Olagunju *et al.*
Food Production, Processing and Nutrition (2024) 6:32
https://doi.org/10.1186/s43014-023-00175-8

Food Production, Processing and Nutrition

RESEARCH

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High-protein, low glycemic index snack from optimized blend of three wholegrains exhibits nutraceutical quality and elicits low glycemic response in diabetic human subjects

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Abstract

Snack products are evolving as new carriers of functional ingredients with nutritional and health-promoting benefits. A blend of whole grains is increasingly being utilized to harness the functional potential of the grain mix. Amaranth, acha, and pearl millet grains flours were optimized using response surface methodology (RSM), to obtain optimum blends (90:5:5 and 47.98:26.68:25.34) with high protein content and low glycemic index. Snack bar products from the blends were labelled MBY and MBZ. A total of 40 diabetic and 10 non-diabetic subjects were recruited. Of the diabetic, about 42% were overweight while 40% were obese, the non-diabetic had normal weights. Each was allowed to consume snacks containing the equivalent of 50 g of carbohydrates. Finger prick was employed to evaluate the postprandial glucose response of snack products while venous blood was evaluated for antioxidant enzymes, carbohydrate-hydrolyzing activities, and insulin using standard methods. Consumption of the multigrain snacks elicited a stable postprandial response (133–141 mg/dL) with 16 and 24% postprandial decline. In addition, snacks had low to intermediate glycemic index (52 and 56) in diabetic and low glycemic index (43 and 45) in non-diabetics; likewise reduced α-amylase/α-glucosidase activities compared to control snacks. Similarly, glutathione level, glutathione peroxidase, superoxide dismutase, and catalase activities in serum from subjects that consumed multigrain snacks were upregulated compared to control and market sample groups. Moreso, snack products promoted a reduction in serum insulin levels in diabetic subjects (45 and 17% for MBY and MBZ respectively). Following the nutraceutical properties displayed by the formulated snack especially MBY, it can be promoted as a functional snack for the management of diabetes while solving the limited snack product choice of diabetes sufferers.

Keywords Multigrain, High-protein snack, Clinical observation, Glycemic regulation, Serum biochemical properties

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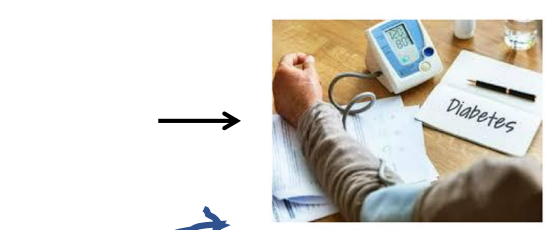
Graphical Abstract



Diabetic patient

Evaluation of postprandial blood glucose & biochemical evaluation

Multigrain snack bar

MBY: 90% amaranth+5% acha+5% pearl millet
MBZ: 47.98% amaranth+26.68% acha+25.34% pearl millet
COB: 100% oat snack bar

- Elicit stable postprandial glucose (up to 24% decline)
- Reduced α-amylase/glucosidase activities
- Upregulation of serum glutathione level, glutathione peroxidase, superoxide dismutase, and catalase activities

Introduction

Diabetes mellitus (DM), which is regarded as the disease of the 21st Century, is a growing epidemic health problem, characterized by elevated blood glucose as a result of either insufficient insulin production by the pancreas or a result of ineffective use of the insulin produced (WHO 2021). Changes in global food systems, the increasing adoption of high caloric intake and low energy expenditure (sedentary lifestyle) have promoted the pervasiveness of obesity and type-2 DM (Hruby & Hu 2015). Improper management of DM has resulted in several macrovascular and microvascular blood vessels initiating nephropathy, ocular disorders, heart attack, stroke and eventually death (Petrie et al. 2018). Obesity and hyperlipidemia are key risk factors of DM, thus, weight management is an essential tool for effective diabetic care and its complications, including cardiovascular diseases such as blood pressure, arteriosclerosis, and glycemic control (Wing et al. 2011).

'High protein' foods contain above 11 g/100 g protein (Ministry of Food and Drug Safety 2019). Interestingly, increased protein intake through high-protein foods complemented with reduced carbohydrate intake has the potential to reduce plasma glucose levels of diabetic individuals (Gannon & Nuttall 2004). Diet as one of the lifestyle interventions for diabetes management cannot be over-emphasized. Several reports have shown the effectiveness of a high-protein plant regimen in reducing the prevalence of diabetes in human subjects, some of which are Malaeb et al. (2019) and Yang et al. (2021). In a similar fashion, low glycemic index foods are reportedly beneficial for blood glucose control (Ojo et al. 2018; Bai et al. 2021). Plant-based diets include products from legumes, whole grains, vegetables and fruits, are nutritious and economically sustainable with diverse product options. Whole grain comprising the bran and germ fractions possess outstanding nutritional and bioactive properties, conferring relevant health-promoting benefits to its consumers and is relevant in weight control (Calinoiu & Vodnar 2018).

Globally, snacks are an important contributor to human's daily energy intake (Smith & Whiting 2019). They are often regarded as junk foods but recently, snack products are evolving as new carriers of functional ingredients with nutritional and health-promoting benefits (Kim et al. 2020). Impressively, the potential of functional foods including functional snack products in controlling hyperglycemia or managing diabetes is being explored (Krawecka et al. 2019; Yang et al. 2021). The consumption of healthy snacks reportedly controls the rise in blood glucose levels while controlling the glycemic response of the subsequent meal (Imai et al. 2018;

Nitta et al. 2019). The diet approach to the management and prevention of type 2 diabetes in some countries is remarkable (Hwalla et al. 2021; Rajput et al. 2022). The glycemic index (GI) measures the dynamics of hydrolysis and absorption of carbohydrates into the bloodstream whereas, glycemic load (GL) predicts the effect of amount of carbohydrate consumed per serving (Krawecka et al. 2019). Overall, both are rating systems to measure the promptness of foods to cause increase in blood glucose levels. Foods with low GI and GL promote low glycemic response to carbohydrate-containing foods and are associated with reduced risk of diseases such as type 2 diabetes mellitus and cardiovascular diseases (Augustin et al. 2015).

Cereal grains are specifically employed for the production of baked products. Multicereal represents a combination of grains and may confer functional characteristics derived from the synergistic contributions of the individual cereal grain. This may be a result of the rich content of dietary fibre, presence of functional phytochemicals and the low glycemic index (Prasadi & Joye 2020). Baked food products especially from white flour with the inclusion of white sugar has been reported to possess relatively high glycemic indices (Olagunju 2019). However, with a careful selection of functional cereals and use of natural sweeteners, resulting snacks may provide potential health benefits to diabetic and normoglycemic individuals. Indigenous wholegrains with health-promoting properties beyond nutrients were carefully selected for this study. The grains have been individually studied and their positive role in the management of diabetes is worthy of endorsement. Amaranth, acha and pearl millet have received popular attention for their antidiabetic properties (Kasozi et al. 2018; Adams & Yakubu 2020; Pei et al. 2022). This property is attributed to the richness in fibre and protein as well as the presence of complex carbohydrates and the low glycemic nature (Saleh et al. 2013; Geetha et al. 2020). Given the respective nutritional relevance and health benefits of the selected grains, developing characteristic snack product from an optimized cereal blend is a step in the right direction to meeting consumers' preference for a functional healthy snack product.

Snacking is a part of the dietary treatment regimen of diabetic patients however, snacks for these group of people remain unavailable in Nigeria. Hence, the study aimed to develop a healthy nutritious snack product from underutilized functional cereals. More importantly, evaluate their postprandial blood glucose effect and glycemic index as consumption of low glycemic index foods may reduce the incidence of serious diabetic complications by abating insulin resistance and promoting strict glycemic control in type 2 diabetes mellitus patients.

Materials and methods

Amaranth (*Amaranthus caudatus*) and pearl millet (*Pennisetum glaucum*) were sourced from *Shasha* market, Akure, Ondo State. Acha (*Digitaria exilis*) was sourced from a local market in Minna, Niger State. The grains were authenticated by a Crop Scientist in the Department of Crop, Soil, and Pest Management, at the Federal University of Technology Akure, Nigeria. Date syrup was purchased from a local spice store. Coconut oil, baking powder and ginger powder were purchased from the supermarket. Soy lecithin was sourced from Qualifirst Foods Ltd, Toronto, Canada. All reagents were of analytical grade.

