Research Article

Banana pseudostem substitution in wheat flour biscuits enriches the nutritional and antioxidative properties with considerable acceptability

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Abstract
Biscuits prepared from composite flours substituted with unconventional plant resources have been considered important for enriching the overall nutritional quality. The present study was undertaken to explore the potential of banana pseudostem flour for the valorization of wheat flour biscuits. At the same time, the study attempts to address the problem of bioresource wastage as huge amounts of banana pseudostem are wasted every year after harvesting of the fruits. In this study, composite flours were prepared by partially substituting wheat flour with banana pseudostem core flour (BPF10, BPF20, BPF30) in different proportions (10, 20, 30% w/w), and biscuits were prepared from these composite flour formulations. Analyses of the physicochemical properties, pasting properties, colour and texture, quantification of phytochemicals and antioxidant properties, and overall sensory evaluation of the flours and biscuits were performed for comparative evaluation. BPF-substituted composite flours showed higher moisture and ash content, pasting temperature and water and oil absorption capacity. BPF-fortified biscuits were found to be rich in ash content, protein, proline, antioxidative phytochemicals, viz. phenols, flavonoids, tannins, ascorbate, and alkaloids, whereas fat, moisture content, and viscosity were comparatively lower than that of the control (wheat flour biscuits). Significant free radical scavenging activities of the BPF-substituted biscuits were also observed. Colour and texture analysis showed desirable changes in lightness (L^*), yellowness (b^*), chroma (C^*), fracturability, and hardness of the BPF-substituted biscuits. Most importantly, considering the sensory characteristics like taste and crispiness, control and BPF10 biscuits were highly comparable. Therefore, the formulation of BPF-substituted biscuits presents an effective way to utilize banana pseudostems, which is also rich in nutraceutical and antioxidative properties.

Keywords Antioxidant · Banana pseudostem · Biscuits · Composite flour · *Musa balbisiana* · Sensory evaluation

1 Introduction

Banana (*Musa* spp.) is widely cultivated for its fruits, and almost all the cultivated species are derived from hybridization of two wild diploid species—*Musa acuminata* and *Musa balbisiana* [1]. Banana fruits are rich in carbohydrates, vitamins, minerals, and several essential amino acids. The fruits are highly valued for fresh consumption as well as for their utilizations in numerous food products like puree, flour, chips, jam, vinegar, wine, juice, etc. [2]. Apart from the fruits, the inflorescence, pseudostem, rhizome and leaves of banana are also consumed locally and

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traditionally in several regions [3]. India is the largest producer of banana in the world with about 31 million tonnes of fruits produced per year [4]. Nearly 1 million hectares of land is under banana cultivation, which is in addition to the plants growing in wild condition [4]. A huge amount of agricultural waste is thus generated every year after the harvesting of fruits in the form of pseudostems and leaves. Therefore, the viable utilization of these agro residues is of utmost importance to reduce wastage.

The banana pseudostem is particularly rich in cellulose, hemicellulose, protein, fat and dietary fibres along with other nutritive elements [5]. The presence of polyphenols and flavonoids, viz. ferulic acid, cinnamic acid, catechin, gingerol, with promising antioxidative activities has been reported [6]. It was shown that the methanolic extract of the pseudostem possesses significant hypolipidemic and antihypercholesterolemic properties [7]. Banana pseudostems also exhibited antimicrobial properties against both gram-positive and gram-negative bacteria and even antitumor activity against hepatocellular carcinoma (HepG-2) and human colon carcinoma (HCT-116) cell lines [8]. Therefore, consumption of banana pseudostem can be beneficial, and hence, materializing its usage in food products appears noteworthy. In this connection, some attempts have been undertaken in the past. Sharma et al. [9] reported the preparation of a functional juice from pseudostem with prebiotic properties containing glucooligosaccharides and D-allulose. Carboxymethylcellulose obtained from banana pseudostem was reported to be used as a natural thickening and gelling agent in food items [10]. Apart from that, the fermentation of banana pseudostem for bioethanol production was also reported [11].

One of the most popular ways of exploiting the underutilized plant resources has been through the preparation of composite flours. Composite flours are mixtures of several flours obtained from cereals, legumes or other plant resources with or without the presence of wheat flours. Composite flours have gained much importance owing to its enriched nutritional profile and better digestibility [12]. Moreover, the usage of composite flours can help overcome the production deficit of wheat in several tropical countries [13]. It could also enhance the scope for utilizing unconventional domestic agricultural resources in flour making, limiting unnecessary wastage at the same time. Preparation of baked products from composite flours has received special attention for the supplementation of good quality proteins, essential amino acids, vitamins, minerals and dietary fibres [14]. In this connection, flours made from banana fruits and peels (ripe and unripe) have been successfully converted into several baked products, viz. slowly digestible cookies, high-fibre bread, snacks, noodles, pasta and cakes [15]. Agama-Acevedo et al. [16] reported the usage of unripened banana flour in cookies that remarkably enhanced the contents of fibre and resistant starch, which in turn reduced starch digestibility and glycemic index. Improved textural and rheological properties were also observed in dough and bread substituted with whole green banana flour [17]. However, to date, very few attempts have been made to transform the pseudostem into edible baked forms. Therefore, the utilization of banana pseudostem into baked products demands special attention. Among the baked products, biscuit or cookie preparations are quite popular due to their ready-to-eat form, palatability, aroma, texture quality and relatively longer shelf life [18].

