Effect of Partially Substitution Wheat Flour with Millet Flour on Butter Biscuit Properties

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This study aims to formulate biscuits incorporating gluten-free millet flour. The wheat flour was substituted by millet flour at 10%, 20%, and 30% (w/w). The physical properties, the proximate composition, and antioxidant activity, including consumer acceptability, were evaluated. The results revealed a significant increase 2-fold in fibre content with slightly increased ash content. Biscuit diameter increased when millet flour increased due to less effect of gluten, relative to increased spread factor. In addition, the biscuit containing 30% millet flour had the darkest colour surface, and its texture was easy to break. There was an increase in the total phenolic content (TPC) (4.76±0.16 mg GAE/g) and antioxidant activity increased to 5.67±0.08 µmol TE/g, and 13.60±1.22 µmol TE/g as measured by DPPH and ABTS assays, respectively. These improved the nutritional values of biscuits when substituted with millet flour at 30%. Furthermore, the most consumer acceptability was observed for the biscuits substituted at this level. Thus, millet flour can be used to substitute wheat flour at 30% in the development of nutritional butter biscuits.

1. Introduction

Recently, a growing need to utilize functional crops has been widely increased to produce nutritious, affordable, and palatable food products. Food technology is necessary to apply for enhancing the nutritional bioavailability, and organoleptic senses and reducing the antinutritional properties. Converting available crops into usable ingredients would be an advantage to substituting wheat flour for bakery products. Millet grains have high nutrients as human foods that present nutrient content slightly higher compared to other staple cereals such as maize, sorghum, and rice (Balasubramanian, Kaur, & Singh, 2014). The nutritional profile of millet possesses comparable contents of 67.5% carbohydrate, 13-19% protein, 5-9% fat, 3-4% fibre, 3.4% minerals, 3.1% calcium, and 2.8% iron (Hama-Ba, 2023; Nambiar et al., 2011). They contain several vitamins and polyphenols including β-carotene, thiamine, riboflavin, niacin, and folate, etc. (Sunil & Venkatachalapathy, 2017). Millet grains are considered gluten-free, which contain higher essential amino acids such as histidine, isoleucine, leucine, and lysine compared to other traditional cereals (Hassan, Sebola, & Mabelebele, 2021). Concerning its rich dietary fibre and phytochemicals, consuming millet products can alleviate obesity-related illnesses, cardiovascular diseases, hyperglycemia, and colorectal cancer (Dias-Martins et al., 2018).

A typical butter biscuit consists of wheat flour, butter, sugar, and eggs as the main raw ingredients. The regular process of making biscuits is classified as mixing, molding, and baking. They are of different shapes, flavors, colours, and some toppings. Butter biscuits are recognized due to their high protein levels, but high levels of carbohydrates and fat. As a consequence, consumption of butter biscuits may lead to an increase in obesity, hypertension, type-2 diabetes, and cardiovascular.

The development of gluten-reduced, fibre-enriched biscuits has gained attention. Based on the previous section, millet is a certain cereal that can be utilized as a promising gluten-free flour to substitute wheat flour by enhancing the nutritional values of the butter biscuits. However, removing gluten from biscuit-making flour affects the physical properties and sensory aspects of biscuits, thus causing low acceptability by consumers (Ari Akin et al., 2022).

Thus, the objective was to develop butter biscuits substituting wheat flour by varying the proportion of millet flour at 10%, 20%, and 30% (w/w), and the substituted millet biscuits were characterized by physicochemical properties, functionality in terms of antioxidant activity, and consumer acceptability.

2. Materials and Methods

Cleaned and dried millet grains were ground using a hammer mill equipped with separate sieving (0.8 mm). Biscuit ingredients: refined wheat flour, sugar, unsalted butter, salt, baking powder, and vanilla were obtained from a local market. All chemicals and other reagents were of analytical grade.

2.1 Substituted Millet Biscuit Formulation and Preparation

The recipe for butter biscuits was optimized by substituting wheat flour at 10%, 20%, and 30% of millet flour along with other ingredients (Table 1). Blending flours, baking powder, and salt were sieved to thoroughly mix. Sugar, vanilla, and egg were mixed in a laboratory mixer at low speed, followed by adding unsalted butter as a shortening agent. Further, the mixture was agitated until appeared creamy or smooth fluffy. Later, the dough was shaped circularly with approximately 10 grams each using a piping bag. The circular-shaped biscuit doughs were baked at 200 °C in a conventional oven for 20 min. The obtained biscuits were cooled and packed in air-tight bags for further analysis.

