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DECREASING THE GLYCEMIC INDEX OF WHITE BREAD USING FINGER MILLET FLOUR AND ITS EFFECT ON POSTPRANDIAL BLOOD GLUCOSE RESPONSE ON WISTAR RATS

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ABSTRACT

Low glycemic index (GI) diets have been recommended for the prevention and treatment of chronic diseases including diabetes, obesity, cancer and cardiovascular diseases. One of the high glycemic index diets consumed daily by many people around the world is the white bread, which is rich in carbohydrates. This study aimed at reducing the glycemic index of white bread by modifying the recipe with finger millet flour and to determine its effect on postprandial blood glucose response on Wister rats. Rats were grouped into five groups (n=5); group I normal control rats feed with white bread only (PC), group II fed with bread made from 15% finger millet composite flour (15%), group III fed with bread made from composite flour of 30% finger millet (30%), group IV fed with standard diabetic bread (commercially available) and group V rats were fed with standard commercial rat feed (NC). The rats were fasted overnight and fed with the bread samples. Blood glucose levels of all the rats were measured using glucometer by pricking the tail vein, at intervals of 0, 30, 60, 90 and 120 mins. From the result obtained, 30% showed lower postprandial blood glucose responses when compared to PC, 15% and NC group ($p \geq 0.05$) and the AUC result showed increased clearance of blood glucose in 30% group when compared with the PC, 15% and NC group. In conclusion, decreasing the glycemic index of white bread with finger millet composite flour can reduce postprandial blood glucose level and also increase the clearance of blood glucose from circulation.

Keywords: Blood Glucose, Finger Millet, Glycemic response, White Bread

INTRODUCTION

The concept of a glycemic index was developed to provide a numeric classification of carbohydrate foods, so that the data would be useful in situations where glucose tolerance is impaired (Jenkins *et al*., 2002). The glycemic index concept was an extension of the dietary fiber hypothesis of Burkitt and Trowell (Burkitt and Trowell, 1977), that postulated about foods that are more slowly absorbed which may have metabolic benefits in relation to diabetes and to the reduction of coronary heart disease (CHD) risk. Glycemic index is the benchmarking of the glycemic response of a fixed amount of available carbohydrate from a test food to the same amount of available carbohydrate from a standard food consumed by the same subject that initially uses the standard "food" as glucose, but later used white bread as the standard (Wolever *et al*., 1985; Jenkins *et al*., 1981). Intrinsic and extrinsic factors that alter the rate of *et al*., 1987). Similarly, evidence has accumulated that a low-GI diet also protects against the development gastrointestinal motility, digestion and absorption, and the nature of the starch, cooking method, particle size, and the presence of fiber, fat, and proteins were all found to result in differences in the glycemic index (Krezowski *et al*., 1986). Several large-scale, observational studies indicated that carbohydrate content is a significant independent predictor of the risk of developing type 2 diabetes the long-term consumption of a diet with a high glycemic load (GL; GI x dietary test (Walever *et al*., 1993; Foster-Powell *et al*., 2002) and cardiovascular disease (Bornat of obesity (Jenkins *et al*., 1988; Liljeberg *et al*., 1992), colon cancer, and breast cancer.

Finger millet or ragi (*Eleusine coracana*) is an important minor cereal in the semi-arid regions of Africa and India (Hilu & Dewet,1976). The millet is unique among the minor cereals because of its superior nutritional qualities and several health benefits (Krishnan *et al*., 2011). It is a rich source of dietary fibre, calcium and phytochemicals with nutraceutical potential (Malleshi & Hadimani, 1993). Despite the fact that the seed coat of the millet is edible, it imparts chewy texture and dark colour to the food products and hence its separation is desired to prepare the product for enhanced consumer appeal (Krishnan *et al*., 2011). The millet flour is prepared almost free from the seed coat by incipient moist conditioning, pulverising and sieving the native as well as the malted millet and also by decortication of the hydrothermally processed millet (Malleshi, 1989; Shobana & Malleshi, 2007)

