# Influence of Whole Wheat Flour Substitution and Sugar Replacement with Natural Sweetener on Nutritional Composition and Glycaemic Properties of Multigrain Bread

### Aderonke Ibidunni Olagunju

Department of Food Science and Technology, Federal University of Technology, Akure, Ondo State 340001, Nigeria

**ABSTRACT:** Prevalence of diabetes mellitus (DM) is rising globally and largely due to dietary lifestyle changes and urbanization. The concept of glycaemic index (GI) as a dietary tool for the management of DM has been recommended by international health organizations. Whole-grain wheat flour (WWF), 'acha' flour (AF), and pigeon pea flour (PPF) were combined in different ratios (80:10:10, 70:15:15, 70:20:10, and 60:20:20) and 100% WWF served as control. Bread was produced from the flour blends using white sugar (sucrose) and/or date palm fruit sugar (DPFS) representing 50 or 100% sugar replacement. Physical attributes, nutritional composition, GIs, and consumer acceptability of the breads were evaluated using standard methods. The multigrain breads had lower values for height, volume, and specific volume, but were most dense than the control [WWF+sugar (WAPC)]. The protein, ash, and crude fibre contents of the breads were significantly improved compared with the control, especially breads containing 100% DPFS, whereas carbohydrate and energy contents were comparable. The breads also contained significant amounts of macro and micro elements and a Na/K ratio of less than 1. Phytate/mineral molar ratios of the bread were lower than the respective critical limits. Multigrain breads showed low GI, especially those with >20% whole wheat substitution and 100% DPFS compared with WAPC, with intermediate GI (65.61) and high glycaemic load (GL). WWF+AF+PPF+DPFS (60:20:20:100) exhibited the highest protein content, a significant fibre content, lowest carbohydrate, GI, GL, and postprandial blood glucose responses, thus may be a suitable dietary guide for sustained health.

Keywords: multigrain bread, date palm sugar, glycaemic index, blood glucose response

## **INTRODUCTION**

The prevalence of diabetes mellitus (DM) is increasing as a result of dietary lifestyle changes, urbanization, and consumption of calorie-rich diets, including an increasing consumer demand for ready-to-eat bakery products. This is generating great medical and research concern and constituting great financial and economic burden. Bread is a common staple food widely consumed by people all over the world. It is a calorie-rich food that represents a major source of energy in human nutrition; moreover, ingestion of carbohydrate is accompanied by increased blood glucose levels and bread has been categorized as a high glycaemic index (GI) food (Hettiaratchi et al., 2009; Lafiandra et al., 2014; Stamataki et al., 2017). Long-term consumption of high GI foods promotes increased insulin demand and can lead to insulin resistance which results in type II diabetes, obesity, and cardiovascular diseases (Augustin et al., 2002; Brand-Miller et al., 2002). The concept of GI as a dietary tool in the management of DM has been recommended by the Food and Agricultural Organizations (FAO) and the World Health Organization (WHO). Bread is known to be a high GI product. The GI database shows breads prepared with refined wheat flour has a mean GI of 75 and a range of 59~89 (The University of Sydney, 2012). However, carbohydrate-rich foods with low glycaemic impact have been shown to be useful for the prevention and management of type II diabetes and other associated diseases (Augustin et al., 2015). White wheat flour is the major raw material for bread, substituting it with other flours have been reported to promote the glycaemic status of bread, and thus reduce postprandial blood glucose responses (Lanzerstorfer et al., 2018). Studies have shown that consumption of whole grain products is associated with reduced risk of oxidative stress related to chronic diseases and age-related disor-

Copyright © 2019 by The Korean Society of Food Science and Nutrition. All rights Reserved.

Received 25 July 2019; Accepted 4 November 2019; Published online 31 December 2019

Correspondence to Aderonke Ibidunni Olagunju, Tel: +234-803-726-7262, E-mail: aiolagunju@futa.edu.ng Author information: Aderonke Ibidunni Olagunju (Researcher)

<sup>☺</sup> This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ders, such as cardiovascular diseases (Ye et al., 2012; Aune et al., 2016). This significant contribution may be attributed to the presence of dietary fiber and antioxidants (Yu et al., 2013). The application of composite flours in the development of food products such as baked goods have been reported by many researchers (Olaoye and Ade-Omowaye, 2011; Ekunseitan et al., 2016; Ayele et al., 2017). Moreover, Olaoye et al. (2007) reported that substitution of up to 15% of composite flour baked products has potential to reduce production cost while enhancing the utilization of local crops.

The development of nutritious, healthy bakery products such as bread is important as the product forms a major part of the human daily diet. This can be achieved by using composite flours comprising of grain crops (cereal and legume grain) and incorporating plant materials rich in antioxidants; low GI can generally reduce overall carbohydrate metabolism. Several reports describing the development of nutritious bread from composite flours comprising wheat and other flours are now available (Olaoye and Ade-Omowaye, 2011; Bhatt and Gupta, 2015; Almoraie, 2019). Studies have evaluated the physicochemical properties and nutritional quality of bread. However, majority of the previous reports did not focus on developing bread with a low dietary GI from wholegrain wheat-based composite flour nor used the selected cereal/legume grains in the current study. Multigrain products are an emerging area of the market, thus there is need to provide useful information on the nutritional composition and GI of multigrain bread so it can be promoted as a potential healthy food choice.

Whole-grain wheat flour comprising of wheat kernel (bran, germ, and endosperm), is an important raw material for baking. It is a rich source of antioxidants and contributes high amounts of fibre, minerals, and vitamins to baked products (Souza et al., 2011), which are lacking or present in very low quantities in refined white flour.

'Acha' is an underutilized cereal widely cultivated in northern Nigeria that is nutritionally superior compared with wheat in terms of protein, amino acids, and minerals, and which evokes low sugar levels upon consumption (Ayo et al., 2007). However, consumption of acha is not widespread and is limited to local Northern dishes (porridge and couscous) and non-alcoholic beverage. Thus, supplementing acha with whole wheat grain flour is a possible means of diversifying its application and promoting cultivation. Recent studies have considered its use in development of nutritious baked goods and their findings elucidates on the potential of 'acha' as a raw material for baking industry (Nanyen et al., 2016; Olagunju et al., 2018).

Pigeon pea is among the most important grain legumes, and is grossly underutilized owing to its hard-to-cook phenomenon. However, pigeon pea is a rich source of protein, B-complex vitamins, minerals, and fibre (Fasoyiro, 2015) and has been reported by several researchers to possess low GI (Panlasigui et al., 1995; Oboh et al., 2010; Devindra et al., 2017). Thus, a blend of the three selected grains will generate a healthier baked product aimed at curtailing the rapidly progressing diabetes menace.

Sugar is the second major ingredient used in formulation of baked products, mainly because it is responsible for promoting golden brown crusts, improved crumb textures and moisture retention in the crumb. Sugar is also an important ingredient for dough fermentation in bread making while imparting sweetness to the baked products. Refined sugar used in baked goods is rich in calories and possesses high GI, and contributes no essential nutrients nor apparent health benefit. Increased intake of sugar has been proven by research to be a major cause of elevated blood glucose concentrations, inducing metabolic problems such as type II diabetes and obesity (Manickavasagan et al., 2013). The recent epidemic of these metabolic diseases has spurred research targeted at improving glycaemic responses of food products, in particular common staples like bread by selecting ingredients without glycaemic contributory potential. Many sugar substitutes are available for use in baking especially for people requiring low-calorie alternatives, such as those diabetes which premeditates limited sugar/calorie consumption. Natural alternatives to refined sugar for use in bakery products include honey, maple syrup, agave nectar, molasses, corn syrup, raw cane sugar, and fruit sugars (Phillips et al., 2009). Date palm (Phoenix dactylifera) is an important sugar substitute, a sweet edible fruit containing more than 70% sugar (mainly glucose and fructose), thus a high energy food source. Date palm is nutritionally beneficial for patients battling with metabolic disorders such as diabetes due to its lower sucrose content, and high contents of fibre, antioxidants, and flavonoids (Hamza et al., 2014). Date palm fruits are also good sources of iron (Fe) and potassium (K), and a minor source of essential amino acids (Hamad et al., 2015). Some studies (Obiegbuna et al., 2013; Bhise and Kaur, 2014; Nwanekezi et al., 2015) have substituted sugar with alternative sweeteners for bread making but did not assess the glycaemic impact. The current research therefore focusses on developing a nutritious and healthy product using composite flours from multigrain crops, and replacing refined sugar with an alternative natural sweetener.