Processing of flour samples

Amaranth, acha, and pearl millet grains were manually sorted, thereafter, thoroughly washed and steeped with distilled water in individual sterile containers. The grains were subjected to fermentation (37 °C/72 h). Note that the steep water was changed daily. It was thereafter drained, allowed to dry in a laboratory oven under convective heat at 45 °C for 6 h, then milled to coarse particle size (212 μm). Flour was packed in screw-capped containers and stored at refrigeration temperature until required for further use.

Multigrain flour blend formulation

Mixture design of response surface methodology (RSM) was employed for the optimization of flour blends, and 14 experimental runs were generated (Table 1). The prefermented coarse flour samples were mixed using a range of 5 to 90% for the flour blend using protein content and glycemic index (GI) as dependent variables. The first round of optimization to maximize protein and minimize the GI yielded the blend 90:5:5 (amaranth:acha:pearl millet), while a second round of optimization to set the protein and GI between 16–18% and 40–45 respectively yielded a blend 47.98:26.68:25.34 (amaranth:acha:pearl millet). The optimized blends were employed in the production of snack bars labeled MBY and MBZ respectively while commercial oat snack bars (COB) served as control.

Production of snack bar

The dry ingredients were weighed into a bowl. On the side, 10 ml of clean water was dispensed into a glass beaker, followed by the addition of 10 g each of date syrup and coconut oil respectively. The glass beaker was heated in a water bath (70 °C) till the mixture was uniformly dissolved. The wet ingredients were then added to the dry ingredients and then completely mixed to form a paste. The dough was rolled to a 3 cm thickness and then transferred to a baking tray (30 cm × 30 cm). The dough sheet

Table 1 Flour composition, protein content, and glycemic index of RSM generated runs

Runs	Amaranth	Acha	Pearl millet	Protein	GI
1	33.333	33.333	33.333	14.29 ± 0.29	43.59 ± 1.09
[a]2	**90.00**	**5.00**	**5.00**	**22.65 ± 0.17**	**42.52** ± 0.58
3	19.167	61.667	19.167	12.35 ± 0.04	47.06 ± 0.48
4	19.167	19.167	61.667	10.55 ± 0.21	52.86 ± 1.11
5	5.00	90.00	5.00	10.84 ± 0.12	47.78 ± 0.63
6	5.00	90.00	5.00	10.71 ± 0.06	52.14 ± 1.80
7	47.50	5.00	47.50	15.89 ± 0.04	43.64 ± 1.03
8	90.00	5.00	5.00	21.13 ± 0.10	41.62 ± 2.24
9	5.00	5.00	90.00	12.31 ± 0.08	58.00 ± 2.16
10	5.00	47.50	47.50	7.93 ± 0.04	38.64 ± 0.50
11	47.50	47.50	5.00	16.24 ± 0.13	43.72 ± 0.76
[a]12	**47.98**	**26.68**	**25.34**	**16.95 ± 0.03**	**43.54** ± 1.06
13	5.00	5.00	90.00	12.65 ± 0.02	56.00 ± 2.24
14	61.667	19.167	19.167	14.14 ± 0.02	48.16 ± 0.36

[a] Indicates the blends selected for production of snack product; GI: glycemic index

was partitioned into a length and width of 6 cm × 2 cm. Baking was carried out in a laboratory oven set at 150 °C for 30 min and the bars separated from each other after baking. The snack bars were cooled and sealed in a polypropylene film; placed in an airtight container and stored at room temperature. Assuredly, the developed multigrain snack was prepared under strict hygienic conditions a day before the evaluation while the commercial product was purchased from Ceci supermarket, Akure. The ingredients and nutritional compositions of the snack bar are provided in Tables 2 and 3. The ingredient for the oat snack (market sample which served as a control) comprised whole grain rolled oat, brown sugar syrup, honey, sunflower lecithin, sodium bicarbonate, salt, and sunflower oil.

Table 2 Formulation of ingredients for snack bar

Ingredients (g/100 g)	MBY (g)	MBZ (g)
Amaranth flour (%)	90	47.98
Acha flour (%)	5	26.68
Pearl millet flour (%)	5	25.34
Date syrup	10	10
Soy lecithin	1	1
Ginger powder	2	2
Baking powder	1	1
Coconut oil	10	10
Vanilla extract	0.5	0.5
Xanthan gum	3	3

MBY: 90% amaranth, 5% acha, 5% pearl millet; MBZ: 47.98% amaranth, 26.68% acha, 25.34% pearl millet

Chemical analysis of snack product

Proximate compositions (total ash - method no.930.22, crude fibre - method no. 950.37, crude fat - method no. 950.36, crude protein - method no. 950.36) of the snack products were determined as described in AOAC (2012). Carbohydrate content was calculated by difference. The proximate composition of the snack product was evaluated, results reported in an earlier study (Olagunju et al. 2022). The carbohydrate content (g/100 g wet weight basis) of each test food was used in estimating the amount equivalent to 50 g carbohydrate. The carbohydrate content of COB was the least (42.34 g/100 g) while MBY and MBZ had 44.95 and 46.83 g/100 g carbohydrate respectively (Table 3 shows dry weight basis composition).

The study was designed to evaluate the potential of multigrain snack products as dietary intervention and as snacks for diabetic subjects. The study was conducted at the Medical Out-Patient Department of the University of Medical Sciences Teaching Hospital (UNIMEDTHC), Akure, and the Federal University of Technology, Akure, Nigeria. The clinical study was carried out following guidelines for human studies with the approval of the Ondo State Health Research Ethics Committee (OSHREC), Ondo State (OSHREC 22/3/2021/316). We hereby certify that the study was performed in accordance with the 1964 Helsinki Declaration and comparable ethical standards.

Basic characteristics and anthropometric measurements

The age, sex, occupation of the patients were documented. The diabetic history (year of diagnosis and treatment commencement) was also documented. The weight of each subject was measured using a digital clinic

Table 3 Proximate composition (dry weight basis) of whole grain snack bar

Constituents (g/100 g)	Samples		
	MBY	MBZ	COB
Ash	3.19 ± 0.00^{a}	2.45 ± 0.41^{b}	1.39 ± 0.01^{c}
Crude fiber	2.08 ± 0.01^{b}	1.08 ± 0.01^{c}	3.54 ± 0.34^{a}
Fat	13.55 ± 0.05^{a}	17.86 ± 0.10^{b}	25.06 ± 0.08^{a}
Protein	21.95 ± 0.66^{a}	17.75 ± 0.19^{b}	3.54 ± 0.34^{c}
Carbohydrate	59.23 ± 0.70^{b}	60.86 ± 0.55^{a}	56.49 ± 0.41^{c}
Moisture	6.96 ± 0.28^{a}	7.03 ± 0.11^{a}	4.06 ± 1.29^{b}
Energy value (kcal/100 g)	446.67 ± 0.62^{c}	475.02 ± 1.04^{b}	464.58 ± 1.52^{a}

Values represent Mean ± SD, values with different superscripts on the same row are significantly different ($P^{<}$ 0.05)

MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); *MBZ* (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet), *COB* Oat snack bar control (control)

weighing scale. Height was measured using a portable calibrated clinic stadiometer (Seca, mLabs209, Germany). The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of participants were measured using a sphygmomanometer.

Experimental procedure

Forty (40) healthy, diabetic, non-smokers (male and female) were recruited from the Medical Out-Patient Department of UNIMEDTHC Akure, Nigeria. Their body weights ranged from 45 to 90 kg (averaging 73.15 ± 12.50 kg) while their body mass index (BMI) ranged from 23 to 35 kg/m^2 (with an average value of 28.36 ± 4.65 kg/m^2). The participants were divided into 4 groups, each group comprising 10 subjects. The inclusion criteria for participation were adults with diagnosis of type 2 diabetes, being older than 40 years of age and with a BMI above 23 kg/m^2. The exclusion criteria include being on medications for any chronic disease conditions or those affecting glucose tolerance, gastrointestinal disorders, pregnancy, breastfeeding, intolerances, or allergies to any ingredient in the snack product. The participants voluntarily gave their consent after being informed about the study. They were properly briefed about the assessment and requested to observe a 12 h overnight fast while avoiding alcohol consumption or engagement in rigorous physical exercise before the test day. The experiment was carried out for non-consecutive days. On the other hand, ten (10) healthy, non-smoking, male and female subjects who have not been diagnosed with diabetes with 45 to 70 kg body weights (average of 63.10 ± 8.45 kg) and 20 to 25 kg/m^2 BMI (averaged 21.56 ± 1.32 kg/m^2) was recruited from among members of staff, Federal University of Technology, Akure.

