

RESEARCH ARTICLE

EVALUATION OF THE NUTRITIONAL, FUNCTIONAL, AND GLYCEMIC PROPERTIES OF COMMERCIALY AVAILABLE WHITE, FINGER MILLET, AND MULTIGRAIN BREADS IN SRI LANKA

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ABSTRACT

With the ongoing increase in metabolic syndrome cases worldwide and the importance of carbohydrate quality for glycemic control, assessing the nutritional and functional qualities of commonly consumed staple foods is of great importance. This study assessed the nutritional composition, glycemic index (GI), functional properties and overall loaf suitability of three commercially available bread types: white, finger millet and multigrain bread for managing metabolic syndrome. While the nutritional parameters were measured using standard AOAC methods, the Folin-Ciocalteu reagent method and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay were used to analyse the total phenolic content (TPC) and antioxidant activity of breads, respectively. Eighteen non-diabetic, healthy participants with an average BMI of  $21.2 \pm 1.7 \text{ kg/m}^2$  underwent GI studies in accordance with ISO 26642:2010 protocols. The studies were conducted after a 10-12-hour fast, measuring the blood sugar response over 2 hours from the time of consumption of bread samples containing 50 g of available carbohydrates. The results indicated that the three breads differed significantly ( $P < 0.05$ ) in specific nutritional properties, including fat, protein, and dietary fiber. White bread, prepared from refined wheat flour, showed the lowest fat ( $2.40 \pm 0.14\%$ ) and ash content ( $1.30 \pm 0.14\%$ ) along with the highest protein content ( $8.10 \pm 0.14\%$ ). Conversely, finger millet bread and multigrain bread contained  $7.20 \pm 0.14\%$  and  $5.40 \pm 0.00\%$  protein and  $3.20 \pm 0.14\%$  and  $4.80 \pm 0.14\%$  fat, respectively. No significant difference ( $P > 0.05$ ) was observed in dietary fiber content between multigrain bread ( $3.40 \pm 0.14\%$ ) and finger millet bread ( $3.10 \pm 0.14\%$ ); however, both were significantly higher ( $P < 0.05$ ) than white bread ( $2.10 \pm 0.14\%$ ). The antioxidant activities were  $0.13 \pm 0.04\%$ ,  $10.09 \pm 0.10\%$  and  $7.76 \pm 0.46\%$  for white, finger millet and multigrain bread, respectively. The TPC was  $3.05 \pm 0.39 \text{ mg GAE/g}$  for white bread,  $3.53 \pm 0.05 \text{ mg GAE/g}$  for finger millet bread and  $4.41 \pm 0.00 \text{ mg GAE/g}$  for multigrain bread, indicating that it was significantly higher ( $P < 0.05$ ) in multigrain bread. Glycemic Index for white, finger millet and multigrain bread were  $63.93 \pm 8.14$  (medium-GI),  $53.50 \pm 4.81$  (low-GI) and  $45.78 \pm 4.14$  (low-GI), respectively. In conclusion, these findings suggest that finger millet and multigrain breads have the potential to serve as healthier bread options for glycemic control and metabolic health in the general population, owing to their lower glycemic impact and higher antioxidant and phenolic content.

Keywords: Glycemic index, Low-GI bread, Antioxidant activity, Total phenolic content, Functional properties, Blood sugar response

INTRODUCTION

Metabolic syndrome is an increasingly prevalent health concern that is associated with several abnormalities, for instance, visceral obesity, fatty liver, insulin resistance, hypertension and cardiovascular diseases (Saklayen, 2018; Noubiap *et al.*, 2022).

Many risk factors are linked with this condition, including lifestyle habits, poor dietary choices, smoking and atherosclerosis (Mohamed *et al.*, 2023). Thus, dietary management plays a pivotal role in the control and inhibition of metabolic syndrome. A low-GI (Glycemic Index) diet has a significant impact on reducing

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conditions such as type 2 diabetes and cardiovascular diseases, which are the core components of metabolic syndrome.

In recent years, the concept of GI has gained vast popularity due to GI labelling and its relevance in the development of dietary guidelines. It measures the blood glucose level after consumption of a food, compared with a reference food, namely white bread or a standard glucose solution containing 50 g of available carbohydrates (Jenkins *et al.*, 2002; Singhania and Sen Ray, 2012). Numerous factors affect the GI of food, including fiber content, fat content, type of sugar present, starch digestibility, protein-starch interactions, and processing conditions (Foster-Powell *et al.*, 2002; Nayak *et al.*, 2014).