Table 1: Ingredients and Percentage Weight (w/w) Used in Formulas.

Ingredient	Formulations of Substituted Millet Biscuits			
	Control	10%	20%	30%
Wheat flour	300	270	240	210
Millet flour	-	30	60	90
Egg	100	100	100	100
Vanilla	10	10	10	10
Baking powder	10	10	10	10
Sugar	180	180	180	180
Salt	0.8	0.8	0.8	0.8
Unsalted butter	227	227	227	227

2.2 Physicochemical Characteristics of Biscuits

Freshly baked biscuits were analyzed for thickness (distance from top to bottom), and width (diameter) was determined using a vernier caliper. The spreading was measured from the ratio of the width and thickness of the biscuits. Hardness was performed by measuring breaking strength using an XT-plus texture analyzer (Lloyd Ltd., Carlisle, UK). It was set at 1.5 mm/s of speed and 15 mm of distance to return to start a cycle. The peak force (N) was measured as an index of biscuit-breaking strength. The colour of baked biscuits was read using a MiniScan colorimeter (Hunter lab, Reston, USA) as L^* (bright-dark), a^* (green-red), and b^* (blue-yellow). Furthermore, the samples were evaluated for moisture content, ash, crude protein (N*6.25), crude fibre, and total lipid content using the AOAC 2005 methods (Latimer Jr., 2023; Moolwong, Klinthong, & Chuacharoen, 2023). Subsequently, a nitrogen-free extract was calculated as an estimate of carbohydrate content.

2.3 Functionality in Terms of Total Phenolic Content (TPC) and Antioxidant Activity

Total phenolic content (TPC) was determined using Folin-Ciocalteau assay according to the method of Chuacharoen et al. (2021) with some modifications. Briefly, 20µL of methanolic extracts were mixed with 1.58mL of distilled water, followed by adding 100 µL of Folin–Cio calteau reagent. Then, 300µL of Na_2CO_3 solution (7%) was added to the mixtures within 8 min, immediately vortexed, and kept for 30 min in darkness.

Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (GENESYS™2, Thermo Fisher Scientific, Waltham, MA, USA). A standard curve was prepared with gallic acid at various concentrations and the results were reported as mg gallic acid equivalent (mg GAE)/g of biscuit.

The antioxidant capacity of biscuits was assessed by DPPH (2,2-diefinil-1-picryl-hydrazine) radical scavenging assay and $ABTS^+$ (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical test according to Moolwong et al. (2023). For the DPPH reaction, 200µL of methanolic extracts and 2.8mL of working $DPPH^{\bullet}$ solution were mixed and kept for 30 min in darkness. The absorbance was read at 515 nm using a UV-Vis spectrophotometer. To determine the quenching of ABTS radical, the radical was generated by dissolving 7mM of $ABTS^+$ with 2.45 mM of $K_2S_2O_8$ followed by storage in darkness at room temperature for 16 h. Then, 50µL of methanolic extract was mixed with 4 mL of working $ABTS^+$ solution thoroughly, followed by incubating for 30 min at 25°C. The absorbance was read at 734 nm using a UV-Vis spectrophotometer. The scavenging activity was calculated based on a standard curve of Trolox and the results were reported as Trolox equivalent (µmol TE)/g of biscuit.

2.4 Consumer Acceptability

A semi-trained panel was used to evaluate the acceptability of substituted biscuits. The experiment consisted of selecting 30 judges (students and academic staff at Suan Sunandha Rajabhat University, Bangkok, Thailand). presenting The objective of the research was explained and the samples were introduced to the panelists. Subsequently, panelists were trained to identify the attributes of desired biscuits before the sessions. Freshly baked biscuits with various millet substituted ratios were served in random code. A 9-point hedonic scale (1-dislike and 9-like) was used to evaluate the sensorial properties of biscuits (appearance, odor, colour, taste, texture in terms of crunchiness, and overall acceptability, respectively).