In the present study, we have attempted to utilize the flour derived from the pseudostem core of *M. balbisiana* Colla (Family—Musaceae), which generally grows in wild conditions. The plant has been less valued for its fruits, and the pseudostem core is often consumed as a vegetable by the local people. However, the consumption of pseudostem is not so popular, and thus, the feasibility of biscuit preparation from banana pseudostem core flour was explored in the present study. In this connection, the preparation of biscuits from the composite flours obtained by mixing wheat and banana pseudostem core flours in different proportions was done and then evaluated in terms of physicochemical, functional, antioxidant and sensory characteristics. The abundance of high protein and lesser fat content, antioxidative phytochemicals and free radical scavenging properties enriched the overall quality of the partially substituted wheat flour biscuits. Also, the colour and textural attributes with considerable acceptance from the volunteers in terms of sensory parameters indicated the feasibility of using banana pseudostem core flour in biscuits.

2 Materials and methods

2.1 Plant material

Fresh pseudostem core of *M. balbisiana* Colla were obtained from the vegetable market (Malda, West Bengal, India) (Fig. 1). The outer sheath of the banana pseudostem was peeled off to obtain the inner central core (diameter < 6 cm), which was then cut into small pieces, and dried in a hot air oven at 50 °C for 48 h (Fig. 1). After drying, the plant sample was milled using a mechanical grinder and sieved through 0.2-mm sieve to obtain a fine powder (Fig. 1). The powder was finally stored in an airtight container at 4 °C for further use.

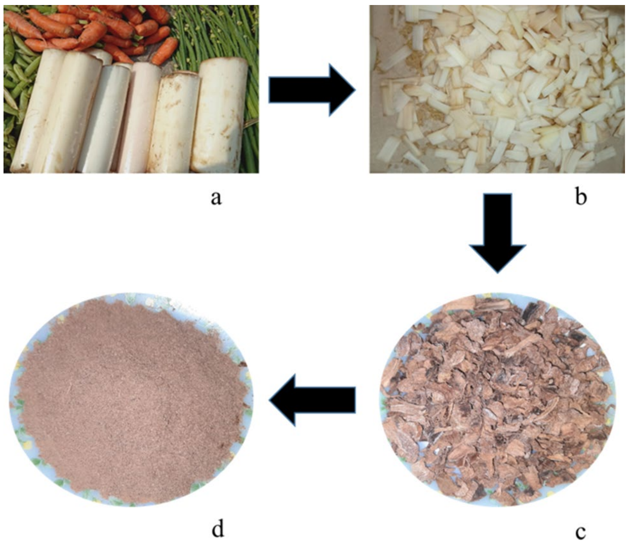

Fig. 1 **a** Banana pseudostems procured from the local market, **b** Pseudostems chopped into small pieces for drying, **c** Dried pseudostem for milling, **d** Pseudostem flour (BPF) sample

2.2 Preparation of composite flour

Refined wheat flour (Aashirvaad, ITC Kolkata, India) containing about 11.8 g protein and 1.7 g fat per 100 g was purchased from a local retail outlet (Malda, West Bengal, India). Banana pseudostem flour (BPF) was mixed to partially substitute the wheat flours by 10, 20 and 30% (w/w). The formulated composite flour samples were again stored in airtight containers and labelled as BPF10, BPF20 and BPF30, respectively.

2.3 Preparation of biscuits

Biscuits were prepared from the control (0% BPF containing wheat flour) and composite flour samples (BPF10, BPF20 and BPF30) following the method given by Kaur et al. [19] with slight modification. Ingredients for biscuit preparation like butter, sugar, salt and sodium bicarbonate were also purchased from a local retail outlet (Malda, West Bengal, India). All the four flour samples (150 g) were mixed separately with butter (40 g), powdered sugar (35 g), salt (1 g), sodium bicarbonate (2 g) and distilled water (10 mL). The mixture was kneaded until a soft and smooth dough was formed. The dough was then rolled on a flat rolling board to a uniform thickness of approximately 0.7 cm using a wooden rolling pin. Circular biscuits of 4.5 cm diameter were cut using a cookie cutter, placed on lightly greased trays and baked at 180 °C for 15 min in a pre-heated convection oven (LG Electronics, India). After baking, all the biscuits were stored in airtight containers for further experiments.

2.4 Physicochemical properties of composite flours and biscuits

The moisture content of the flours and biscuits was analysed according to AACC-approved methods [20]. For determination of ash value, 2 g of sample was weighed in a previously ignited silica crucible and incinerated in a muffle furnace at 600 °C for 5 h [21]. Bulk density and tapped density were determined by taking 10 g of flour samples separately in measuring cylinders. The initial volume was recorded for the calculation of bulk density. The samples were then tapped until no further reduction in volume, which was used for the calculation of tapped density. For the determination of water absorption capacity (WAC) and oil absorption capacity (OAC), 1 g of flour samples was mixed with 10 mL distilled water (for WAC) and 10 mL vegetable oil (for OAC) and were kept at room temperature for 30 min. The mixtures were then centrifuged at 5000 rpm for 10 min. After centrifugation, the supernatant was decanted and the tubes were inverted on a paper towel for 5 min. WAC and OAC were determined by weighing the residues and expressed as percentage of water or oil absorbed per gram of samples, respectively.