Bread is a staple in many diets and contributes substantially to complex-carbohydrate intake. It contains nutrients such as carbohydrates, lipids, protein, vitamins (B vitamins and vitamin E mainly), minerals and trace elements, and can be identified as an inexpensive source of energy (Aghalari *et al.*, 2022). Throughout time, the structure, texture, and composition of bread have evolved to meet the ever-changing needs of consumers. In 2019, the average bread consumption worldwide was reported at 24.5 kg per person per year, and the bread market is projected to record an average per capita consumption of 25.5 kg in 2025, indicating slight growth over recent years (Statista, 2025).

Given bread's basic nature as a food, many attempts have been made to prepare variations to enhance its palatability and nutritional value. Nonetheless, the most common ingredient used to prepare bread remains wheat flour. As a result of its availability, convenience and affordability, it has gained popularity in developing countries, especially in the Asian and African regions. Thus, white bread is the most consumed bread globally. Compared to other types of bread, white bread has low nutritional value due to its low fiber and micronutrient content. Its GI usually varies

from medium to high, which may not be ideal for individuals with metabolic syndrome.

In addition to white bread, a wide range of breads can be found worldwide. Bread varieties with certain functional attributes and health benefits, such as gluten-free bread for coeliac disease and low-calorie bread for obesity management, are gaining consumer attraction. The growing popularity of whole-grain and bran-rich breads is largely attributed to their high dietary fiber content. It can help reduce the risks of cardiovascular disease, colon cancer, and diabetes (Okarter and Liu, 2010).

Another type of bread made with a mixture of grains, seeds and legumes, such as wheat, corn, barley, oats, kidney beans, green gram and other millets, is termed multigrain bread. It can offer a diverse nutrient profile, along with various textures and flavours. The addition of millets, such as finger millet, to bread formulations has drawn interest due to the potential health benefits. The dietary fiber, proteins and essential minerals present make it a desirable ingredient for bread products (Bultosa, 2019).

Studies on the glycemic effects of foods, particularly staples in many diets such as bread and rice, are important for people with medical conditions such as diabetes, abnormal blood glucose regulation, and hypoglycemia. It can help those people avoid foods that can cause a greater rise or a sudden fall in blood glucose levels. Meanwhile, the general population can benefit from preventing such conditions by adopting healthy eating habits and choosing appropriate portion sizes.

In addition to GI, the nutritional quality of bread is influenced by bioactive compounds, such as antioxidants and polyphenols. These compounds can offer protective health benefits by reducing oxidative stress and inflammation. Finger millet, in particular, is recognized for its high antioxidant and polyphenol content, which may contribute to improved metabolic health (Chandra *et al.*,

2016). Measuring these properties in bread products provides a more comprehensive assessment of their functional potential and supports health-based product development for the management of metabolic syndrome.

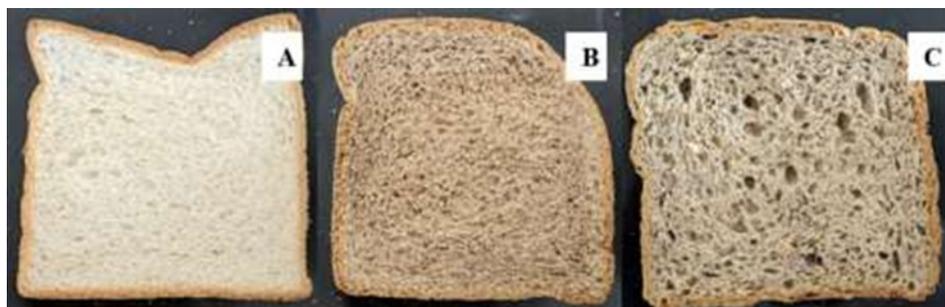
Although novel formulations are continually introduced as healthier alternatives, their health claims must be supported by robust evidence and comply with nutritional labelling standards. Particularly in the Sri Lankan context, there is limited research on determining the GI of different bread formulations. Thus, this research aimed to investigate the *in vivo* GI, total polyphenol content, and antioxidant activity of three commercially available bread types: white bread, finger millet bread, and multigrain bread, to assess their nutritional quality and potential health benefits. This research is novel in combining GI measurement with bioactive compound analysis of breads available in Sri Lanka, thereby addressing a clear

knowledge gap in the local food and nutrition literature.

## MATERIALS AND METHODS

### Preparation of bread samples

The three commercially available bread types investigated in this study, namely white bread, finger millet bread and multigrain bread, are illustrated in Figure 1. Finger millet and Multigrain breads were prepared using the commercially available Kurakkan Bread Mix and Multi-Grain Bread Mix manufactured by AB Mauri Lanka (Pvt) Ltd, Sri Lanka, as per the instructions provided on their labels. The multigrain bread mix contained ingredients such as rice flakes, soybeans, red kidney beans, green gram, corn, lily seeds and white kidney beans. Furthermore, the ingredients used to prepare white bread (low-sugar yeast, fat, dough improver, calcium propionate, and bread softener) were also obtained from the same company. White breads were prepared using standardized formulations to ensure consistency and uniformity throughout the study.



