extract (MFE). Separately, the dry ingredients were mixed in another bowl: 23 g of refined wheat flour, 6.99–7 g of brown top millet flour, 18 g of brown sugar, 1.29 g each of baking powder and baking soda, and 0.13 g of salt.

The wet and dry ingredients were then combined, stirring until just mixed, mixing is done with the help of stainless steel whisker and the batter was allowed to rest for 15 min. The batter was spooned evenly into cupcake liners and baked in a Microwave - oven (IFB 25SC2, Kolkata, India) at 175˚C for 25 min (Fig. 1).

## Optimization of Nutrient-Rich muffins ingredients by using response surface methodology (RSM)

In Design Expert Version 12 software, 20 experiments were conducted using Response Surface Methodology (RSM) to analyze the effects of brown top millet percentage, mahua flower extract percentage, and temperature. Table 2 details the independent variables and their levels, determined from preliminary trials. Table 3 outlines the software parameters with actual levels. Dependent variables included colour, flavour, aroma, body texture, and overall acceptability. RSM facilitated the creation of response surface plots and statistical analysis, employing multiple linear regressions to evaluate first-order effects, two-factor interactions, and second-order polynomial models. The regression coefficients and statistical significance of terms were assessed using ANOVA[17].

## Texture analysis (TA)

Textural Analysis of the Nutrient-Rich muffins samples were evaluated for their textural attributes, as suggested by Yalcin et al.. (2020)[20] using Texture Analyzer TA XT2i (Stable Micro Systems, Godalming, Surrey, UK) fitted with a 25 kg load cell. Triplicate measurements were made for each sample. The samples were tempered at a temperature 20˚C prior testing. The muffins were cut horizontally at the height of the mold, the upper half was discarded and the 2.5 cm-high lower halves were removed from the mold. A double compression test was performed to a height of 1.25 cm (50% compression) with a 75 mm diameter flat-ended cylindrical probe (P/75), at a speed of 1 mm/s with a 5 s waiting time between the two cycles. The parameters obtained from the curves were hardness (N), springiness (mm), chewiness (mJ), resilience and cohesiveness. The textural parameters were

| Serial number | Standard order | Brown top millet (%) | Mahua flower extract (%) | Temperature (˚C) |
|---|---|---|---|---|
| 1 | 11 | 6 | 3.32 | 175 |
| 2 | 13 | 6 | 5 | 166.59 |
| 3 | 6 | 7 | 4 | 180 |
| 4 | 1 | 5 | 4 | 170 |
| 5 | 19 | 6 | 5 | 175 |
| 6 | 10 | 7.68 | 5 | 175 |
| 7 | 7 | 5 | 6 | 180 |
| 8 | 9 | 4.32 | 5 | 175 |
| 9 | 18 | 6 | 5 | 175 |
| 10 | 12 | 6 | 6.68 | 175 |
| 11 | 3 | 5 | 6 | 170 |
| 12 | 17 | 6 | 5 | 175 |
| 13 | 20 | 6 | 5 | 175 |
| 14 | 8 | 7 | 6 | 180 |
| 15 | 4 | 7 | 6 | 170 |
| 16 | 5 | 5 | 4 | 180 |
| 17 | 15 | 6 | 5 | 175 |
| 18 | 14 | 6 | 5 | 183.1 |
| 19 | 2 | 7 | 4 | 170 |
| 20 | 16 | 6 | 5 | 175 |

**Table 3**. Experimental design for analysis and optimization of Nutrient-Rich muffins.

worked out from the force-time curve thus obtained for each sample with force experienced by the probe on Y-axis and time on X-axis[21].

## Determination of phyico-chemical properties

### Estimation of fat content

The fat content of nutrient enriched muffins was estimated by using Soxhlet apparatus (Socs- plus). Soxhlet method is one of the standard methods for analysis of fat in food. The method is recognized by the **AOAC (2000)**[21]. In this method fat content is determined by extracting the fat from sample using solvent extraction. 3 g of sample was weighed into the thimble and inserted in soxhlet extractor. A clean and dry 150 mL Soxhlet flask was accurately weighed and approximately 90 mL of petroleum ether was poured into the flask. The content of the flask was heated at 70–75 °C for one and half hour and then the temperature was increased to 140 °C for evaporation for next one hour. The flask was removed from the Soxhlet extractor and the remaining solvent was evaporated off by placing the flask in an oven at 102 °C until a final weight is reached (1–2 h). Then the flask was cooled in desiccators and the flask contents were weighed. The fat content was calculated by using the following formula.

$$\text{Fat } \% = \frac{W2 - W1}{S} \times 100$$

Where, $W_1$ = Weight of empty flask (g); $W_2$ = Weight of the flask and extracted fat (g); S = Weight of sample.

### Estimation of protein content

Protein estimation was done by Kjeldahl method described by Goyal et al., 2022[22]. Approximately 0.2 g of optimized muffins sample was taken in a clean dry DTL tube (Kjeldahl flask). Thereafter 10 mL pure nitrogen free sulphuric acid ($H_2SO_4$), 4 g of catalyst mixture (pure potassium sulphate crystals and copper sulphate crystals; in the ratio of 5:1) were added into Kjeldahl flask. Then the Kjeldahl flask was transferred to the digestion chamber for digestion of the content. Upon digestion, when the content of the flask became carbon free, the Kjeldahl flask was allowed to cool down. To this flask approximately 400 mL of distilled water is added and then the content transferred to distillation flask. 25 mL of 4% boric acid with Methylene red indicator was taken in a conical flask. Approximately 90 mL of 40% NaOH solution was then added to the distillation flask. The conical flask was placed below the condenser to collect the condensate. The distillation head was fixed on the distillation flask and condenser. The distillation process was continued until about 300 mL distillate was collected in the conical flask.

Following the usual precautions the beaker was removed from the assembly. The evolved nitrogen % was determined by titrating condensate 0.1 N HCL.

Percentage protein was calculated by following formula:

Nitrogen (%) = Sample titre-Blank titre ×Normality of HCL ×14.01 × 100 Wt. of Sample ×Aliquot taken for distillation ×1000.

% Protein = % Nitrogen × 6.25.