Composite flours are mixed flours that include starches and other ingredients which replace wheat partially or totally and are used for the production of bakery and pastry products. It can either be binary or a ternary mixture of flours from crops like soybean, gram, cassava, mung bean, etc. with or without wheat flour (Dendy, 1992). FAO initiated the concept of composite flour technology in 1964, targeting the use of indigenous crops such as millets, legumes, and other root crops in substitution of wheat flour to improve the food availability and food security of the population. The composite flour concept is a growing concept that has gained wider recognition and acceptance among nutrition scientists, being a simple sensible scientific approach in harnessing nutrient sources to meet human needs. It has been used to develop food products for clinical and non-clinical population groups (Zotor *et al.,* 2015). Composite flour is mainly used to enhance the nutritional values, and quality of bakery products, it is also used to prevent the suffering from degenerative diseases associated with the modern lifestyle (Mughal *et al.,* 2019). In view of the foregoing, this study aimed at reducing the glycemic index of white bread by modifying the flour with finger millet flour using a binary mixture technique and to determine its effect on postprandial blood glucose response on Wister rats

METHODOLOGY

Experimental Diets preparation

Diets were prepared starting with the commercially available animal feed (chikun feed), and then substituting it with the bread made from composite flour. The flour was made by substituting 15% and 30% wheat flour with finger millet flour according to the method described by Krishnan *et al*., 2011. While normal bread formulation included 100% refined flour.

Bread formulations

The composite flour of 15% and 30 finger millet flour was used in the baking processes of the test diet. While normal bread formulation included 100% refined flour. The vitamin and mineral premixes were incorporated into the formulations based on the nutrient content of the breads. The bread formulary is as presented in table 1.

Baking procedures

After weighing all ingredients, it was poured into an electric mixing machine (mixer) and was dry mixed for 1 minute, then water was added gradually until it reaches a ratio of 5:3 with the flour, it was mixed for 25 minutes to form the dough. The was removed from the mixer, cut and weighed to a desired weight of 640g. The weighted dough was placed on a rolling machine (roller) to roll it to bread shape, all the rolled dough were arranged in a big baking plate. The dough was rubbed with butter all over and placed into a pan. The dough was allowed to improve using a 0.1g improver (Vizyon bread improver) per 640g dough for about 30 minutes and the bread was put into an oven and heated for 25 minutes at a temperature of 150^{0}C (upper chamber) and 250^{0}C (lower chamber) then it was allowed to cool before usage. The bread was cut into slices sized similarly to rodent feed pellets and were allowed to be oven-dried at 60°C for 24 hr. All the breads used for the research were subjected to proximate analysis to determine its nutritional content and for the purposes of comparison.

Table 1: Ingredients of the experimental diets (breads).

Ingredients	15%	30%	white bread
Hard flour	1275g	1050g	1.5kg
Powdered flour	10g	10g	10g
Finger millet flour	225g	450g	0g
Salt	1.50g	1.5g	4.25g
Sugar	2.50g	2.50g	2.5g
Butter	80g	80g	80g
Improver	2.0g	2.0g	2.0g
Liquid flavour	1.0g	1.0g	1.0g
Preservatives	2.0g	2.0g	2.0g
Milk flavour	3.0g	3.0g	3.0g
Scotch butter	2.0g	2.0g	2.0g
Yeast	20g	20g	20g
Vitamin	premixes	premixes	premixes
Water	700mL	700mL	700mL