Multigrain products are evolving as important components of healthy diets and as an excellent way to increase consumption of whole grains. Thus, in the present study, we sought to evaluate the physical characteristics, nutritional properties, GIs, and consumer acceptability of breads containing substituted flour with date palm fruit sugar (DPFS) partially or totally replacing refined sugar.

### MATERIALS AND METHODS

#### Source of raw materials

Wheat grains (*Triticum aestivum*), pigeon pea (*Cajanus cajan*), and date palm fruit (*P. dactylifera*) were purchased from Oba market, Akure, Ondo State, Nigeria. 'Acha' (*Digitaria exilis*) was purchased from a central market, Minna, Niger State, Nigeria. Other baking ingredients, such as margarine, salt, milk, sugar, ascorbic acid, and yeast were purchased from Ceci supermarkets, Alagbaka, Akure, Ondo State, Nigeria. All chemicals used were of analytical grade.

#### Sample preparation

**Preparation of whole wheat flour (WWF)**: WWF was obtained by cleaning wheat grains to remove dirt, stones and other extraneous materials, milling the wheat grains to powder, and sieving the flour through a 0.35 mm mesh sieve.

**Preparation of DPFS**: DPFS was produced by washing the date palm fruits with clean water to remove adhering dirt, followed by removing the seeds (de-pitting) manually and cutting the fruits into small pieces using a knife. The pulp with pericarp were oven dried at 45°C for 72 h, milled using a professional mill and finally sieved through a 0.35 mm mesh sieve to obtain a fine homogenized flour. **Preparation of acha flour (AF)**: AF was produced using the method of Ayo et al. (2007). Acha grains were winnowed to remove chaff and dust. Adhering dust and stones were removed by washing in water (sedimentation). The washed and destoned grains were dried in a cabinet drier at 45°C for 24 h. The dried grains were milled using a professional mill and the flour was sieved using a 0.35 mm mesh size.

**Preparation of pigeon pea flour (PPF)**: Pigeon pea was processed into flour as described by Olagunju et al. (2018). The seeds were graded, cleaned, and soaked in hot water for 3 h, after which they were dehulled. The dehulled seeds were washed and oven-dried at 50°C for 48 h. The dried seeds were milled using a professional mill, sieved through a 0.35 mm sieve to obtain PPF, and packaged in airtight containers until use.

#### Formulation of composite flours

Composite flour of WWF, AF, and PPF were prepared as shown in Table 1. WWF (100%) served as the control. AF (10~20%) and PPF (10~20%) was used to substitute WWF. A total of four blends were prepared.

#### Production of multigrain bread

Bread was produced using the straight dough method (AACC International, 2000b). WWF-AF-PPF blend was used in the bread following the recipe shown in Table 2. DPFS was used as a replacement for granulated sugar at the following ratios: 0:100, and 50:50 (sugar : DPFS),

 Table 1. Composite flour blend ratios
 (unit: % by weight)

Sample	WWF	AF	PPF
А	100	0	0
В	80	10	10
С	70	20	10
D	70	15	15
E	60	20	20

WWF, whole wheat flour; AF, acha flour; PPF, pigeon pea flour.

Table 2. Bread recipe   (u					
Ingredient	Amount per	680 g loaf			
Flour/flour blend	396.00	100.00			
Sugar/date palm fruit	44.72	11.29			
Margarine	15.00	3.79			
Ascorbic acid	0.10	0.02			
Yeast	2.63	0.66			
Milk	11.29	2.85			
Salt	4.83	1.22			
Water	Varied (250 to 270 mL)	Varied (250 to 270 mL)			

which resulted in substitution of 50% and 100% of sugar, respectively. The dry ingredients were weighed in the required quantities and mixed. Next,  $250 \sim 270$  mL of water was added and mixed thoroughly ( $3 \sim 4$  min) to form the dough. The dough was then kneaded into smooth consistency. Upon completion, the dough was cut in sections of 680 g, moulded and placed into greased baking pans. The dough was proofed at room temperature for 90 min and baked in a pre-heated oven at 230°C for 35 min. Baked bread was left to cool to room temperature before packaging and further analysis.

#### Determination of physical and chemical properties

*Evaluation of physical attributes of multigrain breads*: The freshly baked loaves were allowed to cool and then evaluated for weight and height using the method of See et al. (2007). Loaf volume and specific volume were determined as described by AACC International (2000a). A digital balance (0.01 g accuracy) was used to measure the bread weight. Loaf volume was measured by the seed displacement method with slight modifications whereby sorghum seeds was used in place of rape seeds.

# Determination of proximate compositions of multigrain breads

Loaf samples were analyzed for moisture (air oven method), crude fat (soxhlet extraction method; method no. 950.36), crude protein (Kjeldhal method; method no. 950.36), ash (method no. 930.22), and crude fiber (method no. 950.37) according to the methods described by AOAC (2012). Total carbohydrate (CHO) content was calculated by the difference, as follows:

```
CHO=100-(% moisture+% crude fat+% crude
protein+% ash+% crude fiber).
```

The energy value (kcal/100 g) of the loaves was estimated using the Atwater factors for protein (4 kcal/100 g), carbohydrate (4 kcal/100 g), and fat (9 kcal/100 g).

#### Determination of mineral content of multigrain breads

The contents of the mineral elements sodium (Na), K, magnesium, manganese, Fe, zinc (Zn), and calcium (Ca) were determined using the 210 VGP (Buck Scientific Inc., Norwalk, CT, USA) and FP 902 Flame Photometer (PG Instruments, Leicestershire, UK) by atomic absorption spectroscopy (method no. 999.11) described by AOAC (2012).

# Determination of anti-nutrient composition of multigrain breads

**Determination of oxalate:** One gram of dried and ground bread samples were weighed into a 100 mL conical flask. Next, about 75 mL of 3 M  $H_2SO_4$  was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h, and was filtered using Whatman No. 1 filter paper. About 25 mL of sample filtrates were collected and titrated hot ( $80 \sim 90^{\circ}C$ ) against 0.1 M KMnO<sub>4</sub> solution to the point when a faint pink colour appeared that persisted for at least 30 s (Day and Underwood, 1986).

**Determination of phytate content:** The phytate content was determined according to the method of Wheeler and Ferrel (1971). Four grams of each dried and ground bread samples was soaked in 100 mL of 2 % HCl for 3 h and then filtered through Whatman No. 1 filter paper. Aliquots (25 mL) of filtrate were placed inside conical flasks, and 5 mL of 0.3% ammonium thiocyanate solution was added as an indicator; 53.5 mL of distilled water was then added for proper acidity and the solution was titrated against 0.00566 g/mL of standard FeCl<sub>3</sub> solution that contained approximately 0.00195 g of Fe per mL until a brownish yellow colouration was observed that persisted for 5 min.

**Determination of tamin content**: Approximately 0.2 g of dried and finely ground bread samples were weighed into 50 mL sample bottles; 10 mL of 70 % aqueous acetone was then added and the solutions were properly covered. The bottles were placed in an ice bath shaker and were shaken for 2 h at 30°C. Each solution was then centrifuged, and the supernatants were stored in ice until use. Aliquots (0.2 mL) of each solution were pipetted into test tubes and 0.8 mL of distilled water was added to each. Standard tannin acid solutions were prepared from 0.5 mg/mL of the stock and solutions were made up to 1

mL with distilled water. Folin-Ciocalteu reagent (0.5 mL) was added to the samples and the standards, followed by 2.5 mL of 20%  $Na_2CO_3$ . Solutions were then vortexed and incubated at room temperature for 40 min. The absorbance was read at 725 nm against a blank, and a solution of the same concentration was prepared from a standard tannic acid curve (Makkar, 1993).