**Figure 1: White bread (A), Finger millet bread (B), and Multigrain bread (C)**

### Determination of nutritional composition

The moisture, total carbohydrates, fat, Protein, ash and dietary fiber of each bread type were determined according to standardized AOAC methods (moisture: AOAC 925.10; protein: AOAC 991.20; fat: AOAC 991.36; ash: AOAC 920.153; total dietary fiber: AOAC 985.29; total carbohydrates were calculated by difference) (AOAC International, 2016). Available carbohydrates and energy were determined in accordance with the Codex Guidelines on Nutrition Labelling for all three bread types. The available carbohydrate content was calculated using the subtraction method,

in which dietary fiber was deducted from the total carbohydrate content. Subsequently, the energy value (kcal per 100 g) was estimated using the Atwater general factors, which account for the energy contributions of macronutrients (Capuano *et al.*, 2018).

Additionally, the salt content (as NaCl) was determined using AOAC 17th Edition (2000). The fat fraction of each type of bread, including saturated fat, monounsaturated fat, polyunsaturated fat and trans fat, was calculated using AOAC 996.06. The cholesterol content was determined using GC-FID (Gas Chromatography with Flame

Ionization Detector) and HPLC-DAD (High Performance Liquid Chromatography-Diode Array Detector). For GC-FID, a temperature gradient from 200°C to 300°C at 10°C/min was applied to separate cholesterol, which was detected with a flame ionization detector (Islam and Chun, 2024). For HPLC-DAD, cholesterol was separated on a reversed-phase C18 column using an acetonitrile: methanol mobile phase and detected at 210 nm with a diode array detector (Albuquerque *et al.*, 2016). Sodium content was analyzed by employing AOAC 2011.14.

### Study subjects

Eighteen healthy non-diabetic volunteers, aged 23–28 years, with a body mass index (BMI) of  $21.2 \pm 1.7 \text{ kg/m}^2$ , participated in the glycemic response detection trials. The sample size of 18 was chosen based on guidelines from the FAO/WHO and previous glycemic index studies, which recommend 10–20 participants per test food to obtain reliable *in vivo* GI measurements (FAO/WHO, 1998; Brouns *et al.*, 2005). Exclusion criteria included individuals with allergies or intolerance to any bread ingredient, smokers and those with diabetes, a family history of diabetes, gastrointestinal disease, or cardiovascular disease (Torres *et al.*, 2024). A screening questionnaire was used during recruitment to confirm the above criteria and to collect demographic information, medical history, current medications, and usual dietary habits. Approval for the human trials was sought from the Ethical Clearance Committee, Faculty of Agriculture, University of Peradeniya (Ethical clearance number: ECC/2024/E/025). All participants were provided with detailed information about the study's objectives and methodology in an information sheet before signing a written consent form.

### *In vivo* glycemic index testing

The glycemic index of each bread type was determined following the standard FAO/WHO procedure (FAO/WHO, 1998). Blood glucose levels were obtained on four separate dates for a reference glucose solution and the three bread types. Participants underwent a 10-hour overnight fast and avoided alcohol and

extreme physical exercise before the GI studies. A 100% pure, commercially available glucose powder was used to prepare the standard glucose solution.

During the following weeks, participants were provided with 87.72 g of white bread, 95.78 g of multigrain bread, and 107.99 g of finger millet bread, each portion standardized to deliver 50 g of available carbohydrates, along with 200 mL of purified water. These portion sizes were calculated based on the available carbohydrate content of each bread type, determined by proximate analysis, with dietary fiber subtracted. For example, white bread contained 57 g of available carbohydrates per 100 g. Thus, 87.72 g of white bread provided 50 g of available carbohydrates. Portions for multigrain and finger millet bread were calculated similarly using their respective available carbohydrate contents. This ensured a comparison of glycemic responses using the same amount of available carbohydrates. Participants were given approximately 5–10 minutes to consume each bread portion and drink 200 mL of water.

Finger-prick blood samples were collected using a lancing device (Mega Check TD 5742) before the meal (zero time) and at 15, 30, 45, 60, 90, and 120 minutes after starting to eat the bread samples. The incremental area under the curve (iAUC) for blood glucose was calculated above the fasting (zero-time) glucose concentration, considering only the area above the baseline and excluding any area below it. The GI was then determined using Equation 1. The glycemic load (GL) of each bread sample was subsequently calculated based on the available carbohydrate content in relation to the serving size, as described in Equation 2.

$$GI = \frac{iAUC_{\text{bread}}}{iAUC_{\text{glucose}}} \times 100$$

Eq. 1

$$GL = \frac{GI \text{ of bread} \times \text{Carbohydrates}}{100}$$

Eq. 2

Where; GI: glycemic index of bread, Carbohydrates: Available carbohydrates (g) by serving size, GL: glycemic load, and iAUC = incremental area under the glucose curve

Data were expressed as mean  $\pm$  standard error, and GI values of individuals within  $\pm 2$  standard deviations were considered for the final GI values. The GI classification was determined as follows: low GI ( $\leq 55$ ), medium GI (56–69), and high GI ( $\geq 70$ ) (FAO/WHO, 1998; Somaratne *et al.*, 2017).