Animal collection and grouping

Twenty-five (6 weeks old) Wistar Han rats (Rattus Norvegicus albino rat, average body weight = 66g) of both sexes, were purchased from Animal House Mando, Kaduna State, Nigeria. The animals were subdivided into five (5) treatment groups according to their body weight and were accordingly distributed in a clean aluminium cage, made at Panteka Tudun Wada, Kaduna-South, Kaduna State. The cages contain soft wood chips, which make it suitable for housing the animals for research purposes. The cages were kept in a temperature and humidity controlled room with a 12 hour period of light and darkness in the animal house of Biochemistry department, Kaduna state University, Kaduna. The animals were fed with an adequate supply of commercial feed (chikun feeds) and water until the experimental diet became ready. The animals were allowed 5 days to acclimatise to the new diet before the experiment began. The weight was determined before the start of the experimental diet and also at the commencement of the research. The postprandial blood glucose response was determined at three days interval, after a 12-hour overnight fast, the animals were fed 2 g of the test diets, which was totally consumed within 25 min. Blood samples were taken from the tail vein at fasting (before the consumption of the meal) and at 30, 60, and 90 min and 120 min after the meal to measure serum glucose levels as described by Brites *et al*., (2011).

In the last experimental day, after a 12-hour overnight fast, the animals were weighed, anaesthetised with ethyl ether, and sacrificed by cardiac puncture, and blood was collected for subsequent analysis.

Induction of diabetes mellitus

The method of Reed*et al* (2000) was used to induce type II diabetes in the animals. The animals were given a 10% fructose solution ad libitum for 15 days prior to diabetic induction. All the animals were weighed after 3 days to check the weight gain. The Streptozotocin (STZ) was purchased from Bridge Biotech Ltd. No. 4 Liberty Lane, G. R. A, Ilorin. Kwara State. Before the administration of STZ, all animals were subjected to a day of fasting, a time of low movement and stress. Each animal was given streptozocin (50 mg/kg) dissolved in a citrate buffer with pH 4.4 (20 mg/mL) intraperitoneally. To ensure optimal streptozocin concentration to cause diabetes, the volume of injection was calculated based on the body weight of each animal. To check for diabetes induction 72 hrs. after streptozotocin injection, blood samples were obtained from the animal's tail veins and glucose levels were assessed using a glucometer (Accu-Chek Aviva; Roche diagnostic systems, Mannheim, Germany). The animals were categorised as diabetic if their blood glucose level exceeded 200 mg/dL. Every three days, the animal's blood glucose level was determined to ensure that they are truly diabetics before commencement of treatment.

Management of diabetes mellitus

The rats were sub-divided into 4 treatment groups, each containing 5 rats with alternating 12 hour periods of light and darkness. Each group was fed different types of the test diet, Group 1 normal bread (100% flour), group 2, 15% finger millet composite flour , group 3, 30% FM composite flour and group 4, standard diabetic bread (obtained commercially).

Proximate analysis of the bread produced and the one obtained commercially.

Proximate analysis was carried out to determine, moisture content, fat content, ash content, protein content and total carbohydrate. The proximate analysis was done according to the AOAC, 2000.

Statistical Analysis

The results obtained were submitted to 1-way analysis of variance using SPSS software version 16, where the diet was the single factor. Values are reported as least squares means. When significant effects of diet were observed ($P > 0.05$), the least squares means were compared by Duncan's test. Blood glucose concentration data were analysed with the same model separately for each time point, and the least squares means and SEMs are presented in table 2

RESULTS AND DISCUSSION

The postprandial blood glucose responses of the different breads showed different profiles (Fig. 1). At 0 minutes, animals fed with bread made from 30% composite flour have the highest concentration, while animals fed with normal bread (100% wheat flour) and 15% composite flour have the second and same concentration respectively. At 30 minutes, the animals undergoing treatment with normal feed (negative control) exhibited the highest (Fig. 1) concentration, and animals treated with standard diabetic bread had the lowest values (Fig. 1). But at 60 min, group II and group III were the highest (15% and 30% composite flour) respectively, while group I was the lowest. At 90 min, group III glucose response concentration was the highest and group two is the lowest. Conversely, at 120 min there is significant difference between the glucose response concentration exhibited by group I with all other groups ($P > 0.0001$). The incorporation of finger millet flour led to a significantly ($P > 0.0001$) slower glucose response by the test food when compared to bread made by 100% (group I) refined flour and the standard diabetic bread (group II). The postprandial blood glucose responses results obtained showed that, the consumption of 15% (group II), 30% (group III) bread made from composite flour of finger millet and standard diabetic (Wheat) bread resulted in no significant difference on postprandial blood glucose responses when compared, as shown in figure1 and 2. But, consumption of bread made from 100% refined flour resulted in significant difference on postprandial blood glucose responses ($P < 0.05$) when compared with 15% and 30% bread made from composite flour of FM (fig. 1 and fig. 2).