**Determination of phytate/mineral molar ratio:** The phytate/ mineral mole ratio was determined by dividing the weights of phytate and the investigated minerals by their atomic weights (phytate, 660 g/mol; Fe, 56 g/mol; Zn, 65 g/mol; Ca, 40 g/mol). The molar ratio between phytate and the minerals were obtained by dividing moles of phytate by moles of each mineral.

### Determination of the GI and glycaemic load (GL) of multigrain breads

Fifty-five Wistar albino rats (body weights  $140 \sim 150$  g) were divided into 11 groups (5 rats/group), and were housed individually in metabolic cages in a climate-controlled environment with free access to feed and water. The rats were allowed to acclimatize to the new environment for 5 days. After the adaptation period, the animals were re-weighed and fasted for 12 h (overnight fast). The blood glucose levels of the rats were assessed from the tail vein at time 0. Rats were then fed 2.0 g of the bread samples and glucose (control group), which were consumed within 25 min. Serum glucose levels were measured using an automatic glucose analyzer ('Accu-chek Active' Diabetes monitoring kit; Roche Diagnostic, Basel, Switzerland) at intervals of 15 min for the first half hour, then at intervals of 30 min until 120 min. The glycaemic response was determined as the incremental area under the curve (IAUC) of blood glucose measured geometrically from the blood glucose concentration-time graph ignoring the area beneath the fasting level (Wolever et al., 1991).

$$\frac{\text{IAUC of blood glucose (2 h) for food samples (2.0 g)}}{\text{IAUC of blood glucose (2 h) for glucose (2.0 g)}} \times 100$$

The GL of each bread sample was determined by the method of Salmerón et al. (1997). GL was calculated by multiplying the percentage of the food's carbohydrate content in a typical serving food with its GI value, using the formula below:

$$GL = \frac{\text{Net carbohydrate (g)} \times GI}{100}$$

Net carbohydrate=Total carbohydrates in the food sample.

The animal study was carried out in accordance with guidelines for animal experiments approved by the Ethics Committee of School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria (approval number FUTA/SAAT/2019 /013).

#### Sensory attributes of multigrain breads

Sensory evaluation of the multigrain bread were carried out about 6 h after baking using a 30-member randomly recruited semi-trained panel. The panel (10 male and 20 females) comprised of staff from the Federal University of Technology, Akure, Ondo State, Nigeria and members of community. All participants were 30 to 55 years of age, and freely consented to participating in the sensory evaluation. The criteria for selection included regular consumption of whole wheat bread, were not sick and not hungry. Panelists were subjected to a 30-min briefing about the use of sensory evaluation procedures. The samples were coded with 3-digit random numbers and presented in identical containers in a random order. Panelists were seated in individual sensory booths and were provided with a glass of deionized water to rinse their palates. Water was used to rinse their mouths before tasting each bread sample. Panelists were requested to rate the bread for appearance, taste, texture, flavour, appearance, and overall acceptability by assigning a score based on a nine-point hedonic scale (9, like extremely; 8, like very much; 7, like moderately 6, like slightly; 5, neither like nor dislike; 4, dislike slightly; 3, dislike moderately; 2, dislike very much; 1, dislike extremely).

The sensory study was carried out in accordance with guidelines for human studies approved by the Ethics Committee of School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria (approval number FUTA/SAAT/2019/013).

#### Statistical analysis

Data were generated in triplicate and analyzed by the Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 17.0. Means were compared using Duncan's multiple range test at 95% confidence level. GraphPad Prism 7.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA) was used to calculate incremental area under the curve and other statistical analysis.

# **RESULTS AND DISCUSSION**

#### Physical characteristics of multigrain breads

Physical characteristics of the breads was evaluated to determine the effect of supplementation with AF and PPF on loaf weight, height, volume, and specific volume (Table 3). Results showed that breads made from composite flours, especially those with 50% sugar substitution, were denser (650.15~738.11 g) compared with WAPC (100% whole wheat bread). Both WAPC and breads with 100% sugar substitution were lighter (610.09 g and  $650.08 \sim 658.69$  g, respectively). The observed increase in weight of the composite bread may be a desirable quality as consumers are often attracted to denser bread since it is believed to have more substance and thus be better value for money (Shittu et al., 2007). According to Wu et al. (2009), bread weight is determined by the amount of baked dough, moisture, and CO<sub>2</sub> which diffuses out of the loaf during baking; a reduction in CO<sub>2</sub> retention capacity in composite flours may be responsible for increased loaf weight. However, loaf height, volume, and specific volume was higher for WAPC (6.63 cm, 1,111.67  $cm^3$ , and 1.82  $cm^3/g$ , respectively) than breads with 50% and 100% sugar substitution. The lower values observed for breads with sugar replacement may be attributable to

Table 3. Physical characteristics of multigrain breads made from whole wheat flour substituted with acha and piged	n pea flours
--	--------------

Sample	Height (cm)	Weight (g)	Volume (cm <sup>3</sup> )	Specific volume (cm <sup>3</sup> /g)
WAPC	6.63±0.08ª	610.09±0.58 <sup>f</sup>	1,111.67±0.60 <sup>b</sup>	1.82±0.11 <sup>a</sup>
WDPC	6.30±0.32 <sup>b</sup>	621.26±1.54 <sup>e</sup>	992.82±0.59 <sup>d</sup>	1.57±0.06 <sup>b</sup>
WDPJ	5.23±0.15 <sup>cd</sup>	658.69±0.98 <sup>c</sup>	960.00±1.15 <sup>e</sup>	1.46±0.12 <sup>c</sup>
WSDJ	5.33±0.12 <sup>c</sup>	738.11±0.47 <sup>a</sup>	1,170.00±1.15 <sup>a</sup>	1.59±0.10 <sup>b</sup>
WDPK	4.97±0.09 <sup>de</sup>	646.16±0.22 <sup>d</sup>	945.00±0.87 <sup>f</sup>	1.46±0.12 <sup>c</sup>
WSDK	5.17±0.09 <sup>cd</sup>	674.25±0.72 <sup>b</sup>	1,070.00±1.15 <sup>c</sup>	1.59±0.08 <sup>b</sup>
WDPL	5.08±0.11 <sup>cd</sup>	655.46±0.84 <sup>c</sup>	945.00±0.29 <sup>f</sup>	1.44±0.11 <sup>cd</sup>
WSDL	6.10±0.09 <sup>b</sup>	674.38±0.25 <sup>b</sup>	960.00±1.15 <sup>e</sup>	1.42±0.03 <sup>cd</sup>
WDPM	4.17±0.12 <sup>f</sup>	650.08±0.04 <sup>cd</sup>	710.00±0.58 <sup>f</sup>	1.09±0.09 <sup>e</sup>
WSDM	4.67±0.07 <sup>e</sup>	650.15±0.08 <sup>cd</sup>	910.00±0.58 <sup>e</sup>	$1.40\pm0.06^{d}$

Values are mean±SD of triplicate determination.

Values with different letters (a-f) within the same column are significantly different ( $P \le 0.05$ ).

WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:100); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPL, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50).

reduced availability of yeast food (sugar) for fermentation. The decrease in loaf height may result from the decreased amount of readily available fermentable sugar limiting dough rising. Badifu et al. (2005) reported that dough height to some extent depends on the volume of gas (CO<sub>2</sub>) production during fermentation (proofing), as well as the protein matrix and gluten level. The present study corroborates this report since there was an observable decrease in bread height relative to the increase in flour substitution as a result of gluten dilution/reduction. Also, bread loaf volume may be affected by protein quality and quantity. Gluten development is associated with a mixture of wheat flour and water forming a matrix resulting in increased gas retention, thereby increasing loaf volume and height. The presence of more available gluten protein in WWF is presumed responsible for the greater loaf volume and height observed in WAPC. However, addition of AF and PPF lowered the amount of gluten in the blends, resulting in poor CO<sub>2</sub> retention, and therefore reduced the volumes and heights of the composite breads. Further, blends with increased amounts of PPF showed lower volumes; this may be due to the relatively large particles of legume flours which play a significant role in reducing loaf volume by puncturing gas cells during dough expansion (Chilungo, 2013).