### Preparation of bread extract

Bread extracts for TPC and antioxidant activity assessment were prepared following the method described by Michalska *et al.*, 2007 and Wahyono *et al.*, 2020 with slight adjustments. Bread slices with approximately 1 cm thickness were dried at 40 °C for 5–6 hours and then ground. Five grams of the ground sample from each bread type was accurately weighed and extracted with 25 ml of 80% methanol. Next, the samples were placed on an orbital shaker at 100 rpm for 3 hours. Then each mixture was centrifuged at 4000 rpm for 20 minutes. The supernatant was filtered and kept in amber-colored glass bottles at 4 °C for further studies.

### Determination of total phenolic content

The total phenolic content (TPC) of each bread type was measured using the method described by Wahyono *et al.* (2020) with slight alterations. From previously prepared extracts, 1 ml was transferred to test tubes and diluted 10-fold. Next, 2.5 ml of 10% Folin–Ciocalteu reagent was added to the test tubes and incubated for 10 minutes. Then, 0.8 ml of 2.5% Na<sub>2</sub>CO<sub>3</sub> was added and vortexed. The solutions were incubated at room temperature for 1 hour to develop colour. A blank solution was prepared by substituting the extract with distilled water and processed under identical conditions. The absorbance readings were recorded at 760 nm. A gallic acid standard curve was used to determine the TPC of bread types. The results were expressed as mg GAE (Gallic Acid Equivalent) per gram of dry bread sample.

### Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The antioxidant potential of the samples was determined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Shahidi *et al.*, 2006; Somaratne *et al.*, 2017). A stock solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared by dissolving 4 mg of DPPH powder in 100 ml of 100% methanol. The prepared stock solution was incubated for 3 hours at 4 °C. To each test tube, 1 mL of extract from each bread type was combined with 3 mL of DPPH stock solution, and the mixture was incubated at room temperature for 30 minutes. A blank solution was prepared using 100% methanol. Absorbance values were obtained at 515 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The percentage of DPPH radical scavenging activity was calculated using the following equation 03. The resulting antioxidant activity was quantified by reference to an ascorbic acid standard curve and expressed as micrograms of ascorbic acid equivalents ( $\mu\text{g}$  AAE) per gram of dry bread sample.

$$\% \text{ DPPH Scavenging} = \frac{(C - S)}{C} \times 100\%$$

Eq. 3

Where; C: A Control, and S: A Sample

### Statistical analysis

Data were analysed using Microsoft Excel (2019), SAS (version 9.4), and Minitab (version 22.1.0) to ensure accurate interpretation. One-way Analysis of Variance (ANOVA) was employed to evaluate the significance of differences among bread types in GI, antioxidant activity, and TPC. Tukey's Honest Significant Difference (HSD) test was performed to separate means when significant differences were detected. For all statistical tests, differences were considered statistically significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Nutritional composition

The nutritional composition of white, finger millet and multigrain bread is presented in Table 1. According to the results, three types of bread differed significantly ( $P < 0.05$ ) in certain nutritional properties. Finger millet bread had the highest moisture content among the bread samples, significantly higher ( $P < 0.05$ ) than both multigrain bread and white bread. White bread exhibited the lowest moisture ( $29.10 \pm 0.14\%$ ), fat ( $2.40 \pm 0.14\%$ ) and ash content ( $1.30 \pm 0.14\%$ ) along with the highest protein content ( $8.10 \pm 0.14\%$ ). Finger millet bread and multigrain bread contained  $7.20 \pm 0.14\%$  and  $5.40 \pm 0.00\%$  protein and  $3.20 \pm 0.14\%$  and  $4.80 \pm 0.14\%$  fat, respectively, which were significantly different ( $P < 0.05$ ) from white bread. Dietary fiber content was not significantly different ( $P > 0.05$ ) between

multigrain bread ( $3.40 \pm 0.14\%$ ) and finger millet bread ( $3.10 \pm 0.14\%$ ); yet it was significantly higher ( $P < 0.05$ ) than that of white bread ( $2.10 \pm 0.14\%$ ). The ash content also followed a similar pattern. Among the breads analyzed, white bread had the highest total and available carbohydrate content, which correlated with its elevated energy value. In contrast, finger millet bread showed the least total carbohydrate, available carbohydrates and energy values. Thus, it was observed that, in the presence of other grain-based flours, the carbohydrate content declined, aligning with results from previous research (Devani *et al.*, 2016). Similarly, the protein content was also reduced. Incorporation of finger millet flour can increase the fiber and decrease the carbohydrate content. Ash content and protein also vary accordingly (Rajiv *et al.*, 2011).