Diameter and thickness of the biscuits were recorded using a Vernier caliper. The spread ratio of the biscuits was represented as the factor of diameter and thickness. Total protein content of the biscuits was estimated following the standard protocol [22]. Extraction and estimation of total sugars and reducing sugars were performed following previously described protocols [23, 24]. Extraction and estimation of proline were done following a slightly modified protocol of Bates et al. [25]. 0.5 g of biscuit samples was crushed with 3% aqueous sulfosalicylic acid and centrifuged at 8000 rpm for 10 min. The reaction mixture was prepared by adding 1 mL of extracted solution with 2 mL of glacial acetic acid and 2 mL of acidic ninhydrin. The mixture was then heated in a boiling water bath for 1 h, cooled on an ice bath and finally vigorously shaken with 4 mL of toluene. The toluene layer was separated, and the absorbance was read at 520 nm for the determination of the concentration of proline using a standard curve.

2.5 Pasting properties of the composite flours

Pasting properties of the flour samples were determined using a Rapid Visco Analyser (Model: RVA-Techmaster, Newport Scientific, Australia).

2.6 Colour and texture analysis of the biscuits

Colour of the baked biscuits was read using a Hunter Lab Colorimeter (D-65, Hunter Associated Laboratory, USA). The colorimeter was calibrated by Hunter colour standards before the analysis of biscuit samples [26]. Texture analysis of the baked biscuits was performed in terms of breaking strength analysis using a texture analyser (UTM, Lloyd LR-5 K, Hampshire, UK).

2.7 Quantification of antioxidants and free radical scavenging activity

The quantification of the antioxidants and free radical scavenging assays of the biscuits were performed with the freeze-dried extracts of the biscuit samples. Briefly, the samples were extracted with methanol in a Soxhlet extractor, and the residue was concentrated using a rotary vacuum evaporator. For estimation of total phenol content, Folin–Ciocalteu method was followed [27]. Total flavonoid content was estimated using aluminium chloride method [28]. Estimation of ascorbate was done following the DNPH method with slight modification [29]. 1 g of biscuit sample was crushed with 6% trichloroacetic acid and centrifuged at 10,000 rpm for 10 min. After that, 2 mL of 2% dinitrophenyl hydrazine was added to 4 ml of extract and kept in a boiling water bath for 10 min. After cooling, 5 mL of sulphuric acid 80% v/v) was added and the absorbance was read at 530 nm. The amount of ascorbate was calculated using a standard curve of L-ascorbic acid. Estimation of tannin content was performed following vanillin-HCL assay [30]. Colorimetric estimation of the total saponin content was determined by a vanillin-sulphuric acid method [31]. Estimation of alkaloids was done following a modified protocol of Harborne [23]. Briefly, 5 g of sample was mixed with 200 mL of acetic acid and allowed to stand for 4 h. The extract was filtered and concentrated on a water bath to one-fourth of the original volume. After that, ammonia solution was added dropwise until the precipitation was completed. The precipitate was collected, washed with dilute ammonium hydroxide and then air-dried to obtain the weight of alkaloids.

Free radical scavenging activities of the biscuit samples were expressed as curcumin equivalents, except in case of FRAP assay. DPPH radical scavenging assay was performed following the standard protocol [32]. $ABTS^+$ cation scavenging assay was performed according to a standard protocol of Re et al. [33]. Nitric oxide radical scavenging assay was performed based on Griess-Ilosvay reaction [34]. Hydroxyl radical scavenging assay was performed following a standard protocol [35]. FRAP assay was performed by using ferric chloride method, and the activity was calculated from the standard curve of $FeSO_4$ [36].

2.8 Sensory evaluation of the biscuits

Sensory evaluation of the samples was carried within 24 h of baking following the ethical standards of the Indian Council of Medical Research, New Delhi, India [37]. Approval was obtained from the Internal Ethics Committee of the University of Gour Banga, Malda, and the evaluation was performed adhering to the Declaration of Helsinki. Fifty untrained panellists (22 males and 28 females, age between 20 to 30 years) were recruited from the post-graduate students and research scholars of the University. For the selection of panellists, regular biscuit consumers were given priority, they were well informed and required consent was obtained for their participation. All four biscuit samples were simultaneously placed to all the consumers in a randomized manner. During the procedure, they were instructed to cleanse their mouths in between consuming each sample to minimize any residual effect. The responses of the individuals were obtained concerning physical appearance, colour, taste, aroma, crispiness and overall acceptability of the samples using a nine-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely).

2.9 Statistical analysis

All the experiments were performed in triplicate, except for the texture analysis ($n = 5$), colour analysis ($n = 10$) and sensory evaluation ($n = 50$). The experimental data were subjected to analysis of variance (one-way ANOVA) using statistical analysis software (IBM SPSS Statistics, version 21). Duncan's multiple range tests were used to determine the difference among means at the level of $p < 0.05$.