Specific volume is the volume per unit weight of a loaf, and is generally adopted in the literature as a more reliable measure of loaf size (Shittu et al., 2007). Low specific volumes were observed for the breads (1.09 to 1.82  $cm^{3}/g$ ), which suggests that the decrease in loaf volume was not proportional to the increase in weight. Further, the low observed specific loaf volume could be a result of the minimal hydration capacity of the composite flours. Specific volumes of breads containing 100% sugar substitution [WDPJ (WWF+AF+PPF+DPFS, 80:10:10:100), WDPK (WWF+AF+PPF+DPFS, 70:20:10:100), WDPL (WWF+AF+PPF+DPFS, 70:15:15:100), and WDPM (WWF+AF+PPF+DPFS, 60:20:20:100)] were lower than that for breads with 50% sugar substitution and controls (100% sugar). This confirms that granulated sugar (sucrose) is an important contributor to loaf appearance as it provides the required food for yeast required for dough development. Furthermore, a significant reduction in specific volume (19.78 to 40.11%) was observed as the amount of PPF substitution increased (from 10 to 20%). Addition of non-wheat flour results in gluten dilution and impaired gas retention and thus a lower specific loaf volume. Low specific volumes have previously been reported for white wheat bread containing 100% date palm (Nwanekezi et al., 2015). However, Zafar et al. (2015) reported a 15% reduction in specific volume of whole wheat bread upon substitution of WWF with 25% chickpea. Increased substitution of WWF and total sugar significantly affects the physical characteristics of bread.

#### Proximate composition of multigrain breads

Proximate compositions of breads containing multigrain flour blends are shown in Table 4. Incorporation of DPFS contributed to an increased crude protein content since samples containing 100% DPFS had a higher protein content than those containing 50% sugar replacement. Further, substitution of WWF with >20% AF or PPF resulted in increased crude protein contents (increase of 26 to 55% for 100% DPFS, and 7 to 41% for 50% DPFS). This may be connected to the fact that acha provides equivalent amounts of protein as whole wheat since both are whole grain cereals, whereas pigeon pea is a proteinrich grain legume. However, DPFS contains significant amounts of protein whereas refined sugar lacks protein (Obiegbuna et al., 2013). Overall, the content of ash in bread produced from composite flours was significantly higher than WAPC, although a progressive decline in ash content was observed with increased substitution with AF and PPF. The crude fibre content was significantly higher in bread containing 100% DPFS, especially in composite breads containing high levels (>20%) of nonwheat flour. This may be result of a synergistic effect from fibre contributed by the flour blends and DPFS. The significant crude fibre content is of nutritional advantage in relation to its positive effect for food digestion. A progressive decline in crude fat content was observed with increased AF substitution from 10 to 20% (14 to 7%), however bread containing 100% DPFS had higher crude fat content than bread containing 50% DPFS. This suggests that substitution with AF may result in a lower oil absorption capacity while date palm may increase this property. This result corroborates reports of bread by Nwanekezi et al. (2015). However, Peter Ikechukwu et al. (2017) reported increased fat contents in cookies containing increased amounts of dates. Bread containing composite flours (WWF-AF-PPF) showed higher crude fat contents (14.06 to 18.24%) than WAPC (8.44%). A high fat content serves as a lubricating agent that improves the sensorial quality (softness and aroma) of bread. Moreover, fat is a rich source of energy and is an essential carrier of fat-soluble vitamins (A, D, E, and K). However, the relatively high fat content in multigrain breads does not correlate with the calorie values since the multigrain breads showed lower calorific value; this suggests that fat content is not the singular factor determining calorie content in food. The crude fat content of the multigrain bread was less than the 25% recommended for baked goods, because low fat contents help prevent rancidity in such food products (Ihekoronye and Ngoddy, 1985). The carbohydrate content of whole wheat bread was significantly higher (47.61%) than bread containing AF and PPF substitution (29.73~36.69%). High AF substituted bread (20%) possessed low carbohydrate contents  $(29.73 \sim 31.24\%)$ ; this may be due to the fact that

Sample	Crude protein	Ash	Crude fibre	Crude fat	Moisture	Carbohydrate	Energy (kcal)
WAPC	11.01±0.58 <sup>ef</sup>	2.79±0.01 <sup>bc</sup>	2.08±0.35 <sup>ab</sup>	8.44±0.17 <sup>e</sup>	28.07±0.40 <sup>f</sup>	47.61±1.34 <sup>ª</sup>	310.64±5.63 <sup>bc</sup>
WDPC	12.42±0.01 <sup>d</sup>	$3.85\pm0.00^{a}$	2.55±0.60 <sup>ab</sup>	12.75±0.57 <sup>d</sup>	31.71±0.21 <sup>e</sup>	36.72±1.18 <sup>b</sup>	311.31±3.22 <sup>bc</sup>
WDPJ	10.83±0.00 <sup>f</sup>	4.14±0.15 <sup>a</sup>	1.60±0.42 <sup>c</sup>	17.51±0.14 <sup>ª</sup>	32.28±0.29 <sup>d</sup>	33.65±0.37 <sup>d</sup>	335.51±3.39 <sup>a</sup>
WSDJ	10.22±0.59 <sup>f</sup>	4.22±0.25 <sup>a</sup>	1.48±0.36 <sup>c</sup>	18.24±0.30 <sup>a</sup>	33.56±0.22 <sup>c</sup>	32.28±0.72 <sup>e</sup>	334.16±6.09 <sup>a</sup>
WDPK	13.83±0.11 <sup>c</sup>	3.12±0.11 <sup>b</sup>	3.15±0.55 <sup>ª</sup>	14.38±0.86 <sup>bc</sup>	35.21±0.51 <sup>b</sup>	30.71±0.66 <sup>fg</sup>	307.58±2.40 <sup>c</sup>
WSDK	11.81±0.36 <sup>de</sup>	3.16±0.14 <sup>b</sup>	1.62±0.40 <sup>c</sup>	15.06±0.29 <sup>bc</sup>	37.12±0.18 <sup>ª</sup>	31.24±0.85 <sup>ef</sup>	307.74±2.22 <sup>bc</sup>
WDPL	14.04±0.00 <sup>c</sup>	$2.42\pm0.28^{\circ}$	3.14±0.44 <sup>ª</sup>	14.06±0.33 <sup>cd</sup>	31.02±0.20 <sup>e</sup>	35.12±1.04 <sup>c</sup>	323.18±5.05 <sup>ab</sup>
WSDL	13.48±0.06 <sup>c</sup>	2.93±0.10 <sup>b</sup>	$1.50\pm0.05^{\circ}$	14.33±0.30 <sup>cd</sup>	31.06±0.16 <sup>e</sup>	36.69±0.34 <sup>b</sup>	329.65±4.23 <sup>a</sup>
WDPM	17.10±0.06 <sup>ª</sup>	2.85±0.09 <sup>bc</sup>	2.65±0.48 <sup>ab</sup>	14.06±0.33 <sup>cd</sup>	33.60±0.10 <sup>c</sup>	29.73±0.34 <sup>9</sup>	313.86±5.76 <sup>bc</sup>
WSDM	15.58±0.15 <sup>b</sup>	3.13±0.12 <sup>b</sup>	1.84±0.17 <sup>ab</sup>	15.56±0.32 <sup>b</sup>	31.72±0.27 <sup>e</sup>	32.17±0.50 <sup>e</sup>	331.04±5.88 <sup>ª</sup>

Table 4. Proximate compositions of multigrain breads as affected by flour substitutions, and substitution of sugar with date palm

Values are mean±SD of triplicate determination.

Values with different letters (a-g) within the same column are significantly different ( $P \le 0.05$ ).

WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:100); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:15:15:15:100); WSDL, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50).

acha does not contain equivalent amounts of carbohydrate as whole wheat grain and therefore evokes low sugar. Thus, substitution of WWF had a major effect on the carbohydrate content of bread. Further, it was observed that breads with 100% sugar replacement possess significantly decreased carbohydrate content; overall, a 29, 35, and 37% decrease in carbohydrate content was observed for bread with 20, 30, and 40% non-wheat flour substitution, respectively.