Upon arrival of the study subjects at the test centre, the anthropometric parameters of weight and height were measured following standard procedures and BMI was calculated. The fasting blood glucose was also taken in a relaxed condition using a finger prick blood sample and measured using a Kiptrack blood glucose monitor (MedNet GmbH Borkstraβe 10, 48,163 Münster, Germany). On the first day, participants were given glucose solution (50 g glucose dissolved in 250 mL clean drinking water) and blood glucose readings were taken for 2 h, subsequently, the test food (quantity equivalent to 50 g carbohydrate) was administered to the participants according to their group stated as follows:

Normal control (NC): non-diabetic healthy subjects
Group 1 (G1): diabetic subjects not administered with any test food (CTRL)
Group 2 (G2): diabetic subjects fed with multigrain snack bar from blend 1 comprising 90% amaranth, 5% acha, 5% pearl millet (MBY)
Group 3 (G3): diabetic subjects fed with multigrain snack bar from blend 2 comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet (MBZ)
Group 4 (G4): diabetic subject fed with commercial oat snack bar (COB)

The test food was consumed within 15 min along with 75 cL of drinking water. The subjects maintained a seating position throughout the duration (about 3 h) of the experiment; they were prohibited from eating and drinking till the session ended. Although, light activities such as reading a book, newspaper or mobile phone surfing were allowed. At the end of the study period, 5 mL of blood was drawn from the veins; the blood was centrifuged (2,000 rpm for 10 min) and the resulting serum was stored for subsequent biochemical analysis.

Evaluation of oxidative stress marker in serum

The level of lipid peroxides in the serum was evaluated as described by Ohkawa et al. (1979) and reported as thiobarbituric acid substance (TBARS) level. Exactly 100 µl of blood serum was added to 200 µl of 8.1% sodium dodecyl sulphate (SDS), followed by the addition of 500 µl each of 0.8% thiobarbituric acid and acetic acid solution (2.5 M HCl, pH 3.4) into the test tube. The reaction mixture was heated (100°C) for 1 hr, absorbance taken at 532 nm in a spectrophotometer.

Evaluation of glycemic index and glycemic load of multigrain snack product

The GI of the snack products was evaluated following the World Health Organization guidelines FAO/WHO (1998). After consumption of the test meal, blood glucose concentrations were measured in capillary whole blood obtained by finger prick and measured using Kiptrack blood glucose monitor (MedNet GmbH Borkstraβe 10, 48,163 Münster, Germany) in the fasted state (time 0), after 15 min of complete ingestion of the snack then at 30 min interval for 2 h (30, 60, 90, 120 min). The incremental area under curve (iAUC) for test food and glucose was calculated using the trapezoidal rule of the area under the curve to determine the GI was calculated using the formula below:

$$GI = \frac{Incremental\ area\ under\ 2h\ blood\ glucose\ curve\ for\ food\ samples}{Incremental\ area\ under\ 2h\ blood\ glucose\ curve\ for\ glucose} X\ 100$$

The glycemic load (GL) for each food sample was determined as described by Salmeron et al. (1997). GL

was estimated from the carbohydrate content in a typical serving of the test meal and the GI of the test food using the equation below:

$$GL = \frac{Net\ carbohydrate(g)xGI}{100}$$

Net Carbohydrate = Total Carbohydrate available in a serving of the food sample

Determination of serum antioxidant parameters

Serum glutathione peroxidase (GPx) activity was evaluated following the method of Rotruck et al. (1973). To the prepared buffer, serum, and glutathione were added, respectively, and the mixture was incubated at 37 °C for 10 min. The mixture was centrifuged after the addition of 10% trichloroacetic acid. The supernatant and Ellman's reagent with phosphate buffer were added together and the absorbance was taken at 412 nm.

The reduced glutathione (GSH) level in the blood serum was determined at 412 nm using the method described by Ellman (1959). This involved 100 µL of serum treated with 50 µL of Ellman's reagent (19.8 mg of 5,5′dithiobisnitrobenzoic acid in 100 mL of 0.1% sodium citrate) and 300 µL of phosphate buffer (pH 8.0).

The serum superoxide dismutase (SOD) activity was determined at 480 nm based on the inhibition of the superoxide radical produced in the presence of adrenalin according to the method by Misra and Fridovich (1972).

Catalase activity in serum was evaluated following the method described by Beers and Sizer (1952), 50 µl of serum was added to a reagent mixture comprising 500 µl of 59 mM H_2O_2 and 950 µl of 50 mM phosphate buffer [pH 7.0] before the reaction began. For three minutes, the reaction was allowed to proceed at 25°C while the absorbance was monitored every 150 s (570 nm).

Determination of serum insulin level and carbohydrate (α-amylase and α-glucosidase) enzymes activity

Serum's insulin was quantified using an ELISA kit (Monobind Inc, USA) following the manufacturer's instructions. The absorbance was measured at 450 nm in a microplate reader (BIOBASE-EL10A). The serum α-amylase activity was evaluated using 1% soluble starch solution prepared phosphate buffer as substrate. After incubating the mixture with dinitrosalicylic acid at 100 °C for 5 min and being allowed to cool, the color intensity was measured at 540 nm (Worthington 1993). The serum α-glucosidase activity was determined using 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) prepared in 0.1 M phosphate buffer (pH 6.9) as the substrate, using the method of Apostolidis et al. (2007).

Statistical analysis

Optimization was carried out using Response surface analysis; Design Expert v. 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA). The dataset generated was analysed using SPSS 25.0 for Windows software (SPSS Inc., Chicago, IL, USA), the means were separated using Duncan's New Multiple Range Test (DNMRT) and the analysis of variance (ANOVA) was performed. The incremental area under the curve (iAUC) was calculated and biochemical activities analysed using GraphPad Prism program for Windows (version 8.0; GraphPad Software, Inc., San Diego, CA, USA).

Results and discussion

Basic characteristics

The basic characteristics and anthropometric data of the study group (diabetic and non-diabetic subjects) are presented in Table 4. The non-diabetic subjects were volunteered academic and non-academic staff members of the Federal University of Technology, Akure, Nigeria. They comprise an equal number of males and females aged 39 to 52 years old (average age 44.70 ± 4.94 years). Data showed they had a healthy and normal body weight averaging 63.10 ± 8.45 kg and BMI 21 to 22.81 kg/m^2 (average of 21.56 ± 1.32 kg/m^2). The diabetic subjects were volunteered patients at the out-patients' department of the University of Medical Sciences Teaching Hospital, Akure, Nigeria. Going by the statistics of the diabetic participants, the female gender was more represented than the male counterpart with a sex ratio of 1.2, and their age range was between 46 to 80 years (averaged 65.87 ± 9.69 years), the most represented age group was 51–60 and 71–80 years. About 32% (13 persons) were civil servants (government employees), 17% (7 persons) were entrepreneurs while 50% (20 persons) were retirees thus, about half of the respondents had working functional status. From the information provided by the patients, the diagnostic duration of diabetes was 5 years on average. The average weight was 73 kg and average body mass index (BMI) was 28 kg/m^2. The male had a weight range between 69–90 kg and a BMI of 27.48–31.88 kg/m^2 while female weighed 45–90 kg and BMI 23.48–36.60 kg/m^2. According to the World Health Organization (WHO), BMI cut-offs and their corresponding values are underweight (BMI < 18.5 kg/m^2), normal weight (BMI = 18.5–24.9 kg/m^2), overweight (BMI = 25–29.9 kg/m^2) and obese (BMI ≥ 30 kg/m^2). An earlier study showed a lower cut-off (≥ 23 kg/m^2) as a risk factor for insulin resistance and diabetes (Zafari et al. 2018). It can be deduced that the subjects were majorly overweight and obese. About 42% of the subjects (17 persons) were overweight, 40% (16 persons) were obese while 17% (7 subjects) were at border line of