3 Results and discussion

Biscuits or cookies prepared from different sources have been attempted to introduce novel admixtures of flavour, texture and nutritional qualities. In this connection, the underutilized fruits and vegetables have been explored, owing to their unique nutritional compositions. Asadi et al. (2020) reported enhanced fibre content and antioxidant activities in cookies substituted with flour from fruits of *Manilkara zapota* (Chiku) [38] Physicochemical, nutritional and sensory characteristics of the cookies were found to be improved by using composite flour substituted with amaranth flour [39]. Similarly, low-calorie- and high-fibre-rich biscuits were prepared from wheat flour partially substituted with flour prepared from underground tubers of *Helianthus tuberosus*, an unconventional food crop [40]. Ho and Latif [41] showed that the overall nutritional quality and sensory parameters of cookies can be enriched by blending wheat flour with pitaya or dragon fruit (*Hylocereus undatus*) peel flour. Kim and Park observed desirable changes in quality in terms of colour, texture and sensory attributes in cookies prepared with lotus leaf powder [42]. Pasqualone et al. [43] reported significant increments in phenolic content, antioxidant activities and sensory qualities of biscuits fortified with grape marc extract. In the same way, biscuits prepared from acorn flours showed similar enhancements in nutritional qualities and antioxidative parameters [44]. Taking a cue from these studies, flour prepared from banana pseudostem core was evaluated in terms of their ability to enhance the antioxidative and nutritional attributes of the partially substituted wheat flour biscuits. At the same time, the present study was also attempted towards devising a strategy for the sustainable utilization of banana pseudostems, which are otherwise lost as agricultural waste.

3.1 Physicochemical properties of the composite flour samples

The moisture content of the unsubstituted wheat flour (0% BPF, control) was found to be lowest compared to that of the substituted flours (Table 1). Percentage of moisture content significantly increased with an increasing proportion of banana pseudostem flour (BPF). A similar increase in moisture contents has been observed in cases of composite flours containing rice, cassava and groundnut, which might accelerate microbial growth and affect storage quality. However, further processing into final products can help minimize these adverse effects [45]. Total ash content also significantly increased with the increasing percentage of BPF, compared to control (Table 1). This may be due to the gradual increase in the amount of dietary fibres present in BPF, similarly as reported by the fortification of semolina durum flour with legume flours [46].

High water absorption capacity (WAC) may infer the presence of hydrophilic components, viz. carbohydrates and proteins, and influence the cohesiveness of the food products [47]. In the present study, WAC of the control was lowest (82 ± 3%) among all the flour samples and a significant increase was observed with the substitution with BPF flour (Table 1). Akoja and Coker have also reported a similar increase in WAC of composite flour blended with okra powder and correlated this with enhanced starch and fibre content of the samples [48]. However, subsequent baking at a higher temperature could effectively reduce the water absorption capacity. Oil absorption capacity (OAC) also showed a similar trend, though the control (85 ± 4.6%) and BPF10 (90.33 ± 2.5%) samples did not exhibit any significant differences (Table 1). Bulk density and tapped density were measured to analyse the consolidation and flowability of the flour samples [49]. Moreover, these parameters also affect the hydration rate during baking. Bulk and tapped density were found to be significantly decreased with the increasing amount of BPF, indicating relatively good packaging characteristics of the flour particles and less flowability of the sample (Table 1).

Table 1 Physicochemical and pasting properties of the partially substituted banana pseudostem flour (BPF)

Parameters	Control*	BPF10	BPF20	BPF30
Physicochemical characteristics				
Moisture (%)	$0.12 \pm 0.01^{a**}$	0.1 ± 0.03^{a}	0.13 ± 0.09^{a}	0.14 ± 0.05^{a}
Ash content (%)	1.05 ± 0.13^{a}	1.25 ± 0.53^{a}	1.98 ± 0.33^{b}	2.55 ± 0.1^{b}
Water absorption capacity (%)	82.33 ± 3.06^{a}	106.67 ± 4.73^{b}	122.33 ± 3.51^{c}	137.67 ± 3.79^{d}
Oil absorption capacity (%)	85 ± 4.58^{a}	90.33 ± 2.52^{a}	100.67 ± 4.16^{b}	119.67 ± 0.58^{c}
Bulk density (g/cm^3)	0.53 ± 0.02^{d}	0.5 ± 0.01^{c}	0.45 ± 0.01^{b}	0.43 ± 0.01^{a}
Tapped density (g/cm^3)	0.67 ± 0.03^{c}	0.63 ± 0.05^{b}	0.59 ± 0.01^{ab}	0.53 ± 0.01^{a}
Pasting properties (RVA analysis)				
Peak viscosity (mPa.s)	3154 ± 49.93^{d}	2853.67 ± 59.72^{c}	2539.33 ± 25.66^{b}	2095.33 ± 8.5^{a}
Trough viscosity (mPa.s)	2059 ± 15^{d}	1857 ± 63.02^{c}	1523 ± 38.74^{b}	1332 ± 26.63^{a}
Breakdown (mPa.s)	1095 ± 39.24^{c}	996.67 ± 44.77^{b}	1016.33 ± 31.21^{b}	763.33 ± 23.03^{a}
Final viscosity (mPa.s)	3752.33 ± 19.09^{d}	3146.33 ± 41.68^{c}	2617 ± 21.63^{b}	2333.67 ± 3.21^{a}
Setback (mPa.s)	1693.33 ± 22.01^{d}	1289.33 ± 49.17^{c}	1094 ± 19.92^{b}	1001.67 ± 29.01^{a}
Peak time (min)	6.24 ± 0.1^{b}	6.38 ± 0.08^{b}	6.18 ± 0.08^{b}	5.91 ± 0.14^{a}
Pasting temperature (°C)	68.03 ± 0.06^{a}	70.78 ± 0.49^{b}	71.28 ± 0.06^{b}	72.48 ± 0.98^{c}