**Table 1: Comparative nutritional composition of selected bread types**

Parameter	Unit	White Bread	Finger Millet Bread	Multigrain Bread
Moisture	g/100 g	$29.10 \pm 0.14^c$	$37.80 \pm 0.14^a$	$32.25 \pm 0.21^b$
Total Carbohydrates	g/100 g	$59.10 \pm 0.11^a$	$49.40 \pm 0.10^b$	$55.60 \pm 0.20^c$
Fat	g/100 g	$2.40 \pm 0.14^c$	$3.20 \pm 0.14^b$	$4.80 \pm 0.14^a$
Protein	g/100 g	$8.10 \pm 0.14^a$	$7.20 \pm 0.14^b$	$5.40 \pm 0.00^c$
Dietary Fiber	g/100 g	$2.10 \pm 0.14^b$	$3.10 \pm 0.14^a$	$3.40 \pm 0.14^a$
Ash	g/100 g	$1.30 \pm 0.14^b$	$2.40 \pm 0.14^a$	$1.90 \pm 0.14^a$
Available Carbohydrates	g/100 g	$57.00 \pm 0.14^a$	$46.30 \pm 0.14^c$	$52.20 \pm 0.14^b$
Salt (as NaCl)	g/100 g	$0.71 \pm 0.00^b$	$0.71 \pm 0.01^b$	$1.02 \pm 0.01^a$
Saturated Fat	g/100 g	$1.21 \pm 0.01^b$	$1.37 \pm 0.01^b$	$2.20 \pm 0.08^a$
Trans Fat	g/100 g	ND (LOQ= 0.01)	ND (LOQ= 0.01)	ND (LOQ= 0.01)
Mono-unsaturated Fat	g/100 g	$0.56 \pm 0.03^b$	$1.18 \pm 0.03^a$	$1.27 \pm 0.03^a$
Polyunsaturated Fat	g/100 g	$0.63 \pm 0.01^b$	$0.65 \pm 0.01^b$	$1.32 \pm 0.01^a$
Cholesterol	mg/100 g	ND (LOQ= 0.01)	ND (LOQ= 0.01)	ND (LOQ= 0.01)
Sodium (as Na)	mg/100 g	$431.14 \pm 4.36^b$	$397.27 \pm 5.85^c$	$481.92 \pm 7.52^a$
Energy	kcal/100 g	282.0	242.8	273.6
Energy	kJ/100 g	1195.5	1027.9	1156.8

Data presented as mean  $\pm$  standard deviation. Values with different letters across sample types are significantly different ( $P < 0.05$ ). ND = Not Detected; LOQ = Limit of Quantification (0.01). "ND (LOQ = 0.01)" indicates that the compound was not detected at or above the quantification limit of 0.01.

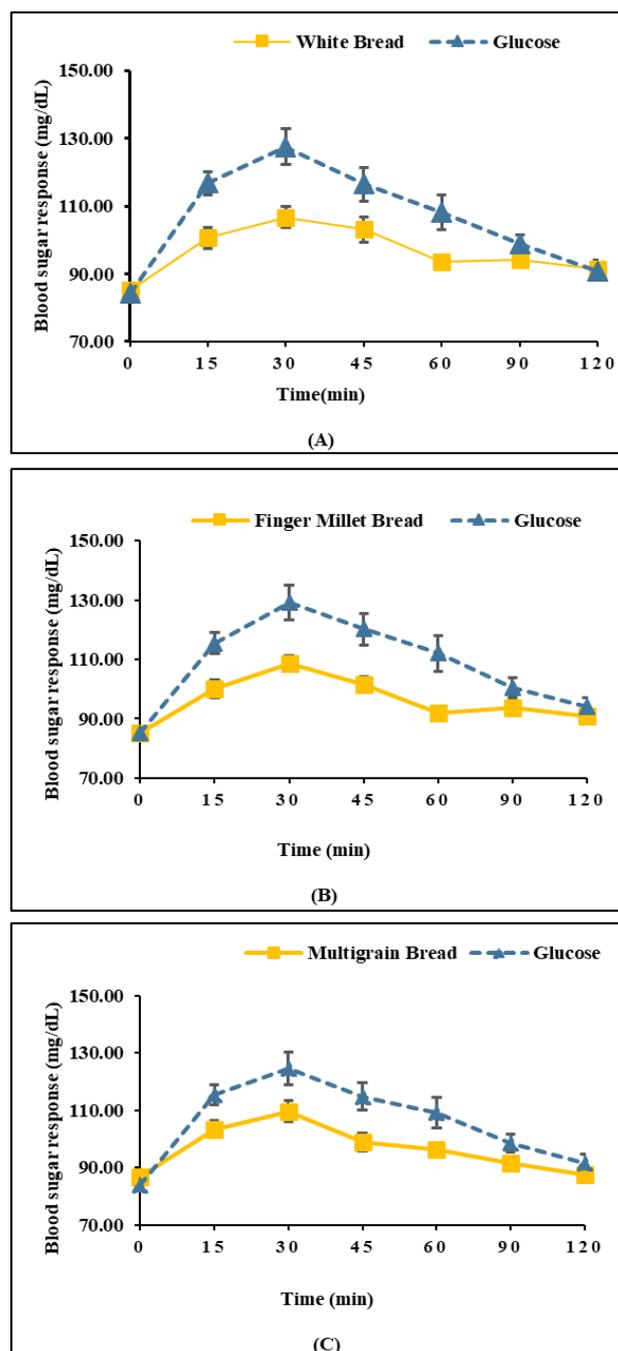
Finger millet bread and multi-grain bread differ significantly ( $P < 0.05$ ) in lipid profile and sodium levels compared to traditional white bread. However, these levels can be affected by ingredient choices and processing methods. Finger millet bread is lower in cholesterol, saturated fat, sodium, and trans-fat. It is rich in beneficial unsaturated fatty