Table 4 Basic characteristics and anthropometric measurements of study participants

Variables	Diabetic ($n=40$)	Non-diabetic ($n=10$)	*P*-value
Gender			
Male	16	5	
Female	24	5	
Age	65.87 ± 9.69	44.70 ± 4.94	0.9898
Anthropometrics			
Weight (kg)	73.15 ± 12.50	63.10 ± 8.45	0.3823
Height (m)	1.64 ± 0.07	1.61 ± 0.11	0.9999
Height (m^2)	2.68 ± 0.22	2.44 ± 0.18[a]	0.9999 NS
BMI (kg/m^2)	28.36 ± 4.65	21.56 ± 1.32	0.0475
Blood pressure			
SBP (mmHg)	169.15 ± 20.02	102.33 ± 25.11	0.009
DBP (mmHg)	86.55 ± 6.60	68.73 ± 12.56	0.01
Fasting blood glucose (mg/dL)	117.85 ± 51.66	117.00 ± 9.87	0.05
Fasting insulin (mlU/mL)	47.10 ± 1.60	21.66 ± 2.15	0.002

BMI Body mass index, *SBP* Systolic blood pressure, *DBP* Diastolic blood pressure, *NS* No significant difference

[a] = < 0.05

normal to overweight presenting BMI of 23.48–24.19 kg/m^2. This corroborates the report that obesity is a common complication in diabetic patients (Yang et al. 2021). It may also be a pointer to the fact that diabetes accompanied by overweight, or obesity may predispose the patient to other diseases. Thus, diabetic patients should consciously consider a weight loss program either following a dietary approach or integrating a routine exercise. The present finding is similar to a report by Wanvoegbe et al. (2016) where 41% of the diabetic subjects were obese while 39% were overweight. Comparing with another study by Lucha-Lopez et al. (2021), the BMIs recorded were significantly lower than the mean values reported for both physically active and sedentary diabetic Spanish women (34.2 and 38.9 kg/m^2 respectively).

The data showed that the non-diabetic subjects had a good control of their blood pressure exhibiting normal blood pressure reading averaged 102/69 mmHg except for one who had a low blood pressure (68/55 mmHg). The subject confirmed that overtime she regularly recorded a low blood pressure. On the contrary, the diabetic subjects showed a poor blood pressure control with an average systolic blood pressure above 150 mmHg and diastolic blood pressure above 80 mmHg. This result agrees with the reports that diabetes and hypertension have strong epidemiological association and are considered common comorbidities (Petrie et al. 2018). The comorbidity of diabetes and hypertension may have been promoted by the overweight/obese status; improper management of these disease conditions may promote risk of other cardiovascular complications such as heart failure, stroke and nephropathy.

The fasting blood glucose (FBG) was 195 mg/dL on average for the diabetic group and 117 mg/dL for the normoglycemic group. According to the American Diabetes Association, the normal to healthy range for fasting blood glucose before meals is < 100 mg/dL while levels above 130 mg/dL is classified diabetes mellitus range. FBG is used as an index for screening for diabetes mellitus, from existing classification and obtained result, FBG of 195 mg/dL is indicative of the diabetic status of the study participants. Although, majority of the subjects had FBG averaging 104–119 mg/dL but some others had very high FBG up to 230 mg/dL which impacted the overall average reported in the study. Nonetheless, on the average, the FBG of the subjects was approximately 137 mg/dL when subjects with extremely high values were excluded. As it stands, the average FBG falls within the range reported by ADA (2020) for diabetes mellitus.

Fasting insulin level is used as a sensitive marker for the assessment of insulin resistance. The fasting insulin (FI) levels were on the average 22 and 47 mlU/mL for non-diabetic and diabetic (overweight/obese) subjects respectively, the FI for the non-diabetic group falls within the normal range whereas, the FI in diabetic subjects was higher. The value obtained (> 30 mlU/mL) may indicate insulin resistance in a diabetic individual than in a normoglycemic individual. This is because similar insulin level is unlikely to comparatively suppress serum glucose in non-diabetic individual at the same rate in diabetic patient. The overweight and obese status in diabetic participants may be implicated to cause an impaired cell response to insulin. Regarding the results of the present finding, the fasting serum insulin

in diabetic participants was higher and doubles those of normal weight non-diabetic subjects which may be a consequence of an impairment in β-cell function in diabetic individuals.

Overall, the prevalence of overweight to obese status, high fasting blood glucose, high blood pressure and abnormal fasting insulin levels observed in diabetic participants show the subjects exhibit risk factors for cardiovascular diseases.

The blood glucose response of healthy non-diabetic subjects who consumed the test samples were recorded over a 2 h period (Fig. 1a). The blood glucose concentration at start of experiment (0 min) was recorded and averaged 99.44 ± 14.35 mg/dL. This is in accordance with the normal range (< 100 mg/dL) set for a healthy subject (American Diabetes Association). After ingestion of the snack products, the blood glucose concentration increased for the different groups with a peak value observed at 30 min followed by a progressive decline till the end of the 2 h study duration. For the diabetic group, the experimental snack products (MBY and MBZ) exhibited low mean postprandial glucose level compared to control sample (COB). On one hand, the group that consumed MBY (G2) exhibited the highest postprandial glucose response within the first 15 min (90.67 to 108.67 mg/dL) however, produced the most significant postprandial reduction in glucose level (33%). This may be associated with the characteristic functional composition of the snack product (high amaranth, protein, and fiber contents). On the other hand, G3 (group that consumed MBZ) took a longer time (30 min) to attain peak postprandial glucose and exhibited a lower increase in blood glucose concentration (13%) compared to MBY. The group that consumed the control sample (G4) exhibited a 23% increase in blood glucose after 30 min and a final 18.69% decline in postprandial glucose after 2 h of consuming product. The subjects' 2 h postprandial blood glucose levels of subjects for MBY are within the normal limits of < 140 mg/dL (Fig. 1b) thus, suggesting the snack product exhibited normal glucose response (Rolfes et al. 2018). MBZ on the other hand, resulted in a postprandial glucose of 141 mg/dL which is fairly within the specified normal range. However, consumption of control sample (COB) as seen in G4 data elicited a postprandial blood glucose of 157 mg/dL which is suggestive of impaired glucose tolerance by subjects. Statistical optimization of preliminarily processed whole grains (amaranth, acha, pearl millet) enhanced the protein content of the snack products (21.95 and 17.75 g/100 g for MBY and MBZ respectively) while the fat content was 17.86 and 13.55 g/100 g respectively (Table 3). These values are significantly ($p < 0.05$) different from the 3.54 g/100 g (protein) and 25.06 g/100 g (fat) recorded for the market control sample (COB), it suffices to say that the nutritional composition of the snack product may have contributed to the observed postprandial blood glucose response.

The incremental area under the curve (iAUC) from postprandial blood glucose reading for diabetic and non-diabetic subjects are presented in Fig. 1c. The diabetic group exhibited high iUAC, G1 (18,378 mg.min/dL) was significantly higher than the values for G2 and G3 (19,258 and 19,852 mg.min/dL respectively) whereas, the non-diabetic group had lower values ranged 10,990 to 15,990 mg.min/dL. The postprandial blood glucose response of diabetic subjects to the multigrain snack showed that significant differences were observed after 15 min and greater differences after 30 and 60 min of test food consumption (Table 5). The group that consumed MBY gave a peak glucose reading of 174 mg/dL at 30 min from a 142 mg/dL baseline and a final 133 mg/dL at 120 min. Group 3 peaked at 60 min (169 mg/dL), from a baseline of 135 mg/dL likewise, G4 peaked at 60 min (176 mg/dL) from baseline of 125 mg/dL to 2 h postprandial blood glucose of 157 mg/dL. The blood glucose reading of G3 and G4 increased steadily for the first 1 h followed by a marginal decline of 16.49 and 10.49% respectively while MBY promoted a 23.92% postprandial decline. Overall, despite the test meal having the same amount of available carbohydrates (50 g), the developed multigrain snack showed stable postprandial blood glucose which was lower than the high postprandial blood concentration recorded in the control sample group (G4). The developed snack bar from underutilized conventional whole grains showed lower blood glucose response than commercial snack bar products. Other studies have been carried out to develop snack products with blood glucose control ability for DM patients and healthy individuals (Manthou et al. 2014; Stamataki et al. 2016; Yang et al. 2021). The snack products developed in those studies also relatively elicited low postprandial glucose response.