*Control, BPF10, BPF20, BPF30—Composite flours prepared by substituting 0%, 10%, 20% and 30% wheat flour with banana pseudostem core flour, respectively

**Values represent mean ± SD ($n = 3$) followed by lowercase letters in superscript. Different letters (a–d) within the row indicate significant differences in the mean values following post hoc analysis by Duncan's test at $p < 0.05$

3.2 Pasting properties of the composite flour samples

Pasting property of flour samples affects the texture and overall digestibility of processed food products and is regarded as an important criterion predetermining quality and aesthetics in the food industry [50]. The pasting property of the composite flour samples is listed in Table 1. Incorporation of BPF into wheat flour significantly reduced all the parameters except pasting temperature, which was found to be significantly higher in the BPF-substituted flours (10%, 20% and 30%). Higher pasting temperature may confer higher water-binding capacity, higher gelatinization tendency and lower swelling property [51]. Concentration-dependent decrease in all the viscosity parameters (peak, trough, breakdown, final and setback) could be due to the complex interaction between proteins, fats and carbohydrates during the heating–cooling phase of RVA. This decrease might be advantageous in further processing of the composite flours due to greater resistance against heating and shearing [52]. Similar changes in pasting properties were also observed in a previous study where wheat flour was substituted with chickpea flour [53].

3.3 Physicochemical properties of biscuits

It was observed that the substitution of wheat flour by BPF enhanced the moisture content. However, after baking, the moisture content of the partially substituted BPF biscuits significantly decreased in comparison with the control sample (Table 2). This indicated an increase in the relative shelf life of the BPF-substituted biscuits post-baking. A similar decrease in moisture content was observed in gluten-free biscuits prepared from buckwheat flour [54]. Ash content in food products gives an idea of the presence of mineral elements [55]. A significant enhancement in ash content of the BPF-fortified biscuits (about 2.1-fold, 2.5-fold and 3.14-fold in BPF10, BPF20 and BPF30, respectively) compared to the control sample was observed (Table 2). In terms of weight loss after baking, BPF20 and BPF30 biscuits showed a significant loss in comparison with the control (about twofold and 1.8-fold, respectively) (Table 2). The weight loss of the BPF biscuits can be attributed to the concomitant loss of moisture during the baking process. The diameter of all the biscuits post-baking remained almost unchanged, whereas the thickness of the BPF biscuits was found to be significantly lower compared to control (about 1.03-fold, 1.07-fold and 1.1-fold for BPF10, BPF20 and BPF30, respectively) (Table 2). Spread ratio of all the BPF biscuits, therefore, showed significant increments (about 1.02-fold, 1.05-fold and 1.09-fold in BPF10, BPF20 and BPF30, respectively) from the control. According to Chauhan et al. [39], such increase in spread ratio could be influenced by the presence of higher amounts of non-wheat proteins in the composite flours, which has also been considered as a desirable trait for biscuits. These findings are in agreement with the results of Bala et al. [56], who also observed similar changes in diameter, thickness and spread ratio of cookies prepared from wheat flour supplemented with cassava and water chestnut flours.

It was also important to assess the nutritional aspects of the biscuits. In this connection, quantification of protein,

Table 2 Physicochemical and functional properties of the banana pseudostem flour (BPF)-substituted wheat flour biscuits

Parameters	Control*	BPF10	BPF20	BPF30
Physical and functional aspects				
Moisture content (%)	$2.24 \pm 0.05^{b**}$	1.93 ± 0.08^{a}	2.02 ± 0.08^{a}	2.01 ± 0.16^{a}
Ash content (%)	0.91 ± 0.05^{a}	1.89 ± 0.15^{b}	2.27 ± 0.2^{c}	2.83 ± 0.12^{d}
Weight loss after baking (%)	18.37 ± 3.73^{a}	24.61 ± 7.08^{ab}	32.88 ± 10.24^{b}	36.03 ± 2.94^{b}
Diameter (cm)	4.62 ± 0.08^{a}	4.63 ± 0.03^{a}	4.57 ± 0.03^{a}	4.55 ± 0.05^{a}
Thickness (cm)	1.02 ± 0.01^{d}	0.99 ± 0.02^{c}	0.95 ± 0.01^{b}	0.92 ± 0.01^{a}
Spread ratio	4.53 ± 0.09^{a}	4.65 ± 0.05^{b}	4.79 ± 0.006^{c}	4.97 ± 0.03^{d}
*Nutritional aspects**** (g/100 g)				
Protein	7.25 ± 0.22^{a}	13.15 ± 0.21^{b}	15.23 ± 0.23^{c}	15.83 ± 0.21^{d}
Total sugars	2.55 ± 0.16^{a}	2.66 ± 0.08^{a}	2.65 ± 0.12^{a}	2.71 ± 0.17^{a}
Reducing sugars	1.01 ± 0.12^{a}	0.83 ± 0.08^{a}	0.83 ± 0.06^{a}	0.94 ± 0.09^{a}
Fats	12.33 ± 1.53^{b}	8.68 ± 0.29^{a}	8.47 ± 0.53^{a}	8.33 ± 1.19^{a}
Proline	0.55 ± 0.08^{a}	1.43 ± 0.03^{b}	1.67 ± 0.02^{c}	1.71 ± 0.06^{c}