acids, hence an excellent choice for cardiovascular and metabolic health. The saturated fatty acid content is around 1.3 g/100 g. The polyunsaturated and monounsaturated fatty acid content ranges around 2.5 g/100 g and 0.7 g/100 g, respectively, according to previous findings (Srivastava and Sureka, 2025). The current

research aligns with these results. Both finger millet and multi-grain bread, when made without processed fat, contain negligible trans-fat and cholesterol. This makes them suitable choices for diabetic management, heart health, and overall wellness. In finger millet bread, the salt content was controlled, and it exhibited lower sodium levels than multigrain bread. Multi-grain breads vary based on formulation. Many commercially available types tend to have higher sodium (300–400 mg/100 g) and saturated fat content, though they are still better than white bread.

### Glycemic index and glycemic load

Determining GI and GL can provide insights into the potential health benefits of food and their role in modulating blood glucose levels. Glycemic index values for food range from 0 to 100. The categorization of food based on the GI values is as follows: high GI food (GI > 70) moderate GI food (GI 55 - 70) and low GI food (GI < 55). The final GI values for white bread, finger millet bread and multigrain bread were  $63.93 \pm 8.14$ ,  $53.50 \pm 4.81$ , and  $45.78 \pm 4.14$ , respectively (Table 2). Thus, both finger millet and multigrain breads were classified as low- GI breads compared to the medium-GI white bread. The blood sugar response over 2 hours during the GI testing is indicated in Figure 2. The peak blood sugar response was observed at 30 min for all three bread types. The GI values were not significantly different ( $P > 0.05$ ) across bread types. According to Sri Lankan food-based dietary guidelines, one serving of bread is approximately 30 g, while 8–13 servings of starchy food are recommended per day. Therefore, the GL was calculated for one serving of each type of bread (1 slice – 30 g). The GL values were 10.93, 7.43, and 7.17 for white, finger millet, and multigrain bread, respectively (Table 2). Hence, white bread was classified as medium GL, whereas finger millet and multigrain breads were low-GL.



**Figure 2: Mean ( $\pm$  SE) change in blood glucose level (mg/dL) over 2 hours following the intake of white bread (A), finger millet bread (B) and multigrain bread (C)**

**Table 2: Glycemic index and glycemic load of breads**

Type of Bread	GI $\pm$ SE	Classification	GL	Classification
White Bread	$63.93 \pm 8.14$	Medium-GI	10.93	Medium-GL
Finger Millet Bread	$53.50 \pm 4.81$	Low-GI	7.43	Low-GL
Multigrain Bread	$45.78 \pm 4.14$	Low-GI	7.17	Low-GL

According to the World Health Organization (WHO), as of 2022, cases of diabetes were reported to be 14% among individuals aged over 18. In 2021, the number of deaths caused directly by diabetes was 1.6 million. Of those, 47% were people below the age of 70. A comprehensive survey encompassing 195 countries in 2015 reported that nearly 604 million adults and 108 million children were classified as obese (Saklayen, 2018). These incidents are reported to have a parallel connection with metabolic syndrome, where it is vital to take necessary actions against this growing health concern.

In relation to the GI of food, low-GI foods are receiving heightened attention for their role in reducing the risk of chronic diseases, including type 2 diabetes and cardiovascular conditions. Thus, the development of multigrain bread, finger millet bread and variations of bakery products is important. Foods with higher whole-grain content and lower GI are often linked to the prevention and management of metabolic syndrome and diabetes (Chlup *et al.*, 2004).