Generally, whole grain cereals are rich in dietary fibre and resistant starch hence, they are considered a relevant material for diabetic patients. In addition, high-protein plant foods promote satiety and fullness which can be useful in suppressing appetite. Therefore, the consumption of high-protein meals is adjudged to regulate blood glucose concentration, hence, may be a safe product for diabetic patients (Russell et al. 2016). It may not be out of place to project that the novel snack product would be relevant for diabetes control owing to its potential to provide valuable nutrients, promote satiety and slow down glycemic release.

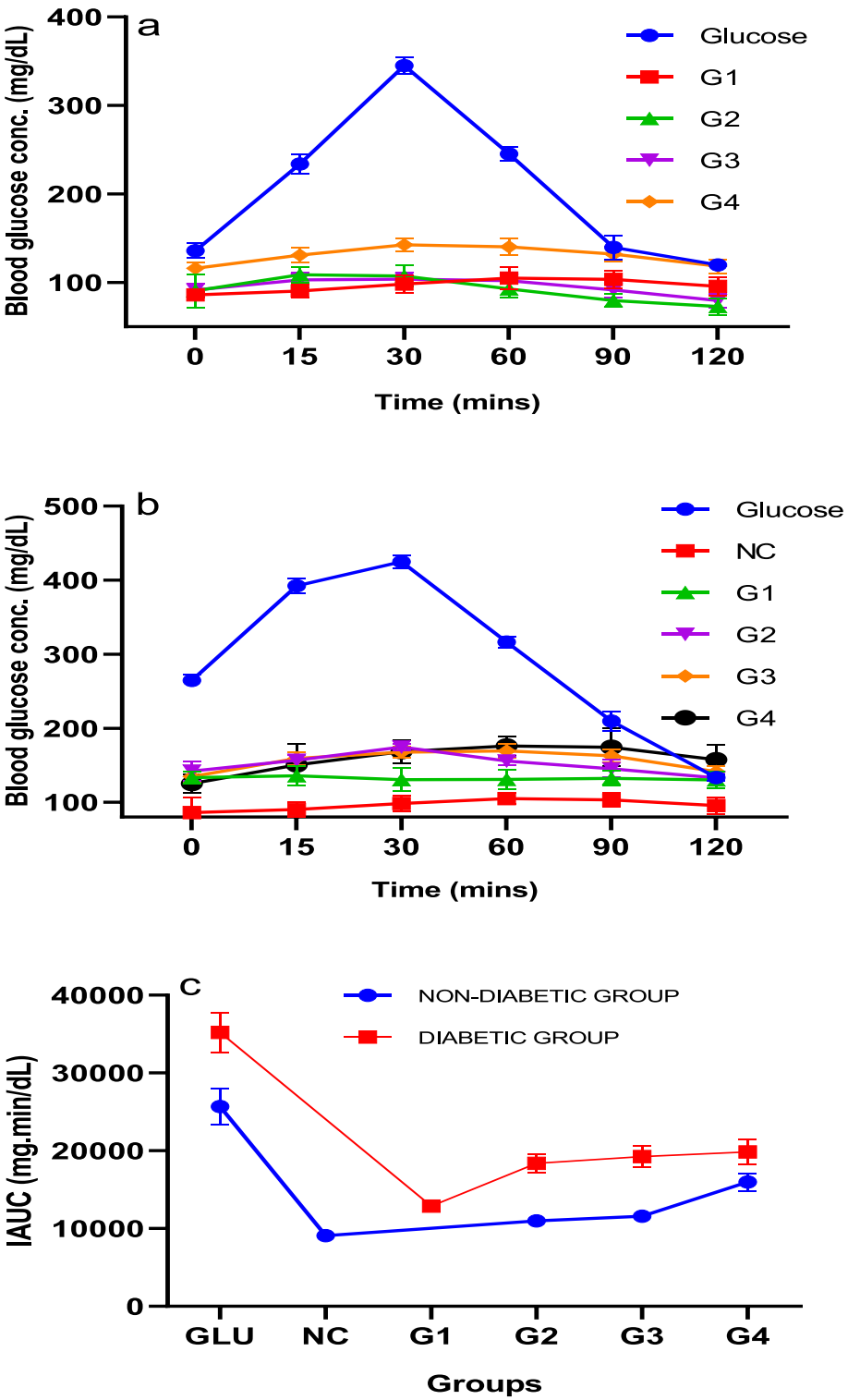


Fig. 1 **a** Blood glucose concentration in non-diabetic subjects. **b** Blood glucose concentration in diabetic subjects. **c** Incremental area under the glucose curve of study participants. Lines represent mean ± SEM ($n = 10$). NC: non-diabetic healthy group (positive control); G1: subjects that did not receive any test food (negative control); G2: subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); G3: subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); G4: subjects fed COB (commercial oat snack)

Table 5 Changes in blood glucose concentration after consumption of whole grain developed snack

Time (mins)	NC	G1	G2	G3	G4
0	86.08 ± 20.15^{d}	133.45 ± 7.68^{b}	142.08 ± 13.26^{a}	134.64 ± 6.94^{b}	125.36 ± 12.97^{c}
15	90.35 ± 9.86^{d}	135.79 ± 13.20^{c}	156.92 ± 7.64^{ab}	159.20 ± 8.50^{a}	150.45 ± 28.30^{b}
30	98.29 ± 10.13^{d}	130.63 ± 15.52^{c}	174.61 ± 4.49^{a}	167.73 ± 7.39^{b}	168.36 ± 16.21^{b}
60	105.00 ± 7.65^{e}	130.98 ± 13.63^{d}	155.85 ± 5.95^{c}	169.27 ± 9.43^{b}	175.91 ± 13.63^{a}
90	103.42 ± 9.20^{e}	132.26 ± 9.85^{d}	145.38 ± 12.09^{c}	162.91 ± 8.53^{b}	174.27 ± 25.93^{a}
120	95.60 ± 11.12^{d}	130.11 ± 10.93^{c}	132.85 ± 4.31^{c}	141.36 ± 7.85^{b}	157.45 ± 20.49^{a}

Values are presented as Mean ± SD; Values with different superscripts on the same row are significantly different ($P^{<}$ 0.05)

NC Non-diabetic healthy subject in a fasted state, *G1* Subjects that did not receive any test food (CTRL), *G2* Subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet), *G3* Subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet), *G4* Subjects fed COB (commercial oat snack)

Table 6 Glycemic index and glycemic of load multigrain snack bars in type 2 diabetic and non-diabetic subjects

Samples	CHO/100 g	Portion size (g)	CHO/portion (g)	Glycemic Index (GI)	Glycemic Load (GL)	Glycemic Index (GI)	Glycemic Load (GL)
MBY	59.23^{a}	84.4^{a}	49.99^{a}	52.19^{c}	23.46^{c}	42.77^{c}	12.67^{c}
MBZ	60.86^{a}	82.3^{a}	50.03^{a}	54.69^{b}	25.60^{a}	45.03^{b}	12.81^{b}
COB	56.49^{b}	88.6^{a}	50.02^{a}	56.38^{a}	23.87^{b}	62.24^{a}	31.13^{a}

Values represent mean of data set; values with different superscripts on the same column are significantly different ($P^{<}$ 0.05)

MBY Snacks bar 1 (snack comprising 90% amaranth, 5% acha, 5% pearl millet), *MBZ* (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); *COB* Oat snack bar control (control), *CHO* Carbohydrate