*Control, BPF10, BPF20, BPF30—Composite flours prepared by substituting 0%, 10%, 20% and 30% wheat flour with banana pseudostem core flour, respectively

**Values represent mean ± SD ($n = 3$) followed by lowercase letters in superscript. Different letters (a–d) with in the row indicate significant differences in the mean values following post hoc analysis by Duncan's test at $p < 0.05$

***Nutritional aspects were calculated per 100 g actual weight of the biscuits fats, proline, total and reducing sugar contents in the biscuit samples was performed. Protein content was found to increase in a concentration-dependent manner in BPF-fortified biscuits in comparison with control (about 1.8–2.2-fold increase) (Table 2). The fat content of all the BPF biscuits was also significantly low compared to control (Table 2). Such high protein and less fat content of the BPF-substituted biscuits can be considered nutritionally beneficial over wheat flour products [57]. Significant increase in proline content was also observed in the BPF-substituted biscuits (about 2.6–3.1-fold increase), referring to the presence of a considerably high amount of free amino acid content in comparison with the control (Table 2). However, no absolute changes in the amount of total and reducing sugars in the BPF-fortified biscuits were observed (Table 2). These changes in amino acid and sugar content were probably caused due to acceleration of Maillard reaction and were also observed in biscuits prepared from composite flours of sunflower seed and wheat [58].

Fig. 2 Physical appearance of biscuits (Control, BPF10, BPF20, BPF30) prepared with unsubstituted wheat flour (0%), and 10%, 20% and 30% (w/w) banana pseudostem core substituted composite flours

3.4 Colour and texture attributes of biscuits

Colour analysis presents an important criterion for the overall acceptability of the baked products for consumption. Maillard reaction between protein and reducing sugars during baking primarily controls the development of colour [59]. In the present study, the colour attributes of the biscuit samples differed significantly, as can be observed superficially (Fig. 2). This was also validated by the colour analysis of the samples. In this connection, *L** value decreased about 1.8-, 1.3- and 1.2-fold in case of BPF30, BPF20 and BPF10 biscuits, respectively, in comparison with control (Table 3). The result is in accordance with that obtained by Sibian and Riar in composite flour cookies prepared by admixtures of kidney bean, chickpea and wheat [60]. Furthermore, significant concentration-dependent decrease in the *b** value was found in the BPF biscuits in comparison with control (Table 3). Colour changes in biscuits or cookies result due to degradation of pigments or formation of any brown coloured pigments during the convection process [61]. Moreover, enzymatic browning of the banana pseudostem flour, which may

Table 3 Colour and texture analysis of the banana pseudostem flour (BPF)-substituted wheat flour biscuits

Parameters	Control*	BPF10	BPF20	BPF30
Colour				
*L**	73.18 ± 3.92^{d}**	52.69 ± 1.6^{c}	49.28 ± 2.73^{b}	40.35 ± 3.27^{a}
*a**	6.92 ± 3.04^{a}	7.12 ± 0.76^{a}	6.31 ± 0.81^{a}	7.22 ± 0.31^{a}
*b**	30.49 ± 3.22^{d}	23.26 ± 2.14^{c}	19.28 ± 0.95^{b}	16.74 ± 1.98^{a}
Δ*E*	–	22.08 ± 3.13^{a}	27.18 ± 4.38^{b}	35.95 ± 4.44^{c}
h(°)	77.66 ± 4.43^{c}	73 ± 0.76^{b}	71.89 ± 1.99^{b}	66.43 ± 2.84^{a}
C	31.35 ± 3.75^{c}	24.32 ± 2.25^{b}	20.29 ± 1.03^{a}	18.25 ± 1.79^{a}
s	0.43 ± 0.07^{ab}	0.46 ± 0.04^{b}	0.41 ± 0.03^{a}	0.45 ± 0.02^{ab}
Texture				
Hardness (*N*)	23.9 ± 9.28^{a}	53.29 ± 13.25^{b}	58.54 ± 4.33^{b}	90.06 ± 14.49^{c}
Fracturability (mm)	3.55 ± 0.82^{b}	2.64 ± 0.89^{ab}	2.4 ± 0.63^{a}	2.87 ± 0.52^{ab}
Stiffness (N/mm)	27,260.18 ± 10,131.69^{a}	74,529.19 ± 9495.35^{a}	61,899.3 ± 9637.79^{a}	97,136.89 ± 13,411.25^{a}

*Control, BPF10, BPF20, BPF30—Composite flours prepared by substituting 0%, 10%, 20% and 30% wheat flour with banana pseudostem core flour, respectively

**Values represent mean ± SD ($n = 10$ for colour and 5 for texture) followed by lowercase letters in superscript. Different letters (a–d) within the row indicate significant differences in the mean values following post hoc analysis by Duncan's test at $p < 0.05$

result due to the oxidation of phenolics by polyphenol oxidase, may also contribute to the browning of the baked biscuits [62]. The increase in brown pigments, in turn, could be attributed to the enhanced antioxidative activity of the baked products [63]. In the present study, the decrease in L^* and b^* values in BPF biscuits led to infer that the incorporation of BPF in wheat flour reduced the lightness and yellowness of the biscuit colour. Significant changes in the colour difference (ΔE) values of the BPF-substituted biscuits from the control also corroborated with the changes in the L^* and b^* values. Such high colour difference values could be developed due to ingredient composition and pigmentation, which in turn depend on the content of reducing sugar, proteins or amino acids present on the surface, baking temperature and time [62]. In our study, the hue values ranged from 66.42° to 77.66°, showing gradual changes from yellow to orange in accordance with the increasing percentage of BPF (Table 3). Similarly, chroma (C^*) of the biscuits was also found to be lowered with increasing concentration of BPF, indicating the decrease in brightness of the BPF biscuits compared to control (Table 3). However, the colour saturation values (s) of all the samples were found to remain almost unchanged. Similar changes were also observed in biscuits made from wheat flour mixed with flours obtained from banana pulp and prickly pear [62].