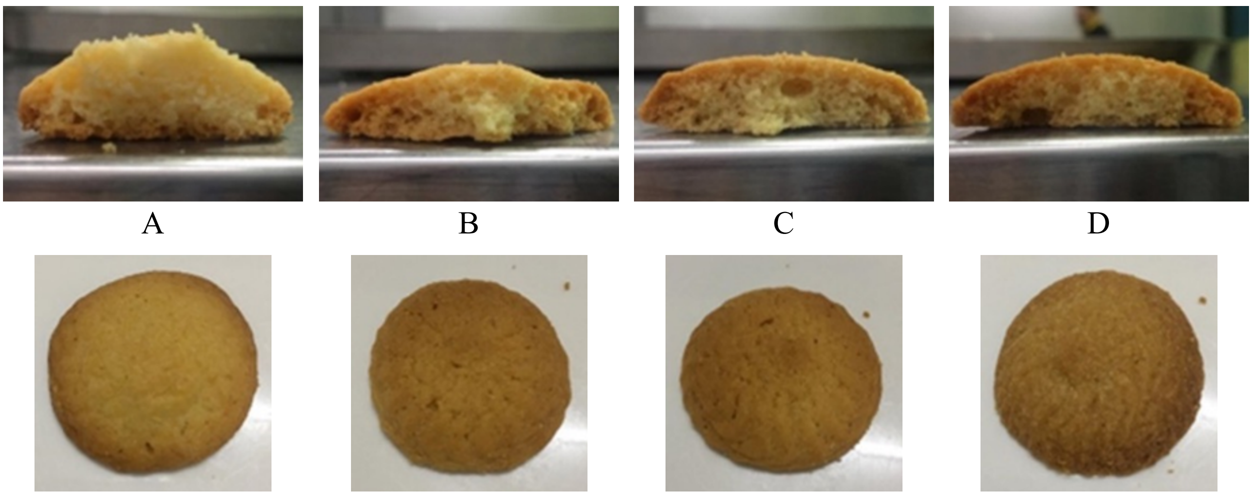
2.5 Statistical Analysis

Experiments were performed in triplicate and values were reported as mean ± SD. Statistical analysis was performed by one-way analysis of variance (ANOVA) using SPSS (version 26, IBM, Armonk, NY, USA). Tukey's HSD test was used to determine the differences between the mean values statistically significant when $p < 0.05$.

3. Results

3.1 Substituted Millet Biscuit Preparation

Substituted butter biscuits with different millet proportions were prepared compared to a biscuit prepared with 100% wheat flour used as the control. The cross-section and top-view of the biscuits were imaged as seen in Figure 1. The control had the highest thickness and smallest width, relative to the significantly smallest spread ratio (Table 2). When millet flour was added, it affected the structure of the biscuits due to its low level of gluten giving a less elastic texture to the dough resulting in denser, stickier, and harder biscuits (Pareyt et al., 2008). Thus, the more millet flour is substituted, the larger spread of biscuits is observed.



A B C D

E F G H

Figure 1: Cross-section of biscuits substituted millet flour at control (A), 10% (B), 20% (C), and 30% (D), and top-view of biscuits substituted millet flour at control (A), 10% (B), 20% (C), and 30% (D).

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3.2 Physical Properties of Substituted Millet Biscuits

The spread ratio is an important factor in determining the behavior of the dough, which reflects to quality characteristics of biscuits. During baking, sugar, and butter were melted making the dough more fluid resulting in the biscuit spread out. Gluten absorbs water to form a matrix that stops the flow of biscuit dough, leading to lower diameters. Thus, increasing millet flour which means reducing gluten in the structure increases width, and decreases thickness, resulting in increasing the spread ratio of the biscuits. The significantly highest spread was found in 30% of substituted biscuits, which was not statistically different from the biscuits of 20%. At 30% of millet flour added did slightly affect the physical characteristics of the biscuits.

Texture results align well with the cross-section pictures of biscuits (Figure A–D). Increased substituted millet flour causes more small holes through the biscuit. The maximum force required to break the biscuits is necessary to observe. Biscuits with the highest substitute (30%) of millet flour showed more fragile behavior, as seen in the lower force applied to break the biscuits. The higher the amount of millet flour replacing part of wheat flour, the lower the gluten content resulting in a lower moisture level, which leads to protein aggregating and hydrating not enough for gluten network formation (Laguna et al., 2011). This leads to the crunchiness of biscuits which may affect consumer preference.