### Estimation of Ash content

The ash content of nutrient enriched muffins was estimated by following the protocol of AOAC (2000)[23]. 3 g of completely homogenized sample was taken accurately in a moisture free crucible. The weight of the crucible was noted. The crucible was placed on hot plate at 130 °C till smoke disappeared. The crucible was then placed in a muffle furnace at 550 °C (6 h). Weight of the cooled crucible was noted down.

$$Ash\ (\%) = \frac{w2 - w1}{w} \times\ 100$$

Where, W = Weight of sample; W1 = Weight of empty silica dish; W2 = Weight of silica dish + Ash.

### Estimation of moisture content

Moisture content of instant dry mix was calculated as per the method of AOAC (2000)[23]. Approximately 5 g of a well- mixed sample was accurately weighed into an empty petri plate. The sample was heated in an oven maintained at 105 + 2 °C for 4 h. After drying, the petri plate was cooled in the desiccator and weighed. Moisture content was calculated as under:

$$\text{Moisture}\ (\%)\ = \frac{w2 - w1}{w} \times\ 100$$

Where, W = Weight of empty petri plate (g); W1 = Weight of empty petri plate + sample (g);
W2 = Weight of petri plate with sample after drying (g).

### Determination of total phenolics and antioxidant activity analysis

*Procedure for DPPH Inhibition method*

Determination of antioxidant activity of the sample was done by DPPH inhibition method[24]. 2 g of sample was taken in 25 mL methanol and kept overnight at room temperature in the dark. 0.2 mL of eluted extract was taken and to it, 1 mL of DPPH solution (80 µg/mL) was added. A control sample was set up with 0.2 mL distilled water as a blank and left at room temperature for 30 min. The sample sets were centrifuged at 1,341 × ***g*** centrifugal force for 15 min (corresponding to 3000 rpm in a rotor with a radius of 10 cm). In a cuvette, 0.5 mL of the centrifuged solution was taken and to it, 1 mL of methanol was added. Absorbance was recorded at 517 nm for both blank and samples using methanol as reference.

% DPPH inhibition = AB-ASAS ×100.

Where, AB = OD for blank, AS = OD for sample.

*Total phenolic content (TPC)*

The total phenolic content was determined by the Folin-Ciocalteu method[25]. 2 g of sample were homogenized in 15 mL of 80% *v/v* aqueous ethanol at room temperature and centrifuged in cold condition at 10,000 rpm for 15 min at 4°C and the supernatant was extracted. The residue obtained was re-extracted twice and the supernatant was poured into petri dishes and evaporated to dryness at room temperature Residue was dissolved in 5 mL of distilled water. 100 µl of this extract was diluted to 3 mL of water and 0.02 mL of the Folin-Ciocalteu reagent was added. After 3 min, 2 mL of 20% sodium carbonate was added and contents were mixed thoroughly and blue colour was developed. The absorbance was measured at 750 nm in UV- Spectrophotometer (Shimadzu, Japan) using gallic acid as standard. The results were expressed as mg gallic acid/g dry material.

*Total flavonoid content (TFC)*

Total flavonoid content (TFC) was determined following the method given by Heimler et al., (2005)[26]. Briefly, 250 µl of sample extract or standard was mixed with 1.25 mL of distilled water which is followed by the addition of 75 µl of 5% sodium nitrite solution. After 6 min, 150 µl of 10% aluminium chloride was added. It was allowed to stand for 5 min before the addition of 500 µl of sodium hydroxide solution. The mixture was brought to 2.5 mL with distilled water, and absorbance was taken immediately at 510 nm using a spectrophotometer. Quercetin was used as a standard, and the results were expressed as µg QE (Quercetin equivalents) $g^{-1}$.

## UPLC (Ultra performance liquid Chromatography)

Phenolic compounds particularly polyphenol found in fruit and herbs can be determined in the form of chromatogram and are identified by their retention time (RT). For UPLC analysis, 100 mg muffin sample was dissolved in 100 mL methanol and kept for 24 h at 25 °C. After 24 h of incubation, 2 mL methanolic samples centrifuged at 10,000 rpm for 10 min and then filtered it with 0.45 µm membrane filter and keep it for estimation for phenolic compound in the sample. Quantification analyses were performed by system Empower™3 software for UPLC analysis of the sample[27].

## Scanning electron microscopic (SEM)

The sample preparation for the studies was done according to the method of (Rajiv et al.., 2011)[28]. The crumb of 20 × 20 mm was cut, defatted with hexane followed by freeze drying (Heto freeze dryer model DW 3, Allerod, Denmark). Leo Scanning electron microscope model 435 VP (Leo Electronic Systems, Cambridge, U.K.) was used in the SEM studies. The prepared samples of crumbs were transferred to the microscope and observed at 15 kV and $9.75 \times 10^{-5}$ torr vacuum.

## Microbial analysis

The microbial analysis involved several key steps to determine the total plate count (TPC) and estimate yeast and mold presence. Initially, plate count agar was prepared and sterilized by autoclaving at 121 °C for 15 min to prevent contamination. In a laminar airflow chamber, Petri dishes were poured with the sterilized media and allowed to solidify. Aseptic inoculation was performed by transferring 0.1 mL of diluted samples ($10^4$ and $10^5$) into nutrient agar plates, which were then incubated at 25 °C for 72 h for TPC assessment and at 30 °C for 4–5 days for yeast and mold analysis using potato dextrose agar. Colony-forming units (CFU) were calculated using the formula CFU = Log10 (A × B), where A represents the number of colonies and B the dilution factor, thus enabling accurate quantification of microbial content in the samples[29].

## Result and discussion

### Optimization of the process for creating nutrient-enriched muffins using brown top millet and mahua flower extract

Design Expert-Version 12 software was used to employ Response Surface Methodology (RSM) to optimize final product. The optimization process involved three variables: Brown top millet flour, Mahua flower extract, and temperature. Twenty different formulations were created by varying the levels of these variables.

The study measured sensory, physicochemical, and textural characteristics of the muffins. By varying the amounts of Mahua flower extract and Brown top millet flour, the primary goal was to create nutrient-stable enhanced muffins. The optimized solution yielded nutrient-enriched muffins with 4.40 g of Mahua flower extract, 6.99 g of Brown top millet flour, baked at 175 °C for 25 min. At these levels, the scores for Colour & Appearance, flavour, aroma, B & T, and O.A. were 7.4, 6.7, 7.6, 7.8, and 7.9, respectively.