The comparatively lower GI observed in multigrain bread is likely due to its higher levels of resistant starch and dietary fiber than in refined wheat-based products. (Chauhan *et al.*, 2017). According to previous research, food products made from multigrain and wheat grains generally range between 44 and 81. This variation can be attributed to the type of grain used, its gluten content and processing methods. A study that included multigrain roti reported GI values ranging from 55 to 62 (Nagaraju *et al.*, 2020). Thus, in addition to bread, wholegrain flour can be a great option for developing pizza, noodles, biscuits, and similar products.

Dietitians often recommend diets rich in millet to diversify refined-flour-based foods within balanced dietary patterns. Incorporation of millets can enhance the functional formulations of bakery products by providing a balanced amino acid composition, antioxidant activity and protein content. Moreover, finger millet is a good source of fiber and minerals, and its

incorporation into the diet has been associated with lower plasma glucose levels and fewer sharp postprandial glucose peaks compared with wheat-based products. (Geetha *et al.*, 2020; Lakshmi Kumari and Sumathi, 2002).

The GI of white bread is usually taken as the standard reference in most test studies. The values were reported between 70 and 100 in most investigations (Chlup *et al.*, 2004; SRV *et al.*, 2022). However, in this analysis, it was classified as around 63, making it a medium-GI product. The possible reasons for this result are high protein levels, which can slow gastric emptying and starch hydrolysis, thereby lowering the postprandial glycemic response (Fardet, 2010; Nilsson *et al.*, 2004). Other possible reasons may include recipe variations, starch retrogradation, and baking and fermentation, which are known to increase resistant starch and lower glycemic response (Fardet, 2010; Livesey *et al.*, 2008).

However, research indicates that preparation steps and storage conditions can modify the glycemic response of foods (Burton and Lightowler, 2008). This questions the accuracy of studies that use white bread as the reference food. Since the conditions related to formulation, preparation, cooking, and storage vary at the commercial scale, comparisons of GI values derived from white bread as a reference standard can be inconsistent. The GI values between participants showed wide significant variability ( $P > 0.05$ ). Variability among individuals within a specific bread type can be due to factors such as glucose metabolism, gastric sensitivity, and gut microbiota composition (Wolever, 2004). Diversity in these factors can accordingly alter individuals' glycemic responses.

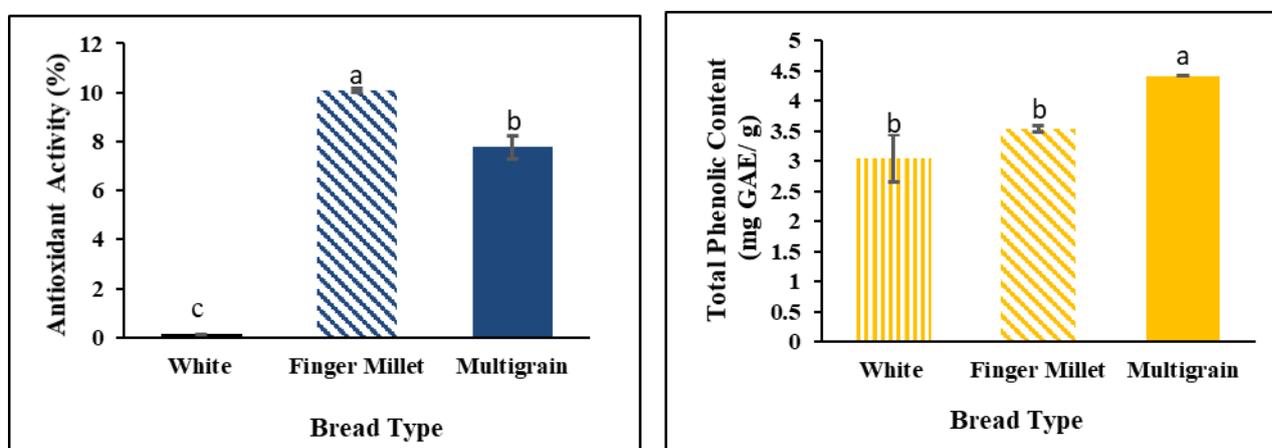
The gut microbiota composition of individuals can affect the capacity to ferment carbohydrates in food, leading to varying glucose absorption rates (Nagaraju *et al.*, 2020). Another critical determinant of the postprandial glycemic response is the gastric emptying rate of everyone (Arunachala *et al.*, 2023). Faster gastric emptying results in a rapid influx of glucose into the small intestine, thereby leading to a higher glycemic

response. (Goyal *et al.*, 2019). Additionally, people have different metabolic responses based on age, genetics, insulin sensitivity, gut microbiota and physical activity levels.