The glycemic index (GI) of snack bar products ranged between 52 and 56 while glycemic load (GL) ranged btw 23 and 26 for diabetic subjects (Table 6). However, for non-diabetic subjects, the GI obtained in response to consumption of a serving of protein-rich snack was 43 and 45 for MBY and MBZ respectively, COB showed GI of 62 while GL for the formulated snacks was approximately 13 and for COB, GL was 31. The international classification of GI is as follows: ≤ 55 (low), 56 to 69 (intermediate), or ≥ 70 (high). The developed snack products had low glycemic indices whereas the commercial sample (COB) possessed an intermediate GI for both groups. Low GI of snacks is indicative of slow release of glucose upon digestion of food thereby controlling glucose release into the blood. This corroborates the result of blood glucose response (Table 5) wherein slow postprandial glucose release was observed especially for G2 who ingested MBY. As is known, several characteristics of food influence its glycemic response; these characteristics include the quantity and type of carbohydrates, presence of other nutrients (such as protein, fat, fibre). Hence, the low GI of the multigrain snack bars may be associated with the low carbohydrate content, the high protein and fibre contents (Table 3) as well as the quality of carbohydrates in the major amaranth grain (Durgadevi & Nazni 2012; Schneider et al. 2015). In addition, the sweetener in the developed snack bar is fruit sugar (fructose); which is reported to have low GI compared to sucrose with high GI; a notable sweetener in commercial snack bars (brown sugar) or control (white sugar) which is sucrose with high GI (Alkaabi et al. 2011; Alalwan et al. 2020). Hence, regular consumption of developed multigrain snack products may depress the surge in postprandial blood glucose as such pivotal in reducing the risk of type 2 diabetes, cardiovascular disease and its accompanying complications. Alkurd et al. (2020) reported intermediate GI (58.94) for multigrain bread from whole wheat and different grains. The approved glycemic load (GL) classification is < 10 for low, 11–19 represents moderate, and > 20 is high. From our result, GL of subjects ranged from 23.46 to 25.60 thus, the snack had a high GL. Although, diets low in GI and GL are relevant to the prevention and management of diabetes, the GL may be significantly reduced by increasing the nutritional components of the snack such as the protein and or fibre contents likewise reduction of the portion size per serving (Brand-Miller 2003).α-amylase and α-glucosidase are key enzymes responsible for the breakdown of starch. The former functions to hydrolyze internal α-1,4 glycosidic bonds, resulting in the production of maltose and oligosaccharides whereas, the latter is a crucial enzyme responsible for catalyzing the final stage of carbohydrate digestion (Kashtoh & Baek 2022). The highest concentrations of amylase are found in the pancreas and salivary glands, although, equally abundant in other organs (Nater et al. 2015). Pancreatic amylase enters the bloodstream

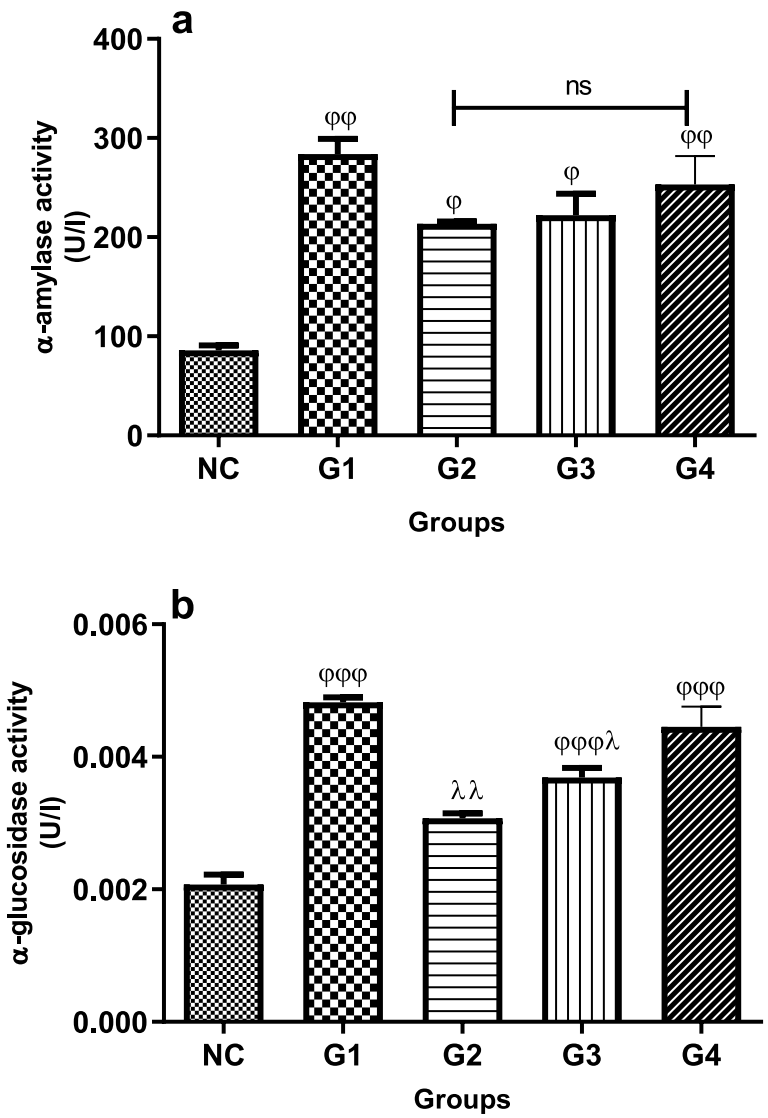


Fig. 2 **a** α-amylase. **b** α-glucosidase activities in serum of experimental subjects. Bars represent mean ± SEM ($n = 10$). Values are statistically different at $^{\varphi}p < 0.05$, $^{\varphi\varphi}p < 0.01$, $^{\varphi\varphi\varphi}p < 0.001$ versus NC and $^{\lambda}p < 0.05$, $^{\lambda\lambda}p < 0.01$ versus G1. NC: non-diabetic healthy group (positive control); G1: subjects that did not receive any test food (negative control); G2: subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); G3: subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); G4: subjects fed COB (commercial oat snack)

and can easily be measured in the blood (Pieper-Bigelow et al. 1990). High amylase activity in serum could be an indicator of a pancreatic disorder especially acute pancreatitis and there exists an established relationship between diabetes and pancreatitis as the former is an etiology to the latter (Hart et al. 2016; Wynne et al. 2019; Richardson & Park 2021). Normal serum α-amylase ranges from 10–150 U/l (Zhuang et al. 2016). According to Fig. 2a, the total serum amylase activity in healthy subjects was low (85 U/l) which is within the normal range. However, significantly high activity was observed in diabetic subjects (283 U/l), which is a potential biomarker of acute pancreatitis in the subjects. Nevertheless, groups that consumed formulated snacks (MBY and MBZ) exhibited 24.73 and 21.72% reduction, respectively. Worthy of note is the fact that there was no significant difference in the α-amylase activity among the groups fed with the multigrain products, but significantly differed (*P* value = 0.0034) from G1 which served as negative control with no dietary treatment. This suggests that consistent consumption of the novel snack may promote a significant reduction in the enzyme. Serum α-glucosidase activity showed similar results although, there was significant difference among the groups (*P* value = 0.0037) however,

no significant difference between diabetic groups and group that consumed COB. The potency of most diabetic drugs is as a result of their α-glucosidase inhibitory activity as the enzyme inhibitor happens to be the most effective in reducing post-prandial hyperglycemia (Hossain et al. 2020). α-glucosidase inhibitors function to delay glucose absorption by decreasing carbohydrate digestion and indirectly decreasing insulin surge. Figure 1b shows that the NC group had the lowest α-glucosidase activity (0.0021 mol/mg protein) while G1 and G4 had the highest (0.0048 and 0.0044 mol/mg protein, respectively). Groups G2 and G3 showed a significant decrease of 35.42 and 22.92% respectively in α-glucosidase activity. This is suggestive that the snack products' carbohydrate digestion may be delayed, conferring a positive effect on glucose absorption.

DM is characterized by persistent hyperglycemia; the condition that promotes biochemical alterations in glucose and lipid peroxidation. Chronic diabetic condition promotes impaired compensatory response to the antioxidant system or increased generation of free radicals thus, diabetic subjects may have a compromised antioxidant defense system. Serum antioxidant status is an important indicator in the control and management of diabetes owing to its role in it plays in the protection against free radicals and oxidative stress. Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are enzymatic antioxidants while reduced glutathione (GSH) is a non-enzymatic antioxidant; all these biomolecules play important synergistic role as a defense mechanism from free radicals thereby protecting living cells/organs from oxidative damage. The results for antioxidant enzyme activities are as shown in Fig. 3a-d.