### Interactive effect of BTM flour and MFE on flavour of muffin

The flavour scores for muffin ranged from 6.87 to 8.1 (Table 4). The highest sensory score for flavour was achieved in trial 5, where the concentrations of BTM flour and MFE were 6 and 5%, respectively, with a baking temperature of 175°C. Conversely, the minimum score was recorded in trial 3, with BTM flour and MFE concentrations of 7% and 4%, respectively, and a baking temperature of 180°C. Figure 2 A indicated that higher concentrations of MFE negatively affect flavour due to increased bitterness and aftertaste. Additionally, a higher baking temperature combined with BTM flour and a lower concentration of MFE appears to mitigate these undesirable flavours. Martínez et al.. (2024)[30] suggest that these variables significantly influence the quadratic interaction effects on flavour.

$$\begin{aligned} Flavour &= 7.036 - 0.1398 * A - 0.0814 * B + 0.0710 * C - 0.1212 * A * B \\ &+ 0.0037 * A * C - 0.0287 * B * C + 0.1750 * A2 + 0.2528 * B2 + 0.0990 * C2 \end{aligned} \tag{1}$$

Where, A is Brown top millet flour, B is Mahua flower extract and C is Temperature.

| Trial No. | Std | BTM flour | MFE | Temperature (°C) | Colour & appearance | Flavour | Body &Texture | Aroma | O.A. |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 11 | 6 | 3.31 | 175 | 6.98 ± 0.03 | 7.68 ± 0.04 | 7.10 ± 0.04 | 7.68 ± 0.05 | 7.12 ± 0.02 |
| 2 | 13 | 6 | 5 | 166.59 | 6.50 ± 0.01 | 7.06 ± 0.03 | 6.50 ± 0.03 | 7.06 ± 0.02 | 7.08 ± 0.02 |
| 3 | 6 | 7 | 4 | 180 | 6.80 ± 0.02 | 8.10 ± 0.03 | 6.80 ± 0.04 | 8.10 ± 0.01 | 7.00 ± 0.04 |
| 4 | 1 | 5 | 4 | 170 | 7.00 ± 0.04 | 7.37 ± 0.02 | 7.20 ± 0.03 | 7.37 ± 0.06 | 7.20 ± 0.04 |
| 5 | 19 | 6 | 5 | 175 | 7.85 ± 0.04 | 6.87 ± 0.02 | 7.90 ± 0.01 | 7.88 ± 0.01 | 8.00 ± 0.02 |
| 6 | 10 | 7.68 | 5 | 175 | 7.39 ± 0.01 | 7.62 ± 0.02 | 7.37 ± 0.01 | 7.62 ± 0.03 | 7.30 ± 0.02 |
| 7 | 7 | 5 | 6 | 180 | 6.90 ± 0.02 | 7.37 ± 0.01 | 6.87 ± 0.03 | 7.37 ± 0.02 | 7.10 ± 0.01 |
| 8 | 9 | 4.31 | 5 | 175 | 7.75 ± 0.01 | 7.37 ± 0.02 | 7.60 ± 0.01 | 7.38 ± 0.01 | 7.40 ± 0.04 |
| 9 | 18 | 6 | 5 | 175 | 7.65 ± 0.03 | 7.00 ± 0.02 | 7.80 ± 0.03 | 7.00 ± 0.01 | 8.06 ± 0.03 |
| 10 | 12 | 6 | 6.68 | 175 | 7.20 ± 0.05 | 7.75 ± 0.02 | 7.40 ± 0.03 | 7.75 ± 0.01 | 7.34 ± 0.04 |
| 11 | 3 | 5 | 4 | 170 | 6.92 ± 0.01 | 7.37 ± 0.03 | 6.80 ± 0.01 | 7.39 ± 0.02 | 7.20 ± 0.03 |
| 12 | 17 | 6 | 5 | 175 | 7.89 ± 0.02 | 7.37 ± 0.01 | 7.87 ± 0.04 | 7.40 ± 0.03 | 7.90 ± 0.05 |
| 13 | 20 | 6 | 5 | 175 | 7.85 ± 0.03 | 7.25 ± 0.02 | 8.00 ± 0.03 | 7.25 ± 0.01 | 7.89 ± 0.04 |
| 14 | 8 | 7 | 6 | 180 | 6.76 ± 0.01 | 7.50 ± 0.04 | 7.00 ± 0.03 | 7.50 ± 0.05 | 7.10 ± 0.01 |
| 15 | 4 | 7 | 6 | 170 | 7.10 ± 0.03 | 7.50 ± 0.02 | 7.10 ± 0.02 | 7.50 ± 0.01 | 6.80 ± 0.04 |
| 16 | 5 | 5 | 4 | 180 | 6.80 ± 0.01 | 7.50 ± 0.04 | 6.83 ± 0.03 | 7.49 ± 0.01 | 6.80 ± 0.02 |
| 17 | 15 | 6 | 5 | 175 | 7.70 ± 0.01 | 6.87 ± 0.02 | 7.92 ± 0.01 | 6.87 ± 0.01 | 8.00 ± 0.03 |
| 18 | 14 | 6 | 5 | 183.4 | 6.50 ± 0.04 | 7.50 ± 0.01 | 6.50 ± 0.02 | 7.50 ± 0.05 | 7.89 ± 0.02 |
| 19 | 2 | 7 | 4 | 170 | 6.90 ± 0.02 | 8.00 ± 0.01 | 7.80 ± 0.03 | 8.00 ± 0.04 | 6.70 ± 0.01 |
| 20 | 16 | 6 | 5 | 175 | 8.00 ± 0.03 | 6.87 ± 0.01 | 8.10 ± 0.01 | 6.90 ± 0.04 | 7.90 ± 0.02 |