Thus, bread containing composite flours as well as partial/complete replacement of sugar with DPFS possessed significant calorie contents (307.58 ~ 335.51 kcal), therefore may be an important baked goods for diabetic control. Significant increases in crude protein, ash, and crude fibre contents and a decrease in carbohydrate content in bread with WWF substituted with AF and PPF. Further, date sugar contributed positively to the chemical composition of the bread developed in this study.

#### Mineral composition of multigrain breads

Significant amounts of both macro and micro elements were observed in the multigrain bread samples (Table 5). The Ca content significantly differed between each bread sample, increasing with increased content of both AF and PPF. Na and Ca levels were elevated in 100% sugar substituted breads, and with increased amounts of whole wheat substituted with AF. The levels of all mineral investigated were higher in the multigrain breads than WAPC. There were no significant differences (P>0.05) in the Mg and Zn contents of breads substituted with AF or PPF. Substitution of flour and sugar improved the mineral content of all breads. A Na/K ratio less than 1 is significant for regulating Na uptake since a high K content promotes beneficial Na uptake, and is therefore protective of cardiovascular function. All samples had ratios of less than 1, indicating that bread consumption

did not contribute to increased Na uptake.

# Anti-nutrient compositions and molar ratios of whole wheat, acha, and pigeon pea breads

The anti-nutrient compositions of the breads are presented in Table 6. Phytate content was significantly higher in bread from either whole wheat grain flour only or blend of whole wheat grain and 20% acha flour (i.e. WDPC, WDPK, WSDK, WDPL and WDPM). This may be attributable to the fact that phytates are generally found in fibre-rich foods such as wheat bran, whole grains, and legumes, and its presence is associated with reduced mineral absorption (Oatway et al., 2001). Phytates, oxalates, and tannins are known to adversely interfere with mineral bioavailability by forming insoluble salts with Zn, Ca, and Fe, and thus preventing their absorption and impair protein digestibility (Gupta et al., 2006). The anti-nutrient content of the breads may not be detrimental to health as the values are far lower than the 80 mg/g critical limit reported by Malomo et al. (2011).

Estimation of the molar ratio of phytate/minerals in foods and diets is used to measure the bioavailability of minerals for the human body. Hence, a summary of the molar ratios is shown in Table 6. Phytic acids markedly decrease Ca bioavailability, therefore phytate/Ca molar ratios have been proposed as indicators of Ca bioavailability. The critical molar ratio of phytate/Ca is 0.24 (Morris and Ellis, 1985). The molar ratios of phytate/Ca in the multigrain breads were lower than the critical value, suggesting that Ca absorption is not adversely affected by phytate in these breads. Phytate is an important inhibitor of Fe absorption in plant foods; our result show that multigrain breads are significant sources of Fe, therefore estimations of phytate/Fe ratios are good indicators of Fe bioavailability. Hurrell (2004) reported that the phytate/Fe molar ratio must be lower than 1 to promote

							(unite n	ig/100 g)
Samples	Na	К	Ca	Mg	Mn	Fe	Zn	Na/K
WAPC	1.20±0.02 <sup>c</sup>	2.41±0.00 <sup>e</sup>	33.99±0.20 <sup>f</sup>	9.25±0.03 <sup>ns</sup>	$0.18 \pm 0.00^{\circ}$	25.23±0.34 <sup>b</sup>	0.14±0.00 <sup>ns</sup>	0.50
WDPC	1.67±0.01ª	4.53±0.11 <sup>b</sup>	35.20±0.00 <sup>e</sup>	9.30±0.05	$0.22\pm0.00^{\circ}$	26.11±0.00 <sup>a</sup>	0.15±0.00	0.37
WDPJ	1.29±0.06 <sup>c</sup>	2.68±0.00 <sup>e</sup>	39.78±0.12 <sup>c</sup>	9.22±0.11	1.65±0.10 <sup>ª</sup>	21.82±0.11 <sup>f</sup>	0.17±0.03	0.48
WSDJ	1.34±0.03 <sup>bc</sup>	5.37±0.09 <sup>a</sup>	37.69±0.26 <sup>d</sup>	9.16±0.09	1.61±0.08 <sup>ª</sup>	20.79±0.20 <sup>9</sup>	0.15±0.09	0.25
WDPK	1.55±0.11 <sup>ab</sup>	2.68±0.10 <sup>e</sup>	40.73±0.32 <sup>b</sup>	9.31±0.00	1.11±0.09 <sup>b</sup>	24.23±0.00 <sup>cd</sup>	0.16±0.03	0.58
WSDK	1.65±0.02 <sup>a</sup>	3.56±0.12 <sup>c</sup>	39.04±0.19 <sup>c</sup>	9.21±0.08	0.98±0.06 <sup>b</sup>	23.03±0.19 <sup>e</sup>	0.15±0.01	0.46
WDPL	1.27±0.09 <sup>c</sup>	2.42±0.02 <sup>e</sup>	47.82±0.33 <sup>a</sup>	9.29±0.08	1.02±0.10 <sup>b</sup>	24.98±0.15 <sup>bc</sup>	0.16±0.00	0.52
WSDL	1.65±0.05 <sup>ª</sup>	3.24±0.07 <sup>d</sup>	47.56±0.11 <sup>ª</sup>	9.33±0.10	0.96±0.07 <sup>b</sup>	23.92±0.20 <sup>d</sup>	0.14±0.03	0.51
WDPM	1.36±0.05 <sup>bc</sup>	3.16±0.11 <sup>d</sup>	47.35±0.08 <sup>ª</sup>	9.19±0.13	1.17±0.00 <sup>b</sup>	21.91±0.64 <sup>f</sup>	0.16±0.03	0.43
WSDM	1.54±0.06 <sup>ab</sup>	3.21±0.06 <sup>d</sup>	47.26±0.51 <sup>ª</sup>	9.23±0.00	$1.00\pm0.00^{b}$	20.83±0.10 <sup>9</sup>	0.16±0.00	0.48

**Table 5.** Mineral compositions of breads made from whole wheat flour substituted with acha and pigeon pea flours

Values are mean±SD of triplicate determination.

Values with different letters (a-g) within the same column are significantly different ( $P \le 0.05$ ).

WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:100); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+PPF+DPFS+sugar (70:15:15:50:50); WDPM, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50:50). <sup>ns</sup>Not significant.

 Table 6. Anti-nutrient compositions and molar ratios of breads made from whole wheat flour substituted with acha and pigeon pea flours

 (unit: mg/g)

Sample	Phytate	Oxalate	Tannin	Phytate/Ca	Phytate/Fe	Phytate/Zn
WAPC	16.89±0.24 <sup>e</sup>	1.54±0.03 <sup>a</sup>	3.75±0.00 <sup>a</sup>	0.030	0.057	11.64
WDPC	18.13±0.48 <sup>cd</sup>	0.61±0.07 <sup>e</sup>	$2.43 \pm 0.00^{d}$	0.031	0.059	11.96
WDPJ	14.83±0.00 <sup>9</sup>	$0.99 \pm 0.05^{\circ}$	3.11±0.11 <sup>b</sup>	0.023	0.058	8.65
WSDJ	$14.42 \pm 0.24^{f}$	0.59±0.03 <sup>e</sup>	3.04±0.15 <sup>bc</sup>	0.023	0.059	9.48
WDPK	18.95±0.00 <sup>a</sup>	1.31±0.03 <sup>b</sup>	3.10±0.18 <sup>b</sup>	0.028	0.067	11.48
WSDK	17.75±0.12 <sup>d</sup>	0.56±0.04 <sup>e</sup>	2.46±0.21 <sup>cd</sup>	0.027	0.066	11.69
WDPL	18.54±0.24 <sup>b</sup>	$0.36 \pm 0.00^{f}$	2.47±0.10 <sup>cd</sup>	0.023	0.063	11.24
WSDL	15.24±0.24 <sup>f</sup>	$0.81 \pm 0.00^{d}$	2.90±0.25 <sup>bcd</sup>	0.019	0.054	10.50
WDPM	18.19±0.24 <sup>cd</sup>	$0.63 \pm 0.05^{e}$	3.75±0.32 <sup>a</sup>	0.023	0.071	11.04
WSDM	14.75±0.05 <sup>9</sup>	$0.81 \pm 0.03^{d}$	1.57±0.14 <sup>e</sup>	0.019	0.060	8.92

Values are mean±SD of triplicate determination.