Another important indicator that impacts consumer perception is the surface colour of biscuits, which is expected in golden-brown colour (Laguna et al., 2011). Biscuits containing higher millet flour displayed lower lightness (L^*) values and yellowness (b^*) values but there was no significant difference in redness (a^*) values. This was due to the colour of the millet flour itself, being darker compared to wheat flour, affecting biscuit colour and having lower L^* values. Thermal processing like baking also has a significant impact on decreasing the brightness of biscuits due to the Maillard reaction and reducing sugars-amino acid interaction, forming brown substances or melanoidins (Laguna et al., 2011; Martínez et al., 2021). As the total lipid contents for all formulations were not significantly different, this reduces the effect of fat content on the structure's biscuit between the formulations. Similar results were observed from the bakery products made with gluten-free millet flour (Krishnan et al., 2011; Pessanha et al., 2021).

Table 2: Physical Properties of Substituted Millet Biscuits at Various Proportions.

Parameter		Formulations of Substituted Millet Biscuits			
		Control	10%	20%	30%
Width (mm)		43.74 ± 0.29^{c}	45.31 ± 1.31^{b}	48.92 ± 1.04^{a}	49.23 ± 0.77^{a}
Thickness (mm)		13.08 ± 0.83^{a}	11.78 ± 0.46^{b}	10.17 ± 0.29^{b}	10.00 ± 0.82^{b}
Spread ratio		3.34 ± 0.51^{b}	3.85 ± 0.98^{b}	4.81 ± 1.03^{a}	4.92 ± 0.35^{a}
Break strength (N)		19.93 ± 0.85^{a}	17.18 ± 1.57^{b}	15.19 ± 0.75^{c}	13.71 ± 0.27^{d}
Colour	L^*	72.38 ± 0.75^{a}	71.90 ± 2.79^{a}	66.06 ± 0.11^{b}	62.38 ± 1.43^{c}
	$a^{*\,ns}$	6.88 ± 0.60	6.81 ± 1.54	6.78 ± 0.16	6.68 ± 0.60
	b^*	29.01 ± 0.45^{a}	27.03 ± 0.93^{b}	25.55 ± 0.42^{c}	23.71 ± 1.79^{c}

Data are presented as mean ± SD (n=3), $^{a-d}$ superscripts are significantly different at $p < 0.05$ in a column, and ^{ns} represents not significantly different at $p < 0.05$.

The proximate composition of the biscuits substituted with different millet flour contents is shown in Table 3. As mentioned above, different dough and biscuit properties were affected by gluten levels. Higher gluten levels reduced the relative level of water availability due to its water absorption being equal to its weight, whereas starch granules absorb water at one-third of the weight (Pareyt et al., 2008). Moisture content values decreased when the ratio of millet flour increased, as gluten content decreased. The biscuits elaborated with millet flour at all levels showed no significant differences in protein and total lipid contents. Although theoretically, millet flour has slightly higher protein and lower fat contents compared to those of wheat flour (Balasubramanian et al., 2014). At these proportions, there was no impact on protein and total lipid contents of biscuits. Regarding phytochemicals, millet flour has a slightly higher bioactive content resulting in increased ash compared to the control. In addition, all of the formulated millet biscuits showed much higher values of fibre, and the highest fibre content was observed in 30% substituted biscuits. Dietary fibre intake benefits the gut microbiome, which plays a key role in immune systems and metabolite production (Zhang et al., 2022). Thus, fortifying biscuits with millet flour is a huge interest in utilizing functional crops to enrich the nutritional values of butter biscuits.

Table 3: Proximate Composition (g/100 g) of Substituted Millet Biscuits At Various Proportions.

Component	Formulations of Substituted Millet Biscuits			
	Control	10%	20%	30%
Moisture	3.00±0.07 [c]	2.49±0.22 [c]	2.25±0.55 [b]	1.96±0.60 [a]
Crude protein [ns]	7.01±0.21	6.98±0.02	6.76±0.11	6.65±0.38
Total lipids [ns]	20.50±0.11	19.21±1.09	19.53±0.55	19.67±0.60
Carbohydrate	65.55±0.21 [a]	65.71±0.90 [a]	64.88±0.11 [b]	63.78±0.38 [b]
Ash	0.80±0.01 [d]	0.92±0.02 [c]	1.01±0.03 [b]	1.09±0.02 [a]
Crude fibre	3.14±0.39 [d]	4.68±1.83 [c]	5.61±0.50 [b]	6.84±0.96 [a]

Data are presented as mean ± SD (n=3). [a-d] superscripts are significantly different at $p < 0.05$ in a column, and [ns] represents not significantly different at $p < 0.05$.