**Table 4.** RSM optimization of nutrient enriched muffin with sensory evaluation.

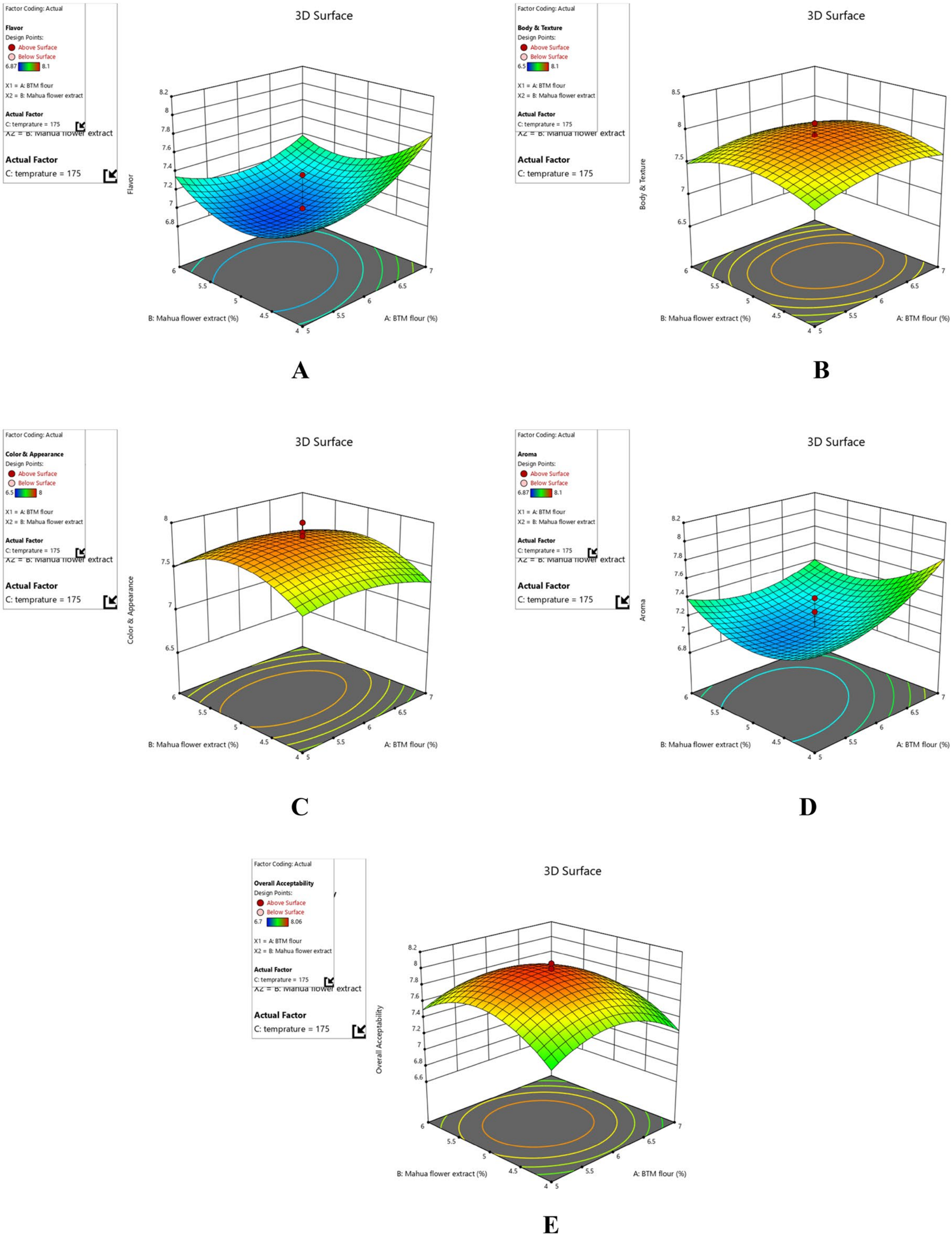


**Fig. 2**. 3D surface plots showing the effects on A) flavour, B) body and texture, C) colour and appearance, D) aroma, and E) overall acceptability of the functional muffin.

According to the above regression Eq. 1, BTM flour and MFE negatively affect the flavour of muffin but temperature shows a positive impact on flavour. If studied with interactive effect BTM flour with temperature and other interaction (BTM with MFE and MFE with temperature) are negative factor for flavour.

## Interactive effect of BTM flour and MFE on body & texture of muffin

The body and texture scores for muffin ranged from 6.5 to 8.1(Table 4). The highest sensory score for body and texture was achieved in trial 20, while the minimum was recorded in trial 2. In trial 2, BTM and MFE concentrations were 5 and 6%, respectively, with a baking temperature of 166.59˚C. In contrast, trial 20 had concentrations of 6% BTM and 5% MFE, with a baking temperature of 175 °C, yielding the highest score for body and texture.

Figure 2B indicates that varying concentrations of BTM and MFE have a positive interaction effect on body and texture. However, changes in baking temperature had a negative impact, with both increases and decreases adversely affecting the outcome. Specifically, in trial 2, a lower temperature resulted in higher moisture content and sogginess, while in trial 18, a higher temperature led to dryness, negatively impacting texture and increasing crust hardness (Ureta et al., 2014).

$$Body\ Texture = 7.9349 - 0.0283*A + 0.0471*B - 0.0292*C + 0.1075*A*B + 0.025*A*C + 0.0425*B*C - 0.1793*A2 - 0.2624*B2 - 0.5276*C2 \quad (2)$$

Where, A is Brown top millet flour, B is Mahua flower extract and C is Temperature.

According to the regression Eq. 2, the body & texture of the product significantly varied at higher MFE concentration. The equation also indicates that variations in BTM concentration and temperature do not enhance sensitivity of body texture.

## Interactive effect of BTM flour and MFE on colour and appearance of muffin

The colour and appearance scores for muffin ranged from 6.5 to 8 (Table 4). The highest and lowest sensory scores for colour and appearance were observed in trials 20 and 2, respectively. In trial 2, BTM flour and MFE concentrations were 6 and 5%, with a baking temperature of 166˚C. Conversely, in trial 20, both BTM and MFE concentrations were 6 and 5%, at a baking temperature of 175˚C.