The reduction in postprandial blood glucose response could be due to the presence of dietary fiber in the FM grains flour that aids in digestion and slow the release of glucose into the bloodstream which could prevent the spiking of blood glucose as reported by (Chandrasekara and Sahidi 2010). Carbohydrates present in finger millet are slowly digested and assimilated as a result of which there is delayed absorption of glucose which ultimately controls the blood glucose levels (Chethan & Malleshi, 2007). Finger millet also contains a high proportion of dietary fiber and resistant starch (RS) which is also known as a functional fiber. Resistant starch (RS) escapes enzymatic digestion and imparts various beneficial effects by preventing several intestinal disorders and also acts as a preventive mechanism for colon cancer by production of metabolites such as butyrate that stabilise colonic cell proliferation.

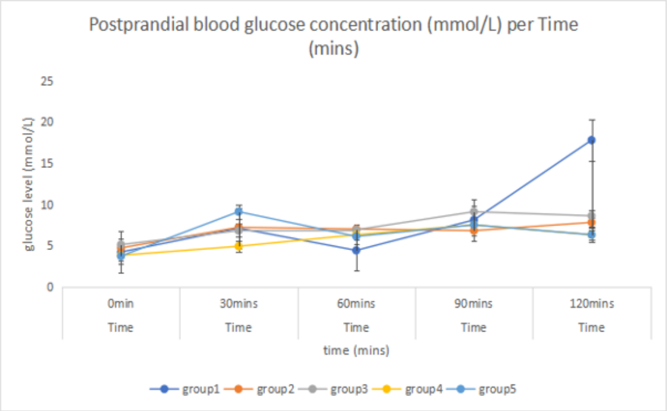


Figure 1: Postprandial blood glucose response of rats consuming bread at intervals of 0 min, 30mins, 60min, 90mins and 120min

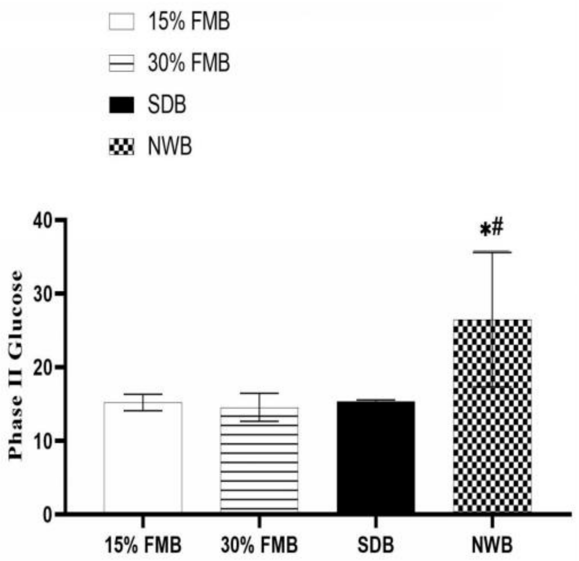


Fig. 2: The effect of 15% and 30% finger millet bread on postprandial blood glucose responses. The experiment was conducted in triplicate and the results are presented as Mean± SD, where $P < 0.05$ was considered statistically significant. * imply significant difference compared to 15% FMB and # implies significant difference compared to 30% FMB