Values with different letters (a-g) within the same column are significantly different ( $P \le 0.05$ ).

WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:100); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDPK, WWF+AF+PPF+DPFS+sugar (60:20:20:50); WDK, WWF+AF+PPF+DPF+SUGAR (60:20:20:50); WDK, WWF+AF+PPF+DPFS+SUGAR (60:20:20:50); WDK, WK+AF+PPF+DPF+SUGAR (60:20:20:50); WDK, WK+AF+PPF+DPF+SUGAR (60:20:20:50); WDK, WK+AF+PPF+DPF+SUGAR (60:20:20:50); WK+AF+

a significant increase in Fe absorption. The multigrain breads had phytate/Fe molar ratios lower than 1, indicating Fe should be bioavailable upon consumption of these breads. The importance of a food as a source of dietary Zn is dependent on the total Zn content as well as the level of other constituents that can affect Zn bioavailability. The formation of insoluble mineral chelates may reduce the bioavailability of dietary Zn, which is dependent on the relative levels of both Zn and phytic acid (Davies and Olpin, 1979). Thus, the phytate/Zn molar ratio is considered a better indicator of Zn bioavailability than total dietary phytate levels in isolation (Oberleas and Harland, 1981). The critical value of phytate/Zn is 15 with adequate availability of Zn at values of less than 10 (Oberleas and Harland, 1981; Woldegiorgis et al., 2015). The phytate/Zn molar ratios of the multigrain breads ranged from 8.65 to 11.96, which is less than the critical value, thus Zn in these breads should be bioavailable. The phytate/ mineral molar ratios observed in this study predict that the multigrain breads contain good amounts of bioavailable Ca, Fe, and Zn.

#### GI and GL of multigrain breads

For the breads investigated in this study, the mean IAUC ranged from 11,970 to 17,498 mg·min/dL. WAPC showed the largest IAUC (17,498 mg·min/dL), whereas significantly lower levels were observed for multigrain breads with WDPM. A previous study by Lanzerstorfer et al. (2018) corroborates our finding through reporting largest mean IAUCs for whole grain rolls. Interestingly, lower mean IAUC values were obtained for breads containing 100% DPFS compared with bread containing 50% DPFS.

Carbohydrate-rich foods raise blood sugar level after ingestion, the extent of which is ranked by the GI of the food; based on the mean IAUC values, the GI for each bread was calculated. According to the GI classification, GI>70 indicates a high GI food; GI>56 to <69 indicates an intermediate GI food; and GI<55 indicates a low GI food. The present results indicate that multigrain bread could be classified as having low a GI ( $44.88 \sim 54.87$ ), with exception of WSDJ which had an intermediate GI (57.72), which may be attributed to the composition of the flour blend (WWF+AF+PPF, 80:10:10) and partial inclusion of white sugar (sucrose) (Table 7). However, the WAPC also showed intermediate GI values (65.61), which is in agreement with the existing GI classification of food products whereby whole-grain wheat bread is classified as a high GI food (Marques et al., 2007). Total substitution of white sugar with DPFS in whole wheat bread positively influenced the GI of the bread since a 15.52% reduction in GI was observed; date palm control bread therefore showed a low GI. Further, WDPM showed the lowest GI, which may be connected to its low carbohydrate content since carbohydrates are key modulators and determinants of postprandial glucose responses which are relative for GI assessment (Lanzerstorfer et al., 2018). The multigrain breads possessed reduced GL compared with WAPC, which has a high GL. The low glycaemic response observed for the multigrain breads may promote a reduction in lifestyle-related complications such as diabetes and other cardiovascular diseases. The low GI val-

 Table 7. Influence of flour and sugar substitutions on glycaemic indexes (GI) and load (GL) of multigrain breads

Sample	IAUC (mg·min/dL)	GI	GL
WAPC	17,498±122 <sup>ª</sup>	65.61±2.17 <sup>a</sup>	41.93±0.84 <sup>a</sup>
WDPC	14,783±107 <sup>c</sup>	55.43±1.63 <sup>c</sup>	26.23±0.27 <sup>c</sup>
WDPJ	14,633±99°	54.87±1.80 <sup>c</sup>	20.15±0.23 <sup>de</sup>
WSDJ	15,398±128 <sup>b</sup>	57.72±1.12 <sup>b</sup>	29.83±0.25 <sup>b</sup>
WDPK	12,173±115 <sup>d</sup>	45.64±1.24 <sup>e</sup>	21.61±0.22 <sup>d</sup>
WSDK	12,870±126 <sup>d</sup>	48.26±0.48 <sup>de</sup>	14.92±0.20 <sup>f</sup>
WDPL	12,255±135 <sup>d</sup>	45.95±2.51 <sup>e</sup>	13.93±0.41 <sup>f</sup>
WSDL	13,103±109 <sup>cd</sup>	49.13±2.24 <sup>d</sup>	22.05±0.38 <sup>d</sup>
WDPM	11,970±96 <sup>d</sup>	44.88±0.36 <sup>f</sup>	13.35±0.18 <sup>fg</sup>
WSDM	12,600±103 <sup>d</sup>	47.24±1.09 <sup>de</sup>	15.20±0.21 <sup>f</sup>

Values are mean±SD of triplicate determination.

Values with different letters (a-g) within the same column are significantly different ( $P \le 0.05$ ).

IAUC, incremental area under the curve; WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:100); WSDJ, WWF+AF+PPF+DPFS+ sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10: 100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPL, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+ PPF+DPFS+sugar (70:15:15:50:50); WDPM, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20: 50:50). ues may be attributed to the whole grain meal flour, which contains high dietary fibre and the pulse grain, which increases flour viscosity and thus delays gastric emptying. The present findings corroborate findings by Lanzerstorfer et al. (2018) who reported significantly low glycemic indices for protein-rich breads.

#### Blood glucose response of breads

The blood glucose response observed over 120 min after ingestion was recorded. A peak in blood glucose concentrations was observed at 15 and 30 min, and a gradual decline progressed thereafter (Fig. 1). WDPM showed the lowest blood glucose response: blood glucose increased by approximately 14% after 15 min, and was almost maintained during the 2 h period (only 6% reduction in mean postprandial blood glucose was observed for this group). The low postprandial response may be attributable to the low carbohydrate (29.73%), and high protein (17.10%) and fibre (2.65%) contents of WDPM. Lanzerstorfer et al. (2018) recommended breads containing low levels of carbohydrates, and high levels of protein and fibre for health-conscious individuals. However, animals fed WDPL showed the fastest decline in blood glucose concentration; initially, a 82.86% spike in blood glucose levels was observed after 15 min, which declined by 45% to the initial blood glucose reading (128 to 70 mg/dL). The postprandial reduction in glucose level may be due to the high levels of protein and crude fibre, and low level of carbohydrate (14.04, 3.14, and 35.12%, respectively) in the sample. Interestingly, this blend uniquely comprises



**Fig. 1.** Effect of bread administration on blood glucose response of normal rats over 120 min period. Values are mean±SD of replicate determination. WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80: 10:10:100); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50), WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPL, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+PPF+DPFS+sugar (70:15:15:50: 50); WDPM, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50).