The concentration of BTM flour and MFE positively affected colour and appearance (Fig. 2C.). However, a decrease in temperature from 175˚C to 166˚C adversely impacted these attributes, resulting in a raw, lighter colour instead of the desired golden brown[31]. The interaction effects of these variables were significant in their quadratic terms. Optimal colour and appearance were achieved when the concentration of mahua flower extract was between 4.5% and 5.5%, and the concentration of brown top millet flour was between 5.5% and 6.5%. Acceptability diminished if the concentrations of either ingredient fell outside these optimal ranges.

$$Colour\ and\ Appearance = 7.8268 - 0.0487*A + 0.0402*B - 0.0483*C + 0.0175*A*B - 0.0275*A*C - 0.0075*B*C - 0.1125*A2 - 0.2823B2 - 0.4908*C2 \quad (3)$$

Where, A is Brown top millet flour, B is Mahua flower extract and C is Temperature.

According to the regression Eq. 3 the colour and appearance of the product improve with an increase in the concentration of MFE. However, an increase in the concentration of BTM and temperature reduces the colour and appearance quality. While the individual concentrations of BTM and MFE have a positive effect, the combined effect of BTM with temperature and MFE with temperature has a negative impact.

## Interactive effect of BTM flour and MFE on aroma of muffin

The Aroma scores for muffin varied between 6.5 and 8.1 (Table 4). The highest sensory score for Aroma was achieved in trial 3, while the minimum was observed in trial 17. In trial 17, the concentrations of BTM flour and MFE were 6% and 5%, respectively, with a baking temperature of 175˚C. In contrast, trial 3 used 7% BTM flour and 4% MFE, with a baking temperature of 180˚C.

The aroma is enhanced by the functional components in BTM flour and the natural sweetness of mahua flower extract (Fig. 2D). Baking at a slightly higher temperature promotes Maillard browning, which contributes to the development of complex flavours and aromas[32]. Figure D illustrates that increasing the concentrations of both BTM flour and MFE improves the Aroma score. Conversely, reducing the quantities of these ingredients leads to a lower Aroma score.

$$Aroma = 7.0479 + 0.1379*A - 0.0792*B + 0.0688*C - 0.125*A*B + 6.71386E - 17*A*C - 0.03*B*C + 0.1727*A2 + 0.2487*B2 - 0.0949*C2 \quad (4)$$

Where, A is Brown top millet flour, B is Mahua flower extract and C is Temperature.

According to regression Eq. 4, BTM and temperature both enhance the aroma of the product, whereas MFE decreases aroma sensitivity. When considering the combined effects of all factors, MFE with temperature and BTM with MFE have a negative impact on aroma, while BTM with temperature has a positive effect.

## Interactive effect of BTM flour & MFE on overall acceptability of muffin

The Overall Acceptability scores for muffin varied between 6.7 and 8.06 (Table 4). The highest sensory score was recorded in trial 9, where BTM flour and MFE concentrations were 6 and 5%, respectively, with a baking temperature of 175 °C. Conversely, the minimum score was observed in trial 19, where the concentrations of BTM flour and MFE were 7 and 4%, respectively, and the baking temperature was set at 170˚C. Figure 2E illustrates that overall acceptability increases when MFE concentration is between 5 and 5.5%, with BTM flour concentration at 6%. Acceptability declines when these concentrations deviate from these optimal ranges.

$$Overall\ Acceptability = 7.9691 - 0.0635*A + 0.0673*B + 0.1070*C - 0.0125*A*B + 0.1375*A*C + 0.0375*B*C - 0.2860*A2 - 0.3231*B2 - 0.2382*C2 \quad (5)$$

Where, A is Brown top millet flour, B is Mahua flower extract and C is Temperature.

| Attributes | Brown top millet flour | Dried Mahua flower |
|---|---|---|
| Moisture (%) | 11.46 ± 0.55 | 11.6 ± 1.05 |
| Carbohydrates (%) | 69.2 ± 1.66 | 70 ± 1.85 |
| Protein (%) | 11.5 ± 0.45 | 6.5 ± 0.66 |
| Fat (%) | 2.61 ± 0.36 | 0.9 ± 0.42 |
| Ash (%) | 2.1 ± 0.77 | 2.5 ± 0.40 |
| Reducing sugar (mg/mL) | 0.58 ± 0.20 | 77.04 ± 1.55 |
| DPPH (%) | 51.24 ± 1.55 | 88 ± 0.54 |
| TPC(mg GAE/g) | 3.24 ± 0.85 | 20.5 ± 1.55 |
| Ascorbic acid(mg/100mL) | – | 40 ± 0.81 |

**Table 5**. Nutrient analysis of the Raw Ingredient. The data is presented as the mean ± standard deviation (n=3).

| Constituents | Amount in/100 g or as % of the sample | |
|---|---|---|
| | Control | Optimized muffin |
| Moisture (%) | 19.4 ± 0.51 | 20.25 ± 1.06 |
| Protein (%) | 3.36 ± 0.51 | 7.35 ± 0.49 |
| Fat (%) | 16.95 ± 0.64 | 21.47 ± 0.66 |
| Ash (%) | 1.06 ± 0.42 | 1.9 ± 0.71 |
| Carbohydrate (%) | 41.25 ± 1.77 | 50.20 ± 0.28 |
| % DPPH Inhibition Activity | 11.98 ± 1.30 | 37.84 ± 1.41 |
| TPC (mg GAE/gm) | 6.59 ± 0.69 | 12.15 ± 0.24 |
| TFC(mg QE/g) | 64.20 ± 1.70 | 65.65 ± 1.63 |

**Table 6**. Nutrient analysis of the optimized and control muffin. The data is presented as the mean ± standard deviation (n=3).

According to the equation, overall acceptability is positively influenced by the percentage concentration of MFE and temperature, but negatively affected by the concentration of BTM. The combination of temperature with BTM and MFE also has a positive effect. However, increasing the combined concentrations of BTM and MFE negatively impacts overall acceptability.

*Proximate analysis of different ingredients in muffins*
Table 5 shows the proximate composition of brown top millet and mahua flower extract used in the manufacturing of nutrient enriched muffins.