Catalase is an important enzyme responsible for the neutralization of hydrogen peroxide (H_2O_2), a non-radical reactive oxygen species (ROS), hence, maintaining an optimum level of H_2O_2 in the cell. Figure 2a presents the serum catalase activity in the different groups which ranged from 2.28 to 9.92 μmole H_2O_2.consumed/min/mg protein. G1 and G4 showed the lowest activity (2.28 and 3.03 μmole H_2O_2.consumed/min/mg protein respectively). The significantly reduced CAT activity in the G1 group's serum suggests overwhelming oxidative stress as a result of a diabetic condition, thus, indicating increased production of oxygen-free radicals, which could be linked with persistent hyperglycemia, otherwise known as high blood glucose (Takemoto et al. 2009). The notable low levels of the enzyme in G1 corroborates other reports that catalase levels are decreased in diabetic subjects compared to non-diabetic subjects (Ezeiruaku et al. 2016). This may not be unconnected to certain pathophysiologies of the DM disease. Significant ($p < 0.01$, $p < 0.001$) improvement in CAT activity was observed in G2 and G3 groups, the groups showed relative comparisons between groups with a relative P value of 0.006. The increased serum CAT activity by the experimental groups especially G2 is suggestive of the ability of the snack to promote cellular defense against oxidative damage.

NC group had a high SOD activity (95.24 U/l) inferring the availability of sufficient activities of SOD enzyme in the non-diabetic healthy individual (Fig. 3b). The SOD enzyme is responsible for the removal of superoxide radicals which are usually produced during any oxidation reaction process. However, the G1 and G4 groups showed a reduction in the activities of this enzyme (35 and 42% respectively) suggesting that substantial concentrations may have been used up in the process of combating oxidative stress as a result of the diabetic condition. G2 and G3 groups on the other hand showed upregulated levels of the enzyme (up to 115 and 87 U/l respectively) activity. Although, there was no significant difference between the study groups and normal control but, a significant difference within the group existed (P value = 0.005).

GSH is an important antioxidant with relevance in protecting cells against oxidative damage and eventually chronic disease. As seen for other antioxidant enzymes, the NC presented the highest GSH level (1.62 mg/g protein) while G1 had the least level (0.36 mg/g protein) of GSH content (Fig. 3c). Interestingly, there was no significant difference in the levels of GSH content (0.70 to 0.82 mg/g protein) within the experimental groups (G2, G3, G4) whereas, significantly different from NC and G1 as the former had very high GSH level while the latter had very low levels (P value = 0.0002). GSH can be obtained from the consumption of foods rich in sulfur-containing amino acids moreover, these amino acids are vital for the maintenance of GSH homeostasis. Grains, however, have little glutathione precursor amino acids; this may be the reason for the low GSH levels in the snack-fed group. Overall, the significantly low levels of each antioxidant enzyme in the diabetic subjects as compared to normal healthy subjects is a pointer to the prevalence of oxidative stress and indicative of impaired antioxidant status in diabetic patients. However, the groups that consumed the experimental multigrain snack exhibited significant levels of upregulation in the antioxidant profile enzymes. The current finding corroborates the results of Karn et al (2016) in which consumption of a multigrain-formulated diet exhibited a positive effect on diabetic animals with respect to antioxidant status.

Diabetes mellitus is associated with increased free radical activity and is characterized by alterations in carbohydrate, protein, and lipid metabolism. Hyperlipidemia is common in patients with type 2 diabetes, the condition may induce increased production of lipid peroxides

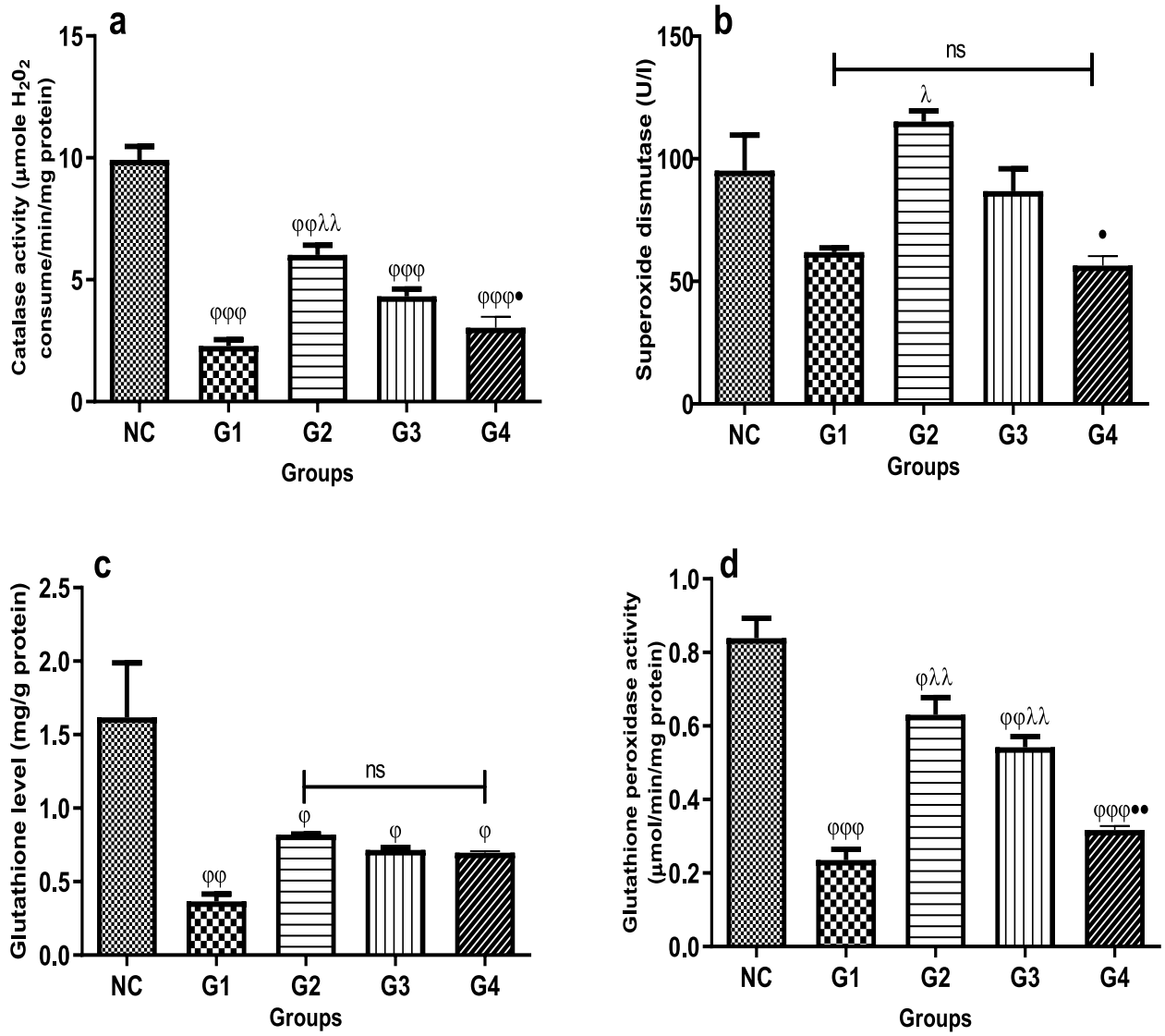


Fig. 3 **a** Catalase activity. **b** Superoxide dismutase activity. **c** Glutathione level. **d** Glutathione peroxidase activity in subject serum. Bars represent mean ± SEM ($n = 10$). Values are statistically different at $^{\varphi}p < 0.05$, $^{\varphi\varphi}p < 0.01$, $^{\varphi\varphi\varphi}p < 0.001$, ns versus NC and $^{\lambda}p < 0.05$, $^{\lambda\lambda}p < 0.01$, ns versus G1, $^{\bullet}p < 0.05$, $^{\bullet\bullet}p < 0.01$ versus G2. NC: non-diabetic healthy group (positive control); G1: subjects that did not receive any test food (negative control); G2: subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); G3: subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); G4: subjects fed COB (commercial oat snack), ns: no significant difference

which is an indication of a decline in the cellular antioxidant defense mechanism. Lipid peroxides were measured as thiobarbituric acid reactive substances (TBARS). The TBARS level in the serum of subjects was found to be significantly ($P < 0.05$) elevated in G1 (diabetic untreated group) compared to control subjects (Fig. 4). TBARS levels increased twofold (0.36 mmol/mg protein) in diabetics compared to healthy controls (0.15 mmol/mg protein). The high lipid peroxidation in diabetic subjects evidenced by elevated TBARS may exacerbate the occurrence of vascular complications. The ingestion of a multigrain experimental diet, however, promoted a reduction (47% for MBY, 28% for MBZ) in the TBARS level which showed no significant difference from the NC group.