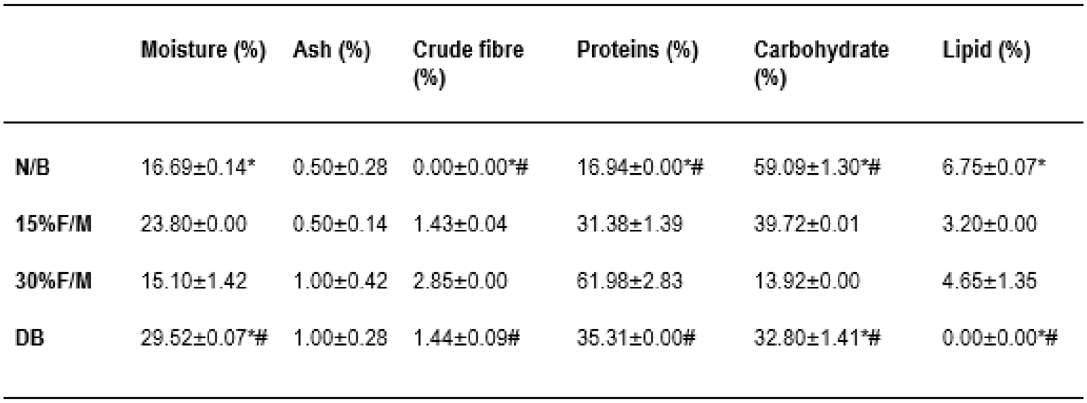
Table 1: Postprandial blood glucose response of rats consuming the bread at interval of 0 min, 30mins, 60min, 90mins and 120min

Groups	0 mins	30min	60min	90mins	120 mins
NWB	4.2 ± 0.2	7.1 ± 0.1	4.4 ± 0. 12	8.1 ± 0.01	17.8 ± 0.02
15%	4.2 ± 0.02	7.2 ± 0.01	7.0 ± 0.01	6.8 ± 0.1	7.8 ± 0.12
30%	5.1 ± 0.11	6.8 ± 0.12	6.9 ± 0.13	9.1 ± 0.1	8.6 ± 0.2
DB	3.8 ± 0.12	4.9 ± 0.2	6.3 ± 0.11	7.5 ± 0.2	6.3 ± 0.2
NF	3.7 ± 0.1	9.1 ± 0.11	6.1 ± 0.2	7.5 ± 0.1	6.3 ± 0. 12

The Proximate analysis of bread made from composite flour enriched with 15% and 30% finger millet, wheat flour bread and standard diabetic bread were obtained and presented in table 2. From the results obtained, the moisture content of the breads ranged from 15.09% in 30%, 16.69% in normal bread (100%wheat flour), 23.80% in 15% and 29.47% in standard diabetic bread. This result is in agreement with what was obtained by Bashir *et al.* (2022), where it was found that composite flour had lower moisture content and also had better shelf stability than the 100% wheat flour. The lower moisture content values observed in bread made from composite flour (30%) in this study imply that the bread enriched with finger millet could be stored for a reasonably longer period without spoiling. Moisture content of food samples is the main determinant of food spoilage. It is well established that low moisture content of food samples reduce the activities of microorganisms and increase the shelf life of the food products. In contrast, high moisture contents in food products facilitate the activities of microorganisms, and thereby reduce the nutritional quality and shelf life of the food products (Alozie *et al*., 2009).

The ash content of bread made from the composite flour and that of standard diabetic bread was higher than that of the normal white bread, this could be due to higher amount of minerals in finger millet and other constituents of diabetic bread. Similar results were also observed by Rajiv *et al*. (2011) where addition of finger millet flour increased the ash content and lowered protein content in muffins. Also Adeleke & Odedeji (2010) obtained similar results on bread made from wheat and sweet potato flour blends. The ash content increased significantly ($p < 0.05$) with increasing levels of FM flour. Which is also similar to the result obtained by Bansee *et al.* (2016) in which an increase in ash content was observed in bread samples enriched with FM flour, in their study on quality of white bread enriched with finger millet flour. The results show similarity with the report of Ramashia *et al.* (2021) that ash content of native finger millet ranges from 3.71± 0.14 to 5.55 ± 3.47%. This suggests that finger millet flour could probably provide essential, valuable and useful minerals needed for good development of the body.