A concentration-dependent rise in hardness and stiffness of the BPF-substituted biscuits in comparison with control was observed (Table 3). Significant increments in the hardness of BPF biscuits could be attributed to its lower moisture contents, which may in turn affect the acceptability in terms of enhancing the crunchiness [54]. Previous studies with beetroot leaf powder-substituted wheat flour cookies also showed similar enhancements in hardness and stiffness [61]. According to De Simas et al. [64], the hardness of the cookies results due to extensive hydrogen bond formation between starch and protein during dough making and baking. Several other factors, viz. higher protein and fibre content, and higher water absorption capacity, may be responsible for increased hardness. Also in our study, 2.27–3.56-fold enhancements in stiffness of the BPF-substituted biscuits from the control sample were observed; however, the changes were not significant. Further, no significant changes in fracturability except for BPF20 biscuits were observed, where it showed a significant decrease (about 1.5-fold) from control (Table 3).

3.5 Antioxidant properties

The presence of phenolics, flavonoids and other antioxidant molecules in baked products have been considered advantageous owing to their nutraceutical properties [65]. In the present study, the substitution of BPF in the wheat flour biscuits gradually enhanced the total phenolic and flavonoid contents in a concentration-dependent manner (Table 4). BPF30 biscuits were found to contain the highest amount of phenol and flavonoid (292.5 mg gallic acid equivalent/100 g and 209.93 quercetin equivalent/100 g), that accounted for about 2.6- and 2.1-fold increase from the control. Similarly, tannin content was found to be significantly increased in all the BPF-substituted biscuits (Table 4). This type of enhancement in total phenolic content in the biscuits prepared from unconventional flours of purple sweet potato and kale has been reported previously [66]. A significant increase of about 1.05-fold and 1.15-fold in ascorbate content was observed in BPF20 and BPF30, compared to control (Table 4). Alkaloid content also gradually increased in the BPF-substituted biscuits; however, only BPF30 biscuits showed a significant increase (about 2.7-fold) from the control (Table 4). On the other hand, a concentration-dependent reduction in the saponin content was observed in all the BPF-substituted biscuits (Table 4).

The presence of antioxidant molecules in the baked products is often associated with an increase in the free radical scavenging properties [67]. The enhancement of free radical scavenging properties in the biscuits would therefore be of immense importance from the viewpoint of consumption. In the present study, DPPH radical scavenging activity of the biscuit samples increased in a concentration-dependent manner with an increasing amount of BPF; however, the control sample did not exhibit any activity (Table 4). In ABTS cation scavenging assay, BPF10 showed about 1.9-fold increase from that of the control (Table 4). A further increase was also found in BPF20 and BPF30 samples (about 2.9-fold in both cases). The pattern of scavenging followed almost a similar trend in cases of NO and OH scavenging assay (Table 4). Also, a concentration-dependent increase in FRAP activity was observed with the substitution of BPF, but significant increase was observed only in BPF20 and BPF30 samples (2.6- and 3.2-fold increase from the control, respectively) (Table 4). Previous studies have reported the enhancements in free radical scavenging activities of wheat flour cookies supplemented with tomato powder and crude lycopene [68]. Similarly, Jan et al. [63] also observed similar increments in antioxidative activities of gluten-free cookies prepared from raw and germinated *Chenopodium album* flour.

3.6 Sensory evaluation of the biscuits

Sensory evaluation of the biscuit samples for the assessed parameters more or less varied significantly (Fig. 3). Individually, the physical appearance of the BPF biscuits showed a decrease of 1.07-, 1.19- and 1.2-fold (BPF10,

Table 4 Quantitation of some antioxidant molecules and free radical scavenging properties of the banana pseudostem flour (BPF)-substituted wheat flour biscuits