Sample	Colour	Appearance	Flavour	Texture	Taste	Overall acceptability
WAPC	7.79±0.15 <sup>ª</sup>	7.66±0.18 <sup>a</sup>	7.48±0.21 <sup>ª</sup>	7.52±0.27 <sup>ª</sup>	7.65±0.30 <sup>ª</sup>	7.90±0.22 <sup>ª</sup>
WDPC	7.70±0.19 <sup>a</sup>	6.69±0.24 <sup>b</sup>	7.61±0.25 <sup>b</sup>	7.00±0.31ª	6.83±0.18 <sup>b</sup>	7.20±0.22 <sup>ª</sup>
WDPJ	6.34±0.29 <sup>b</sup>	6.38±0.23 <sup>b</sup>	6.10±0.40 <sup>b</sup>	5.66±0.32 <sup>bc</sup>	5.72±0.34 <sup>bc</sup>	6.17±0.33 <sup>b</sup>
WSDJ	6.76±0.31 <sup>b</sup>	6.38±0.31 <sup>b</sup>	6.31±0.32 <sup>b</sup>	6.34±0.32 <sup>b</sup>	6.31±0.42 <sup>b</sup>	6.55±0.36 <sup>b</sup>
WDPK	6.34±0.23 <sup>b</sup>	6.24±0.30 <sup>b</sup>	$5.52 \pm 0.30^{bc}$	5.31±0.30 <sup>c</sup>	5.34±0.29 <sup>bc</sup>	5.86±0.28 <sup>bc</sup>
WSDK	6.45±0.29 <sup>b</sup>	6.62±0.26 <sup>b</sup>	5.93±0.28 <sup>b</sup>	6.17±0.26 <sup>bc</sup>	5.62±0.33 <sup>bc</sup>	5.97±0.33 <sup>bc</sup>
WDPL	6.59±0.30 <sup>b</sup>	6.31±0.28 <sup>b</sup>	5.41±0.36 <sup>bc</sup>	5.97±0.32 <sup>bc</sup>	5.21±0.35 <sup>c</sup>	5.85±0.31 <sup>bc</sup>
WSDL	6.72±0.28 <sup>b</sup>	6.45±0.30 <sup>b</sup>	5.55±0.33 <sup>bc</sup>	6.03±0.39 <sup>bc</sup>	$5.52 \pm 0.40^{bc}$	5.93±0.31 <sup>bc</sup>
WDPM	6.34±0.25 <sup>b</sup>	6.45±0.23 <sup>b</sup>	5.48±0.33 <sup>bc</sup>	$5.86 \pm 0.25^{bc}$	$4.86 \pm 0.37^{\circ}$	5.75±0.36 <sup>bc</sup>
WSDM	6.72±0.15 <sup>b</sup>	6.76±0.20 <sup>b</sup>	5.69±0.38 <sup>bc</sup>	6.31±0.31 <sup>b</sup>	5.21±0.38 <sup>bc</sup>	6.00±0.26 <sup>b</sup>

Table 8. Sensory characteristics of breads made from whole wheat flour substituted with acha and pigeon pea flours

Values are mean±SD of triplicate determination.

Values with different letters (a-c) within the same column are significantly different ( $P \le 0.05$ ).

WAPC, whole wheat flour (WWF)+sugar (100:100); WDPC, WWF+DPFS (100:100); WDPJ, WWF+acha flour (AF)+pigeon pea flour (PPF)+date palm fruit sugar (DPFS) (80:10:10:10); WSDJ, WWF+AF+PPF+DPFS+sugar (80:10:10:50:50); WDPK, WWF+AF+PPF+DPFS (70:20:10:100); WSDK, WWF+AF+PPF+DPFS+sugar (70:20:10:50:50); WDPL, WWF+AF+PPF+DPFS (70:15:15:100); WSDL, WWF+AF+PPF+DPFS (60:20:20:100); WSDM, WWF+AF+PPF+DPFS+sugar (60:20:20:50).

equal amounts of AF and PPF. As expected, all multigrain breads showed lower mean postprandial glucose levels than the control (whole-grain wheat bread).

# Sensory characteristics of whole wheat-acha-pigeon pea bread

The mean sensory (colour, appearance, aroma, mouthfeel, and overall acceptability) scores of the bread is presented in Table 8. There was no significant difference (P>0.05)in colour and appearance of the multigrain breads, but the mean scores of all composite, sugar replaced breads were numerically lower than WAPC. As expected, breads containing 50% sugar replacements were rated better in terms of texture, taste, and overall acceptability than 100% DPFS breads, and the mean scores decreased as the proportion of substitution of WWF with other grain flours increased. This could be due to the newness of multigrain breads to the consumers, since the consumers may not accustomed to consuming breads containing non-wheat or legume flours. Overall, the acceptability of the breads were rated above 5.70 on a nine point hedonic scale and were therefore largely acceptable to the consumers. Previous studies have reported lower sensory scores (but with no significant difference) for palatability of bread containing chickpea flour at  $15 \sim 30\%$  (Hefnawy et al., 2012) and 25~35% (Zafar et al., 2015). Menon et al. (2015) reported lower scores for composite flour breads containing refined wheat, high protein soy, sprouted mung bean, and mango kernel flours, which significantly differed from scores of standard bread.

Bread of an acceptable quality was successfully produced when WWF was substituted with AF and PPF, and when sugar was partially or completely replaced with date palm. Use of date palm fruit pulp meal as a sweetening agent to replace refined sugar in the breads improved the nutritional values (increased protein, ash, crude fibre, and mineral element contents, and decreased carbohydrate content). Further, the multigrain breads had low glycemic indices and (especially WDPM) reduced postprandial blood glucose responses. Overall, AF and PPF, and DPFS could serve as economic substitutes for wheat flour and sugar, respectively, and provide a healthier product for health-conscious individuals.

### AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

#### REFERENCES

- AACC International. AACC method 10-05.01. Guidelines for measurement of volume by rapeseed displacement. Approved Methods of Analysis. 11th ed. American Association of Cereal Chemists, St. Paul, MN, USA. 2000a.
- AACC International. AACC method 10-09.01. Basic straightdough bread-baking method—long fermentation. Approved Methods of Analysis. 11th ed. American Association of Cereal Chemists, St. Paul, MN, USA. 2000b.
- Almoraie NM. The effect of walnut flour on the physical and sensory characteristics of wheat bread. Int J Food Sci. 2019. 2019: 5676205.
- AOAC. Official methods of analysis. 19th ed. Association of Official Analytical Chemists International, Rockville, MD, USA. 2012.
- Augustin LS, Franceschi S, Jenkins DJA, Kendall CWC, La Vecchia C. Glycemic index in chronic disease: a review. Eur J Clin Nutr. 2002. 56:1049-1071.
- Augustin LSA, Kendall CWC, Jenkins DJA, Willett WC, Astrup A, Barclay AW, et al. Glycemic index, glycemic load and glycemic response: an International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). Nutr Metab Cardiovasc Dis. 2015. 25:795-815.
- Aune D, Keum N, Giovannucci E, Fadnes LT, Boffetta P, Greenwood DC, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific

mortality: systematic review and dose-response meta-analysis of prospective studies. Br Med J. 2016. 353:i2716.