## Physicochemical content of control and optimized muffin

The analysis of the control muffin (C) compared to the optimized muffin (O) revealed several differences (Table 6). The moisture content was 19.86% ± 0.51% for the control and 20.25% ± 1.06% for the optimized muffin, with the latter showing slightly higher moisture likely due to the water-holding capacity of date fruit fiber concentrates used in the optimized formulation[33]. In terms of protein, the control muffin had 3.36% ± 0.51% while the optimized version had 7.35% ± 0.49%, benefiting from the inclusion of dry fruits, millet, cereals, and pulses which enhance protein content[34]. The fat content was 16.95% ± 0.64% in the control muffin versus 21.47% ± 0.66% in the optimized muffin; this higher fat content contributes to a richer flavour and better texture[35]. The ash content also differed with the control muffin containing 1.06% ± 0.42% and the optimized muffin 1.9% ± 0.71% (Table 6), the elevated ash content in the optimized muffins can be attributed to the rich mineral composition of brown top millet and mahua flower extract, which surpasses that of refined wheat flour. Their incorporation enhances the nutritional profile by increasing essential mineral levels (Rustagi et al., *2024*). Carbohydrates were more abundant in the optimized muffin, containing 50.2% ± 1.61% compared to 41.25% in the control. Regarding antioxidant properties, the DPPH inhibition activity was notably higher in the optimized muffin (37.84%) compared to the control (11.98% ± 0.923%) (Table 6). The total phenolic content (TPC) was significantly greater in the optimized muffin (12.15% ± 0.17%) compared to the control (6.59% ± 0.90%). Similarly, the total flavonoid content (TFC) in the optimized muffin (65.65 ± 1.63 mg QE/g) was slightly higher than that of the control (64.20 ± 1.70 mg QE/g) (Table 6). Brown top millet and Mahua flower extract-enriched muffins showed a marked increase in antioxidant activity, as evidenced by higher TPC, TFC, and DPPH radical scavenging capacity compared to the control. This is primarily due to the rich concentration of bioactive compounds in mahua flower extract, which possesses potent antioxidant properties (Singh *et a.*,2021). The antioxidant activity of browntop millet and mahua muffins is supported by various studies highlighting the beneficial properties of millet phenolics and their processing effects. Browntop millet, like other millet varieties, contains significant polyphenols that exhibit strong antioxidant capabilities, which can be enhanced through specific processing methods.

Browntop millet is rich in polyphenols such as catechin and ferulic acid, which contribute to its antioxidant activity[36]. Studies indicate that millet flours, including browntop millet, demonstrate effective DPPH radical scavenging, with varying levels of antioxidant activity depending on the millet type[37].

Mahua (*Madhuca longifolia*) is known for its rich nutritional profile, including antioxidants that can complement the health benefits of millet in muffins. Synergistic Effects: Combining mahua with browntop millet in muffins may enhance the overall antioxidant capacity, providing a functional food option that supports health.

In contrast, while browntop millet and mahua muffins show promising antioxidant activity, the bioavailability of these compounds can be affected by processing methods and the presence of bound phenolics, which may limit their efficacy in some cases[36,37].

## UPLC analysis of dried Mahua flower

A 10 µl aliquot of Mahua extract was injected into the chromatographic column. The resulting chromatogram, as depicted in Fig. 3, displays a total of five distinct peaks. Vanillin (RT-0.458 min), p-hydroxybenjoic acid (RT-1.479 min), gallic acid (RT-1.884 min), caffeic acid (RT- 2.338 min) and myrecitin (RT-6.44 min) were observed in optimized muffin (Valvi et al., 2023). All the detected compounds possess potential antioxidant, anti-inflammatory, antimicrobial and anticancerous properties.

These results corroborate previous findings by Singh et al. (2021)[38], who also observed a high concentration of gallic acid in methanolic extracts of Mahua flowers using HPLC analysis. Compared to conventional HPLC, UPLC provided sharper peaks and better separation with a run time reduced to 10 min, enhancing throughput and accuracy.

The high gallic acid content aligns with the known antioxidant and antimicrobial properties attributed to Mahua flowers. This supports traditional uses of the flower in ethnomedicine for treating inflammation and infections[38]. Moreover, the presence of quercetin and rutin suggests potential cardiovascular and anti-diabetic benefits, as these compounds are known for their free radical scavenging and vasoprotective effects.

It is notable that the UPLC fingerprinting of Mahua flower extracts offers not only a rapid method for quality control but also a foundation for nutraceutical product development. Quantitative differences observed in this study compared to others may be due to factors such as geographic location, stage of maturity, or extraction method, which are known to influence phytochemical content[39].

UPLC method proved efficient in detecting and quantifying key phenolic constituents in Mahua flower, with gallic acid being the most abundant. These findings support the potential of Mahua flower as a valuable source of bioactive compounds for food and pharmaceutical applications.

## Principal component analysis (PCA)

In this study, PCA was carried out to determine the relationship between various variables, including Moisture, TPC (Total Phenolic Content), protein, carbohydrate, ash, fat, DPPH Inhibition Activity, and TFC (Total Flavonoid Content). Each red line in the plot corresponds to one of the variables (moisture, TPC, etc.). The First Component (x-axis) and Second Component (y-axis) represent the first two principal components, which explain the majority of the variance in the data set (Fig. 4). Variables with lines pointing in similar directions are positively correlated, while those pointing in opposite directions are negatively correlated. Variables like TPC, Moisture, and Protein are closely grouped, suggesting they contribute similarly to the principal components and are likely correlated with each other. Fat, Ash, and DPPH Inhibition Activity are in the opposite direction from the TPC and Moisture, indicating a negative correlation with those variables. Carbohydrate and TFC seem to be closer to the origin, suggesting that they have a moderate contribution to the first two components.