Parameters	Control*	BPF10	BPF20	BPF30
*Antioxidant biomolecules***				
Total phenol (mg gallic acid equiv./100 g)	113.75 ± 5.5[a]***	171.81 ± 20.9[b]	269.07 ± 8.64[c]	292.51 ± 7.25[d]
Total flavonoid (mg quercetin equiv./100 g)	97.59 ± 5.67[a]	154.49 ± 6.75[b]	193.11 ± 6.53[c]	209.94 ± 4.82[d]
Tannin (mg tannic acid equiv./100 g)	34.03 ± 2.19[a]	66.46 ± 5.29[b]	80.05 ± 2.94[c]	73.51 ± 3.32[c]
Ascorbate (mg/100 g)	74.49 ± 0.83[a]	71.75 ± 1.93[a]	78.74 ± 0.99[b]	85.31 ± 2.22[c]
Saponin (mg diosgenin equiv./100 g)	509 ± 68.71[a]	395.82 ± 52.67[b]	157.64 ± 23.41[c]	68.84 ± 12.62[d]
Alkaloid (mg/100 g)	26.67 ± 5.77[a]	36.67 ± 11.55[a]	53.33 ± 20.82[a]	73.33 ± 15.28[b]
*Free radical scavenging*****				
DPPH (mM CE/g)[5]	n.d	0.44 ± 0.14[a]	1.31 ± 0.21[b]	2.08 ± 0.19[c]
ABTS (mM CE/g)	4.62 ± 0.19[a]	7.46 ± 0.87[b]	9.85 ± 0.04[c]	9.66 ± 0.04[c]
NO (mM CE/g)	4.77 ± 0.13[a]	4.99 ± 0.09[ab]	5.1 ± 0.13[b]	6.41 ± 0.11[c]
OH (mM CE/g)	0.9 ± 0.82[a]	12.7 ± 1.38[b]	9.64 ± 1.17[c]	14.99 ± 0.43[d]
FRAP (mM Fe^{2+}/g)	0.21 ± 0.07[a]	0.35 ± 0.08[ab]	0.41 ± 0.09[b]	0.48 ± 0.09[b]

*Control, BPF10, BPF20, BPF30—Composite flours prepared by substituting 0%, 10%, 20% and 30% wheat flour with banana pseudostem core flour, respectively

**Quantification of antioxidant biomolecules was done per 100 g actual weight of the biscuits

***Values represent mean ± S.D. ($n = 10$ for colour and 5 for texture) followed by lowercase letters in superscript. Different letters (a–d) with in the row indicate significant differences in the mean values following post hoc analysis by Duncan's test at $p < 0.05$

****Free radical scavenging activities were performed from the freeze-dried methanolic extracts and finally calculated as mM curcumin equivalent (CE) or mM Fe^{2+} per 1 g actual weight of the biscuits

BPF20, BPF30, respectively) from control. Colour and aroma of the BPF biscuits also showed a similar pattern of decrease from the control sample. Taste attribute of the BPF20 and BPF30 biscuits declined by about 1.06- and 1.17-fold, respectively. More importantly, the taste attribute of the BPF10 biscuits did not change significantly from the control. In terms of crispiness, a similar trend was observed, where BPF10 biscuits did not show any observable difference from the control. Also, the overall acceptability of the BPF biscuits showed a significant decrease from the control. However, only 1.04- and 1.08-fold decrease in the overall acceptability of the BPF10 and BPF20 biscuits from the control seems reasonable. These results are in accordance with the previous studies with banana pseudostem incorporated wheat flour cookies by Ambrose and Lekshman [69].

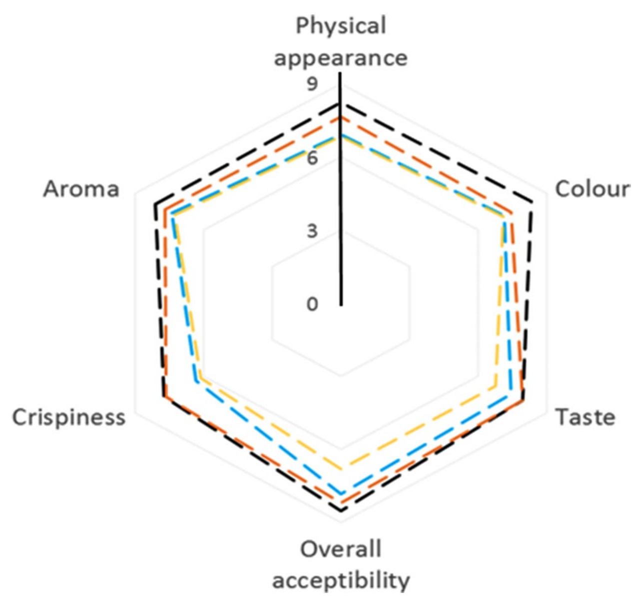

Fig. 3 Radar plot depicting the sensory characteristics of biscuits (— — Control, — — BPF10, — — BPF20, — — BPF30) prepared with unsubstituted wheat flour (0%), 10%, 20% and 30% (w/w) banana pseudostem core flour-substituted composite flours. Values represent the average of 50 replicates based up on a nine-point hedonic scale

4 Conclusion

The biscuits supplemented with BPF constitutes several bioactive compounds like phenolics, flavonoids, ascorbate, proline, etc. and also showed promising free radical scavenging properties. High protein, low fat and low sugar content of the BPF biscuits are equally important for their dietary and health benefits. Low moisture and high ash content of the biscuits along with high pasting temperature and low viscosity of the BPF-substituted flours also confer relatively longer shelf life and good storage characteristics. Moreover, sensory characteristics of the biscuits were moderately acceptable, especially the BPF10 and

BPF20 biscuits. Enrichment in the taste and overall acceptability could be enhanced with some modifications in the biscuit composition. Further studies on the simulated gastrointestinal digestion of the BPF biscuits in future will be carried out to present an accurate picture of the nutritional and health benefits. This study therefore presents a feasible option for the utilization of banana pseudostem waste in a very simple and effective manner.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All the necessary guidelines for sensory evaluation have been followed, and approval was obtained from the Internal Ethics Committee of University of Gour Banga, Malda, India.

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