- Ayele HH, Bultosa G, Abera T, Astatkie T. Nutritional and sensory quality of wheat bread supplemented with cassava and soybean flours. Cogent Food Agric. 2017. 3:1331892. Available from: https://doi.org/10.1080/23311932.2017.1331892
- Ayo JA, Ayo VA, Nkama I, Adewori R. Physiochemical, *in-vitro* digestibility and organoleptic evaluation of "acha" wheat biscuit supplemented with soybean flour. Niger Food J. 2007. 25: 77-89.
- Badifu GIO, Chima CE, Ajayi YI, Ogori AF, Ogori AF. Influence of mango mesocarp flour supplement to micronutrient, physical and organoleptic qualities of wheat-based bread. Niger Food J. 2005. 23:59-68.
- Bhatt SM, Gupta RK. Bread (composite flour) formulation and study of its nutritive, phytochemical and functional properties. J Pharmacogn Phytochem. 2015. 4:254-268.
- Bhise S, Kaur A. Baking quality, sensory properties and shelf life of bread with polyols. J Food Sci Technol. 2014. 51:2054-2061.
- Brand-Miller JC, Holt SH, Pawlak DB, McMillan J. Glycemic index and obesity. Am J Clin Nutr. 2002. 76:281S-285S.
- Chilungo S. Physicochemical properties and baking qualities of baked wheat products supplemented with cassava and pigeon pea flours. Master's thesis. Michigan State University, East Lansing, MI, USA. 2013.
- Davies NT, Olpin SE. Studies on the phytate: zinc molar contents in diets as a determinant of Zn availability to young rats. Br J Nutr. 1979. 41:590-603.
- Day AL, Underwood RA Jr. Quantitative analysis. 5th ed. Prentice-Hall, Englewood Cliffs, NJ, USA. 1986. p 701.
- Devindra S, Shilpa C, Charu K, Aruna T, Prasad GBKS. Estimation of glycemic carbohydrate and glycemic index/load of commonly consumed cereals, legumes and mixture of cereals and legumes. Int J Diabetes Dev Ctries. 2017. 37:426-431.
- Ekunseitan OF, Obadina AO, Sobukola OP, Omemu AM, Adegunwa MO, Kajihausa OE, et al. Nutritional composition, functional and pasting properties of wheat, mushroom, and high quality cassava composite flour. J Food Process Preserv. 2016. 41:e13150. Available from: https://doi.org/10.1111/ jfpp.13150
- Fasoyiro S. Pigeon pea: processing, nutritional composition, products and recipes. LAP Lambert Academic Publishing, Saarbrücken, Germany. 2015. p 1-72.
- Gupta S, Jyothi Lakshmi AA, Prakash J. *In vitro* bioavailability of calcium and iron from selected green leafy vegetables. J Sci Food Agric. 2006. 86:2147-2152.
- Hamad I, AbdElgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, et al. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) Cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 2015. 20:13620-13641.
- Hamza AM, Collins A, Ado SG, Ikuenobe CE, Ataga CD, Odewale JO. Proximate compositions evaluation and variability among cultivars of date palm (*Phoenix dactylifera* L.) in Nigeria. Int J Plant Soil Sci. 2014. 3:248-259.
- Hefnawy TMH, El-Shourbagy GA, Ramadan MF. Impact of adding chickpea (*Cicer arietinum* L.) flour to wheat flour on the rheological properties of toast bread. Int Food Res J. 2012. 19:521-525.
- Hettiaratchi UP, Ekanayake S, Welihinda J. Glycaemic indices of three Sri Lankan wheat bread varieties and a bread-lentil meal. Int J Food Sci Nutr. 2009. 60(Suppl 4):21-30.
- Hurrell RF. Phytic acid degradation as a means of improving iron absorption. Int J Vitam Nutr Res. 2004. 74:445-452.
- Ihekoronye AI, Ngoddy PO. Integrated food science and technology for the tropics. Macmillan Publishers, London, NY, USA. 1985. p 1-386.

Lafiandra D, Riccardi G, Shewry PR. Improving cereal grain car-

bohydrates for diet and health. J Cereal Sci. 2014. 59:312-326.

- Lanzerstorfer P, Rechenmacher E, Lugmayr O, Stadlbauer V, Höglinger O, Vollmar A, et al. Effects of various commercial whole-grain breads on postprandial blood glucose response and glycemic index in healthy subjects. Austin J Clin Med. 2018. 5:1031.
- Makkar HPS. Antinutritional factors in foods for livestock. BSAP Occas Publ. 1993. 16:69-85.
- Malomo SA, Eleyinmi AF, Fashakin JB. Chemical composition, rheological properties and bread making potentials of composite flours from breadfruit, breadnut and wheat. Afr J Food Sci. 2011. 5:400-410.
- Manickavasagan A, Mathew TA, Al-Attabi ZH, Al-Zakwani IM. Dates as a substitute for added sugar in traditional foods—a case study with idli. Emir J Food Agric. 2013. 25:899-906.
- Marques C, D'auria L, Cani PD, Baccelli C, Rozenberg R, Ruibal-Mendieta NL, et al. Comparison of glycemic index of spelt and wheat bread in human volunteers. Food Chem. 2007. 100:1265-1271.
- Menon L, Majumdar SD, Ravi U. Development and analysis of composite flour bread. J Food Sci Technol. 2015. 52:4156-4165.
- Morris ER, Ellis R. Bioavailability of dietary calcium: effect of phytate on adult men consuming nonvegetarian diets. In: Kies C, editor. Nutritional Bioavailability of Calcium. American Chemical Society, Washington, DC, USA. 1985. p 63-72.
- Nanyen D, Dooshima IB, Julius A, Benbella I. Nutritional composition, physical and sensory properties of cookies from wheat, acha and mung bean composite flours. Int J Nutr Food Sci. 2016. 5:401-406.
- Nwanekezi EC, Ekwe CC, Agbugba RU. Effect of substitution of sucrose with date palm (*Phoenix dactylifera*) fruit on quality of bread. J Food Process Technol. 2015. 6:484.
- Oatway L, Thava V, Helm JH. Phytic acid. Food Rev Int. 2001. 17: 419-431.
- Oberleas D, Harland BF. Phytate content of foods: effect on dietary zinc bioavailability. J Am Diet Assoc. 1981. 79:433-436.
- Obiegbuna JE, Akubor PI, Ishiwu CN, Ndife J. Effect of substituting sugar with date palm pulp meal on the physicochemical, organoleptic and storage properties of bread. Afr J Food Sci. 2013. 7:113-119.
- Oboh H, Osagie A, Omotosho A. Glycemic response of some boiled legumes commonly eaten in Nigeria. Diabetologia Croatica. 2010. 39:125-131.
- Olagunju AI, Omoba OS, Enujiugha VN, Aluko RE. Development of value-added nutritious crackers with high antidiabetic properties from blends of *acha* (*Digitaria exilis*) and blanched pigeon pea (*Cajanus cajan*). Food Sci Nutr. 2018. 6:1791-1802.
- Olaoye OA, Ade-Omowaye BIO. Composite flours and breads: potential of local crops in developing countries. In: Preedy VR, Watson RR, Patel VB, editors. Flour and Breads and Their Fortification in Health and Disease Prevention. Academic Press, San Diego, CA, USA. 2011. p 183-192.
- Olaoye OA, Onilude AA, Oladoye CO. Breadfruit flour in biscuit making: effects on product quality. Afr J Food Sci. 2007. 1:20-23.
- Panlasigui LN, Panlilio LM, Madrid JC. Glycaemic response in normal subjects to five different legumes commonly used in the Philippines. Int J Food Sci Nutr. 1995. 46:155-160.
- Peter Ikechukwu A, Okafor DC, Kabuo NO, Ibeabuchi JC, Odimegwu EN, Alagbaoso SO, et al. Production and evaluation of cookies from whole wheat and date palm fruit pulp as sugar substitute. Int J Adv Eng Technol Manag Appl Sci. 2017. 4:1-31.
- Salmerón J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. J Am Med Assoc. 1997. 277:472-477.

- See EF, Wan Nadiah WA, Noor Aziah AA. Physico-chemical and sensory evaluation of breads supplemented with pumpkin flour. ASEAN Food J. 2007. 14:123-130.
- Shittu TA, Raji AO, Sanni LO. Bread from composite cassavawheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. Food Res Int. 2007. 40:280-290.
- Souza EJ, Guttieri M, Sneller C. Nutritional profile of whole-grain soft wheat flour. Cereal Chem. 2011. 88:473-479.
- Stamataki NS, Yanni AE, Karathanos VT. Bread making technology influences postprandial glucose response: a review of the clinical evidence. Br J Nutr. 2017. 117:1001-1012.
- The University of Sydney. Glycemic Index Database. 2012 [cited 2019 Aug 13]. Available from: https://www.glycemicindex. com/about.php
- Wheeler EL, Ferrel RE. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. 1971. 48:312-320.
- Woldegiorgis AZ, Abate D, Haki GD, Ziegler GR. Major, minor and toxic minerals and anti-nutrients composition in edible

- mushrooms collected from Ethiopia. J Food Process Technol. 2015. 6:430.
- Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. Am J Clin Nutr. 1991. 54:846-854.
- Wu KL, Sung WC, Yang CH. Characteristics of dough and bread as affected by the incorporation of sweet potato paste in the formulation. J Mar Sci Technol. 2009. 17:13-22.
- Ye EQ, Chacko SA, Chou EL, Kugizaki M, Liu S. Greater wholegrain intake is associated with lower risk of type 2 diabetes, cardiovascular disease, and weight gain. J Nutr. 2012. 142:1304-1313.
- Yu L, Nanguet AL, Beta T. Comparison of antioxidant properties of refined and whole wheat flour and bread. Antioxidants. 2013. 2:370-383.
- Zafar TA, Al-Hassawi F, Al-Khulaifi F, Al-Rayyes G, Waslien C, Huffman FG. Organoleptic and glycemic properties of chickpea-wheat composite breads. J Food Sci Technol. 2015. 52: 2256-2263.