Moisture vector aligns with the direction of the First Principal Component, indicating that as the addition of BTM flour and MFE increases, the moisture content of the optimized muffin also rises. This is likely due to the lower dry matter content in the added ingredients compared to the control muffin sample. The TPC (Total Phenolic Content) showed a significant increase in bioactive compounds, such as phenolics and flavonoids, with the incorporation of BTM flour and MFE. This is in conformity with previous findings of Loncar et al., (2022)[40]

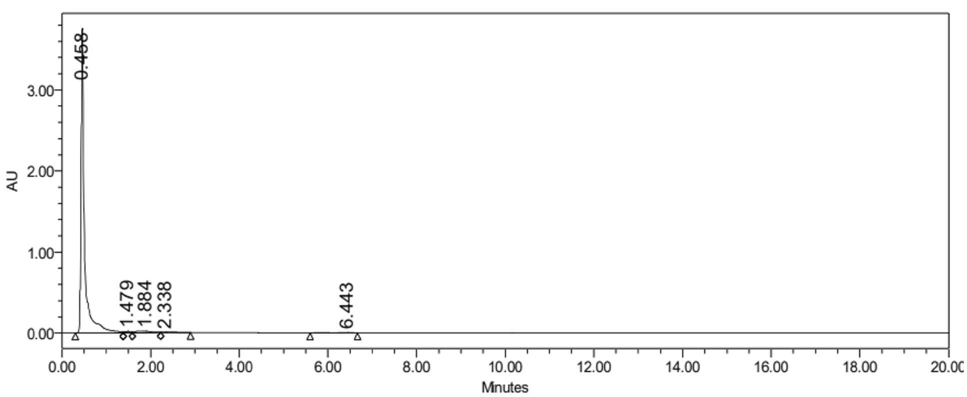


**Fig. 3**. UPLC Analysis of Dried Mahua Flower.

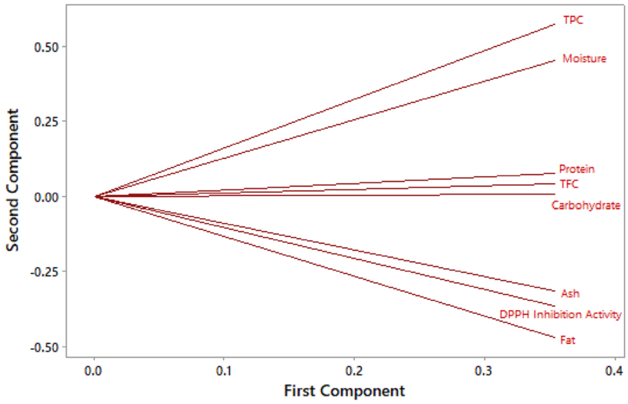


**Fig. 4**. PCA biplot illustrating the relationships between muffin samples based on their chemical composition and other parameters.

| | Control | Optimized muffin |
|---|---|---|
| **Height(mm)** | 33.6 | 27.0 |
| **Weight Loss (%)** | 14.86 | 16.40 |

**Table 7**. Muffin height and weight loss (%).

on apple powder-enriched muffins, which reported an increase in cellulose and other bioactive compounds. This enhancement is further supported by the DPPH Inhibition Activity, which reflects an increase in antioxidant activity. The DPPH vector suggests that the optimized muffin has improved health benefits, particularly an enhanced capacity to neutralize free radicals and prevent oxidative damage, thus elevating its functional quality.

In contrary, addition of BTM flour and MFE, showed no significant ($p<0.05$) variation in protein and carbohydrate. Consistent with the results from Loncar et al.[40], where protein and starch content decreased with the addition of apple fruit powder, it is possible that BTM flour and MFE reduce protein and carbohydrate levels in the optimized muffin. The Ash and Fat vectors point in a similar direction, away from TPC, suggesting that while ash and fat content may increase with the addition of BTM flour and MFE, the increase is less substantial compared to bioactive components like TPC and TFC. This trend mirrors findings from Loncar et al.[40], where fat content decreased with the addition of apple fruit powder. The similar ash and fat content between the control and optimized muffins implies that the overall dry matter or non-bioactive components remain relatively unchanged.

## Muffin weight loss and height (%)

The height of baked muffins was significantly reduced when BTM was substituted for RWF as illustrated in Table 7. The control muffins (without BTM) started out at 33.6 mm in height. But with BTM optimization, the height dropped to 27.0 mm. additionally the baking loss increased from 14.86% in the control muffins to 16.40% in the optimized muffins. According to earlier research on replacing wheat flour with resistant starch[41], Finger millet flour[42], and rice and quinoa flour[43], these results are similarly consistent. The reduction in muffin height with BTM substitution could be due to fewer air cells and the batter's reduced air bubble retention capacity. Moreover, the limited stability of gas cells caused by gluten dilution[7] likely contributed to the poor leavening of the muffin batter during the process of baking.

## TPA of muffins

Substituting Refine wheat flour (RWF) with Brown top millet (BTM) resulted in muffins that were noticeably firmer than the control batch. Table 8 illustrates that incorporating BTM flour into the recipe increased the muffins hardness, springiness, resilience, cohesiveness, and chewiness compared to the control. Optimized muffin showed more hardness and springiness than control sample. However, resilience, cohesiveness and chewiness were similar to control sample.

**Hardness** increased with higher millet flour substitution, likely due to the higher dietary fiber and lower gluten content, which reduces the gas-holding capacity of the batter. The fibrous nature of millet absorbs more water, forming a denser crumb structure. This is in line with the findings of **Goswami et al. (2015)**[44], who observed increased hardness in barnyard millet-enriched muffins.

**Springiness** and **cohesiveness** declined slightly with higher millet levels, indicating reduced elasticity and structural integrity, which is attributed to the absence of gluten network formation. Muffins with higher millet content tend to be more crumbly and less elastic, which may affect consumer perception negatively if not balanced with other softening agents like fat or hydrocolloids.

| Parameters | Optimized | Control |
|---|---|---|
| Hardness cycle 1 | 6.03 N | 4.85 N |
| Hardness cycle 2 | 5.09 N | 4.02 N |
| Resilience | 0.20 | 0.19 |
| Cohesiveness | 0.51 | 0.46 |
| Springiness | 8.17 mm | 7.70 mm |
| Chewiness | 25 mj | 17.00 mj |