Table 2: showing Proximate analysis of 15% & 30% finger millet enriched bread, normal bread and standard diabetic bread



	Moisture (%)	Ash (%)	Crude fibre (%)	Proteins (%)	Carbohydrate (%)	Lipid (%)
N/B	16.69±0.14*	0.50±0.28	0.00±0.00*#	16.94±0.00*#	59.09±1.30*#	6.75±0.07*
15%F/M	23.80±0.00	0.50±0.14	1.43±0.04	31.38±1.39	39.72±0.01	3.20±0.00
30%F/M	15.10±1.42	1.00±0.42	2.85±0.00	61.98±2.83	13.92±0.00	4.65±1.35
DB	29.52±0.07*#	1.00±0.28	1.44±0.09#	35.31±0.00#	32.80±1.41*#	0.00±0.00*#

The crude fiber content of the FM incorporated bread was found to be higher than that of the diabetic bread and 0.00 crude fibre was obtained from normal white bread. This may be due to higher crude fiber in the FM than that of the white flour which is also similar to the result found by Desai *et al*. (2010) and Chhavi and Sarita (2012) in which nutrient composition of bread formulated using FM flour contained significantly higher calcium, soluble dietary fibre and phyto-chemicals in their study on evaluation of composite millet breads for sensory and nutritional quality and glycemic response. This was also resonated by Bansee *et al*. (2016) where the white bread enriched with the FM had higher crude fiber and calcium. Also the result is similar to what was obtained by Mudau *et al*. (2021) that the fiber content increased significantly ($p < 0.05$) with increasing levels of FM flour which ranged from 2.14% to 3.02% for (10%) and (40%) FM composite flour bread.

The protein content of the breads was found to be between 16.94% in normal whole wheat bread and 61.98% in bread enriched with 30% finger millet, which is opposite to what was obtained in a study done by Malomo *et al*. (2011), where protein content decreased with the increased level of finger millet in comparison to the control (normal white) bread. Similar results were also observed by Rajiv *et al*. (2011) where addition of finger millet flour increased the ash content and lowered protein content in muffins. However, it was not in agreement with what was obtained by Mudau *et al*., (2021) where Protein content in the bread samples ranged from 6.75% to 8.14%. Bread made from 100% wheat flour had significantly ($p < 0.05$) higher protein content than bread made from composite flour. The decrease in protein content could be due to low protein content and non-gluten protein of FM flour which might have diluted the protein in wheat flour thereby resulting in low protein level of composite flour bread (Ijah *et al*., 2014).

The carbohydrate content of diabetic bread was found to be low compared to the normal white bread, this may be due to the composite nature of its flour. Also, Finger millet incorporated bread has shown a decreasing trend with the increased proportion of FM in comparison to the control bread. This could be attributable to the less amount of carbohydrate in finger millet seed than that of the wheat, which is in line with the results found by Chhavi and Sarita (2012) that the control (refined wheat flour) bread contained significantly higher carbohydrate, physiological energy and starch, in their study on evaluation of composite millet breads for sensory and nutritional quality and glycemic response. This result was found not to be in agreement with what was found by Mudau *et al*., (2021) where the Carbohydrate contents increased with increasing levels of FM flour substitution varying from 51.67% to 54.47%. Finally, the fat content was found to be lowered with the substitution of FM as compared to the control bread, this result is similar to what was obtained by Bansee *et al*. (2016) in their study on quality of white bread enriched with finger millet flour where fat content was also found to be decreased with the increased substitution level of FM flour as compared to the control bread. Mudau *et al*. (2021) reported otherwise, that the fat content of the bread increased significantly from 2.30% to 3.17% with increasing levels of FM flour substitution

Conclusion

In conclusion, it was found that there was increased in nutrient content of bread made from composite flour formulated, particularly sample that contain 30% finger millet enriched bread had significantly the highest protein content of 61.98%, fibre (2.85%) and lower moisture and carbohydrate content of 15.09% and 13.92% respectively. The bread made from composite flour has an effect on postprandial blood glucose responses when compared to bread made from 100% wheat flour and standard diabetes bread. Thus, this study suggested that the use of food substance made from composite flour may have a significant effect in management of diabetes, obesity and other cardiovascular diseases.

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