Significant ($P < 0.01$) insulin spikes (up to 78 mIU/mL) were observed in diabetic subjects (Fig. 5). These may be a consequent result of type 2 diabetes condition associated with the buildup of glucose in the bloodstream. This is suggestive of hyperinsulinemia which is a risk factor pivotal for promoting insulin resistance and the development of type 2 diabetes (Janssen 2021). The established

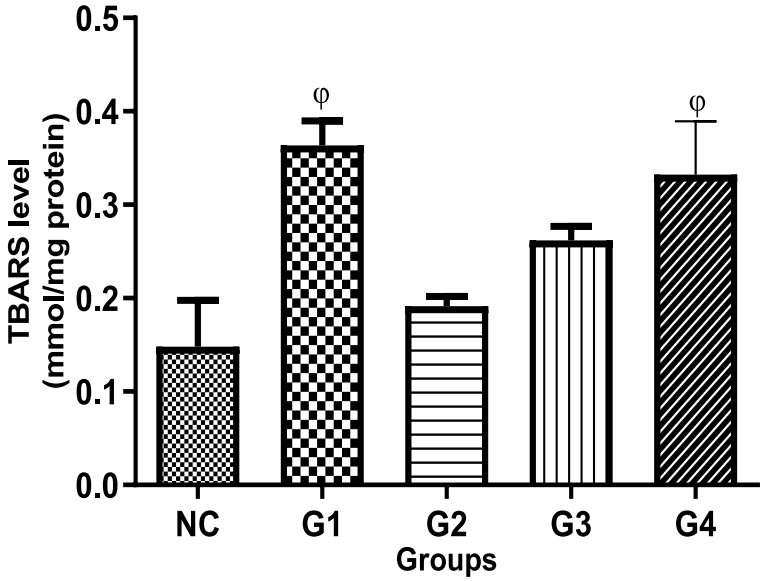


Fig. 4 Thiobarbituric acid reactive substance levels in serum of human subjects. Bars represent mean ± SEM ($n = 10$). Values are statistically different at $^{\varphi}p < 0.05$ versus NC. NC: non-diabetic healthy group (positive control); G1: subjects that did not receive any test food (negative control); G2: subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); G3: subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); G4: subjects fed COB (commercial oat snack)

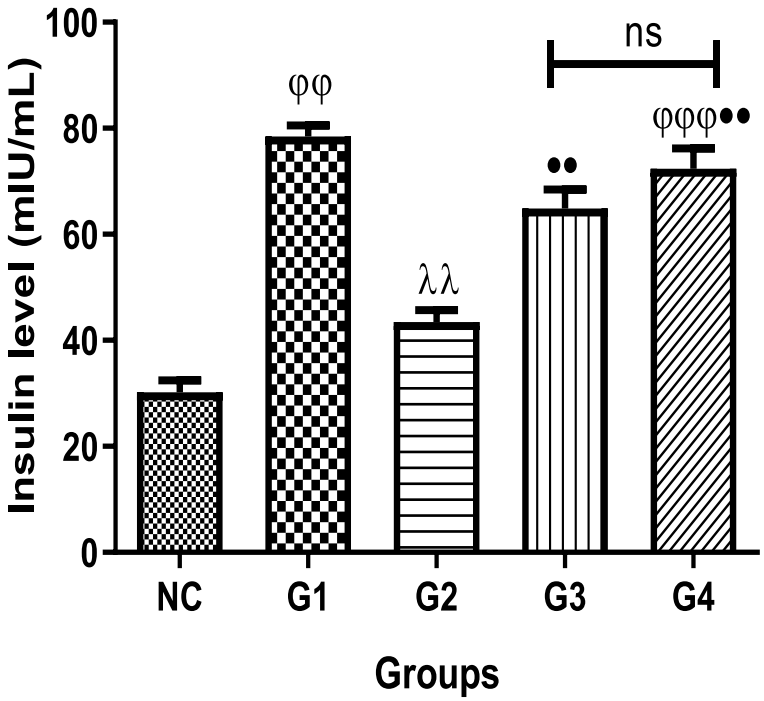


Fig. 5 Insulin levels in serum of human subjects. Bars represent mean ± SEM ($n = 10$). Values are statistically different at $^{\varphi\varphi}p < 0.01$, $^{\varphi\varphi\varphi}p < 0.001$, $^{\lambda\lambda}p < 0.01$, ns versus G1, $^{\bullet\bullet}p < 0.01$ versus G2. NC: non-diabetic healthy group (positive control); G1: subjects that did not receive any test food (negative control); G2: subjects fed MBY (snack comprising 90% amaranth, 5% acha, 5% pearl millet); G3: subjects fed MBZ (snack comprising 47.98% amaranth, 26.68% acha, 25.34% pearl millet); G4: subjects fed COB (commercial oat snack), ns: no significant difference

overweight and obese status of the participants (diabetic subjects) as depicted by the BMI (Table 4) is a pointer to the correlation of obesity with hyperinsulinemia/insulin resistance. Figure 4 showed that the consumption of the protein-rich, low GI multigrain snack regulated the secretion of insulin in diabetic subjects. The decreased serum insulin levels in groups that consumed test meals may be attributed to the low glycemic index of the snack product thus, offering a lower prevalence of metabolic effects. This may suggest that the multigrain snack did not rapidly increase the blood glucose levels of the subjects especially sample MBY comprising 90% amaranth, 5% acha, and 5% pearl millet as G2 showed the lowest insulin level. The high content of dietary fiber in amaranth grain may have contributed to this outcome. This result corroborates an earlier report that a low glycemic index meal decreases insulin resistance and insulin levels (Gao et al. 2019). Similar to our findings, Sobhana et al (2020) reported that consumption of multigrain roti promoted reduced serum insulin in type 2 diabetic participants. G2 and G3 exhibited postprandial insulin concentrations averaging 43 and 65 mIU/mL respectively. The level of insulin may be sufficient to effectively remove glucose from the bloodstream.

Conclusions

Varied reports have shown that there is an existing limitation in the diversity and suitability of snack products for diabetic patients. The developed high protein, low glycemic index snack product is a convenient nutritious and healthy product with potential suitability for this class of patients. The outcome of this study may serve as basic data for the development of novel products for individuals requiring postprandial blood glucose control especially diabetic and pre-diabetic. Dietary intervention such as the consumption of multigrain snack bars especially MBY (an optimized blend of amaranth, acha, and pearl millet; 90:5:5) with low glycemic index and potential nutraceutical properties may be a long-term dietary nutrition or integral self-care approach alongside lifestyle changes for effective diabetes management.

Acknowledgements

The authors express profound gratitude to all the diabetic patients and non-diabetic subjects who consented and participated in the clinical experiment. Also, immense appreciation to the members of staff, Medical Out-Patient Department of the University of Medical Sciences Teaching Hospital, Akure, Nigeria for their technical support during the study. Finally, to Mrs. Kikelomo Paseda for her technical support in navigating the study protocol.

Authors' contributions

AI, SA, IS conceptualized and designed the experiment. AI, TI, IS performed formal analysis. AI, IS, SA collated the data. AI, TI, SA performed statistical analysis of data. AI compiled the first draft. AI, IS, SA, ET, AC revised the draft. AI, TI sourced the required funds. All authors read and approved the final draft.

Funding

This research did not receive any funding from the public, private nor government agency.

Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The subjects consented to participate in the study, the clinical study was carried out following guidelines for human studies with the approval of the Ondo State Health Research Ethics Committee (OSHREC), Ondo State (OSHREC 22/3/2021/316). We hereby certify that the study was performed in accordance with the 1964 Helsinki Declaration and comparable ethical standards. In addition, the clinical study was conducted in accordance with the guidelines as outlined by the Ethics Committee of the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria (approval number FUTA/SAAT/2021/024).

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 30 March 2023 Accepted: 30 May 2023
Published online: 02 March 2024

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