**Table 8**. Texture analysis of control and nutrient rich muffin.

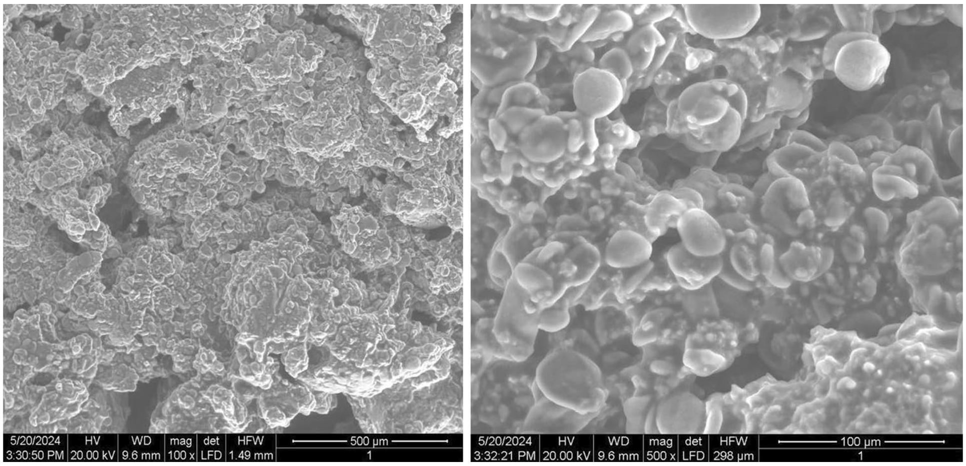


**Fig. 5**. Microstructure of nutrient enrich muffin.

**Chewiness** increased proportionally with millet content, as the muffins became firmer and less springy. This could be perceived either positively or negatively, depending on the target demographic and product positioning (e.g., health-focused consumers may prefer denser textures).

**Resilience**, a measure of how well a muffin recovers from deformation, was also lower in millet muffins, confirming the reduction in elasticity.

These results suggest that while millet flour enhances the nutritional profile of muffins—especially in terms of dietary fiber, minerals (iron, calcium), and antioxidants—it also significantly alters the texture. An optimal substitution level (e.g., 30–50%) may provide a balance between nutrition and acceptable texture. Texture modification strategies such as the addition of emulsifiers (e.g., lecithin), hydrocolloids (e.g., xanthan gum), or partial incorporation of gluten-containing flours may help restore the desirable crumb characteristics.

## SEM

Micrographs (Fig. 5) of BTM muffins containing 7% BTM reveal gelatinized starch granules embedded in a disrupted protein matrix. Brown top millet starch granules are also present, and the gluten matrix appears notably disrupted. Compound BTM starch granules are visible, intertwined within the irregular gluten matrix. Overall, the microstructure of BTM flour muffins shows increased density with fewer air pockets compared to conventional muffins. According to[45], bran particles can potentially disrupt the coherence of the matrix. According to[46] the granular microstructure of brown top millet flour was displayed by SEM pictures, exhibiting irregular and spherical particles with sizes ranging from 2.34 µm to 12.4 µm.

## EDX

BTM is a rich source of various minerals per 100 g of grain[47],. The SEM-EDX analysis of the optimized muffin sample revealed (Fig. 6) the weight percentages of elements as follows: C (54.0%), O (44.7%), Na (0.7%), Al (0.3%), P (0.0%), S (0.1%), and K (0.2%).

## Conclusion

The integration of Mahua flower extract (MFE) and browntop millet (BTM) flour into muffin formulations demonstrates a promising approach toward developing functional bakery products with enhanced nutritional and bioactive profiles. The optimized formulation—incorporating 6% BTM and 5% MFE, baked at 175 °C—not only improved the proximate composition, total phenolic content, and antioxidant activity, but also achieved high sensory acceptability, indicating strong potential for consumer acceptance.

UPLC profiling revealed the presence of key phenolic constituents such as p-benzoic acid, caffeic acid, mericitin, and catechin, which are known for their antioxidant, antimicrobial, and therapeutic properties,

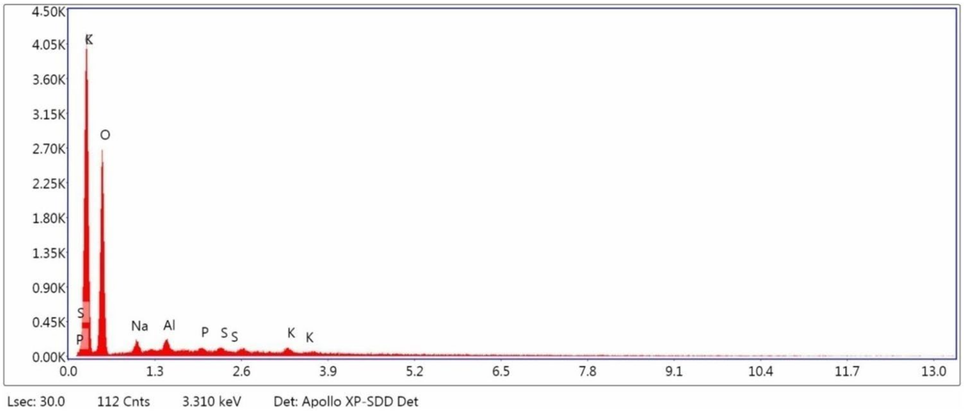


**Fig. 6**. EDX Analysis of nutrient enrich muffin.

thereby validating the functional enrichment of the product. Furthermore, SEM-EDX analysis indicated superior microstructural integrity, improved hygroscopicity, and the presence of essential minerals like potassium, sulfur, and phosphorus, supporting both the quality and nutritional density of the optimized muffins.

The product maintained its sensory and physicochemical stability over 28 days of storage, suggesting its viability as a shelf-stable functional food. This research contributes to the valorization of underutilized bioresources such as Mahua flower and millet, aligning with goals of sustainable nutrition, food diversification, and health-focused product innovation.

Future work should focus on scaling up the formulation, conducting in vivo bioavailability studies, and performing consumer-based market analysis to facilitate commercial translation. The successful integration of traditional bioresources with modern food technology exemplifies a novel path for developing next-generation, nutrient-enriched bakery products aimed at preventive health and functional food markets.

Given the growing demand for functional foods and gluten-free alternatives, this formulation presents a promising direction for value-added bakery product innovation. Future research should focus on optimizing textural properties through the use of natural binders or gluten substitutes, evaluating shelf-life stability under commercial conditions, and assessing consumer acceptance across diverse demographic groups to support large-scale application and market adoption.

## Data availability

Data will be made available on request to the Corresponding author (Abhishek Dutt Tripathi).

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SB: Investigation, formal analysis, writing original draftMA: Formal analysis, writing and reviewing original draftADT: Investigation, formal analysis, writing and reviewing original draft, supervisionAA: Investigation, formal analysis, writing and reviewing original draft, supervisionAN: Formal analysis, writing and reviewing original draft.

## Declarations

### Competing interests
The authors declare no competing interests.

### Additional information
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