### Antioxidant activity and total phenolic content

As per the DPPH assay, the antioxidant percentages were  $0.13 \pm 0.04\%$ ,  $10.09 \pm 0.10\%$ , and  $7.76 \pm 0.46\%$  for white, finger millet, and multigrain bread, respectively (Figure 3). The percentages differed significantly by bread type ( $P < 0.05$ ). Using the ascorbic acid standard curve for white bread, the antioxidant activity was not detected within the tested range, indicating minimal activity

and therefore cannot be considered significant. This is most likely attributed to the fact that refined wheat flour used to make white bread has already had the bran and germ removed. This is where all the natural phenolics, vitamins such as vitamin E, and other antioxidants are found in high concentration (Zhou *et al.*, 2020; Adom and Liu, 2002). For finger millet bread, the reported AAE was  $15.25 \mu\text{g AAE/g}$ , and for multigrain bread,  $4.4 \mu\text{g AAE/g}$ . The TPC was  $3.05 \pm 0.39 \text{ mg GAE/g}$  for white bread,  $3.53 \pm 0.05 \text{ mg GAE/g}$  for finger millet bread, and  $4.41 \pm 0.00 \text{ mg GAE/g}$  for multigrain bread (Figure 3). Hence, TPC was significantly higher ( $P < 0.05$ ) in multigrain bread.



**Figure 3: Antioxidant activity (%) and total phenolic content of white, finger millet, and multigrain breads.**

Data are expressed as mean  $\pm$  standard deviation (SD). Bars with different letters denote significant differences among bread types ( $P < 0.05$ ).

Multigrain bread is rich in antioxidant compounds such as phenolic acids, flavonoids, phytic acid and vitamin E. Ferulic acid and p-coumaric acid are the major phenolic compounds present in multigrain breads, contributing substantially to their antioxidant activity (Angioloni and Collar, 2011). These compounds are rich in the bran and germ layers of various grains. The bread-making process can further enhance the release and availability of these acids. Moreover, breads made from whole wheat flour tend to have a greater amount of ferulic acid when compared to those made from refined flour. The values can range from 2.10 to 2.35 mg Ferulic Acid Equivalents (FAE)/g for whole wheat-based products, depending

on the specific grain used and the processing conditions (Yu *et al.*, 2013).

Incorporation of various types of grains enhances the functional attributes of multigrain bread. For example, red kidney beans have been reported to enhance the overall antioxidant activity of bakery products upon addition (Olagunju *et al.*, 2021; Roy *et al.*, 2020). Furthermore, soybeans provide isoflavonoids, which have good antioxidant properties (Mohammed Ali *et al.*, 2024). They can also enhance the protein and ash content of the final product, thereby improving the overall nutritional profile.

Finger millet or ragi is very well known for its high content of phenolic compounds in the form of flavonoids and other phenolic acids, which are also accountable for its antioxidant activity. The seed coat of millet is particularly rich in polyphenols, which possess both antioxidant and antimicrobial activities. The quantity and composition of these compounds, however, can vary depending on the millet variety and growth conditions. Generally, dark-colored millet varieties contain higher levels of phenolic compounds compared to light-colored types. (Xiang *et al.*, 2019).

Depending on the methodology used to detect the TPC and antioxidant value, variations in results can be observed. Thus, the use of different standards, such as the Gallic acid standard curve and the ascorbic acid standard curve, can introduce variability compared to previous research findings. Furthermore, high baking temperatures and the use of refined flour can further reduce the antioxidant content, leading to variations in commercially available products. Yet still, the finger millet and multigrain breads provide significantly higher ( $P < 0.05$ ) values compared to white bread in terms of antioxidant activity and TPC.

## CONCLUSION

In risk groups and patients with metabolic syndrome, the choice of healthier bread varieties could be notably important for regulating blood glucose concentrations, lipid profiles, and even abdominal adiposity. Results from the current study show that multigrain and finger millet breads are lower-GI and lower-GL bakery products and may better regulate postprandial blood sugar levels than wheat-based white bread. Moreover, these breads showed relatively higher levels of antioxidant activity and total phenolics, indicating their potential health benefits in managing and even preventing the development of metabolic syndrome. However, the current study is limited in terms of a relatively small sample size, the absence of a randomized crossover design and possible variations arising from commercial baking conditions. Thus, future research is

needed to examine long-term regulation of glycemic response and to formulate approaches to improve the nutritional and health benefits of such bakery products.

## AUTHOR CONTRIBUTION

GMS and BDRP conceptualized the research project, designed the experiment, and provided supervision and manuscript editing. TMPMT conducted the laboratory experiments, collected and analyzed the data, and prepared the original manuscript. MRMPJ and SADAD provided manuscript editing and supervision.

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