A significant increase in total phenolic content in biscuits containing millet flour was observed, relative to the antioxidant activity of the biscuits. %DPPH radical scavenging activity was increased from 0.47 to 5.67 μmol TE/g when the millet flour content increased to 30%. Similarly, the quenching ability of ABTS radical was also enhanced from 1.25 to 13.60 μmol TE/g by adding more millet flour to the biscuits. The more millet flour substitutes, the higher the content of TPC increases. Millet flour is not only high in fibre, but also contains bioactive polyphenolic compounds (Jacob et al., 2024). Furthermore, the Maillard reaction occurs between amino acids and sugar during the baking process, associated with the increment of antioxidant activity, similar to what was found by others (Hussain et al., 2020; Marak et al., 2019; Sharma, Saxena, & Riar, 2016).

Table 4: TPC and Antioxidant Activity of Substituted Millet Biscuits at Various Proportions.

Parameter	Formulations of Substituted Millet Biscuits			
	Control	10%	20%	30%
TPC (mg GAE/g)	1.19±0.08 [c]	1.74±0.04 [c]	3.24±0.01 [b]	4.76±0.16 [a]
DPPH* scavenging activity (μmol TE/g)	0.47±0.02 [d]	2.10±0.04 [c]	3.45±0.11 [b]	5.67±0.08 [a]
ABTS* quenching ability (μmol TE/g)	1.25±0.05 [d]	3.45±0.10 [c]	8.04±0.11 [b]	13.60±1.22 [a]

Data are presented as mean ± SD (n=3). [a-d] superscripts are significantly different at $p < 0.05$ in a column, and [ns] represents not significantly different at $p < 0.05$.

One of the most important requirements for food products is to evaluate consumer acceptability. Participants were asked individually to identify their preferences on the following parameters: appearance, odor, colour, taste, texture (in terms of crunchiness), and overall acceptability. Table 5 indicates the values related to the sensory evaluation of the biscuits. It was shown that millet flour substitution had no significant effect on the odor and colour aspects of biscuits. The colour of biscuits was changed significantly by substituting millet flour as evaluated by the colorimeter, but the biscuit colour was still within a range of consumers' acceptability. Unlikely, the highest score of appearance and taste attributes were observed in the biscuits with the highest millet flour (30%), due to it being recognized as highly palatable (Hama-Ba, 2023). Crunchiness is an important factor in the enjoyment of biscuits (Tunick et al., 2013). Using sensory analysis including biting force and sound intensity can assess the true perception of the crunchiness of the biscuits. The finding showed the biscuit at a 30% level of millet flour had the highest score in crunchiness, which was relative to the highest score of overall acceptability. Thus, the wheat flour substitution with millet flour at a ratio of 30% can be accepted in biscuit production with the highest consumer acceptability.

Table 5: Consumer Rating of Substituted Millet Biscuits at Various Proportions.

Sensory attributes	Formulations of substituted millet biscuits		
	10%	20%	30%
Appearance	6.53±0.73 [b]	6.67±0.66 [b]	7.23±0.72 [a]
Odor [ns]	6.73±0.78	6.80±0.71	6.97±0.76
Colour [ns]	6.53±0.62	6.67 ±0.71	6.87±0.72
Taste	6.50±0.77 [b]	6.83±0.74 [b]	7.37±0.61 [a]
Crunchiness	6.53±0.68 [b]	7.09±0.62 [a]	7.27±0.63 [a]
Overall acceptability	6.70±0.74 [b]	6.93±0.69 [b]	7.40±0.72 [a]

Data are presented as mean ± SD (n=3). [a-b] superscripts are significantly different at $p < 0.05$ in a column, and [ns] represents not significantly different at $p < 0.05$.

4. Conclusion

This research provides beneficial knowledge to support the substitution of gluten-free millet flour for wheat flour by enhancing the nutritional properties and functional aspects of biscuits. The results revealed that with the highest level of millet flour in which decreased gluten amounts, biscuit diameter increased, related to increased spread factor. In addition, the biscuit colour turned darker, and the texture was easy to break. The contents of fibre and ash were enhanced in the biscuits with the millet incorporation, similar to total phenolic content, DPPH, and ABTS results indicating a significant increase in antioxidant activity by increasing the level of millet flour in biscuit formulas. Furthermore, the consumer evaluation suggested that the substitution of millet flour at a high level up to 30% of butter biscuit production did not adversely affect its sensory attributes. Thus, this research confirmed the potential of utilizing millet flour as a functional ingredient for the production of butter biscuits.

Conflict of Interest

The authors declare no conflict of interest in this research concerning research and authorship.

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