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Effect of Malted African Breadfruit (*Treculia africana*) Seed Flour Inclusion on *In-vitro* Glycemic Index, Starch and Protein Digestibility of Fibre Rich Snack Bars

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OPC and NTU designed the study, supervised the research work. Author EAP carried out the laboratory work performed the statistical analysis and managed the literature searches and wrote the first draft of the manuscript. Authors NVE and UME wrote the protocol. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to elucidate the suitability and utilization of malted African breadfruit (*Treculia africana*) seed flour in snack bars production. Malted African breadfruit seeds, maize and coconut were processed to flour and evaluated of their proximate composition, phytochemical composition and particle sizes. Six (6) products were developed from the flour blends in the respective ratio of 0:95:5 (T_0), 20:75:5 (T_{20}), 25:70:5 (T_{25}), 30:65:5 (T_{30}), 35:60:5 (T_{35}) and 95:0:5 (T_{95}). Soluble dietary fibre (SDF = 5.15 – 3.15%) decreased while insoluble (IDF = 7.23 – 19.23%) and total dietary fibre (TDF = 12.33 – 22.39%) increased significantly ($p<0.05$) with increasing malted African breadfruit inclusion. *In vitro* glycemic index (IVGI) and starch digestibility (IVSD) decreased significantly ($p<0.05$) from 57.30 – 45.65% and 57.48 – 31.44% respectively, with increasing substitution of malted African breadfruit seed flour. A negative correlation was observed

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between the TDF and IVGI content of the snack bars. *In vitro* protein digestibility ranged from 68.19 to 87.45%. With reference to standard classifications, the formulated malted African breadfruit seed based snack bars could be referred to as 'high fibre' and 'low glycemic' foods, and may have positive health benefit to the consumers, especially the diabetics and those interested in weight management.

Keywords: Malted African breadfruit seeds; snack bars; total dietary fibre; starch digestibility; glycemic index.

1. INTRODUCTION

"Snack bars, known commonly as cereal bars, are convenient alternatives as nutritious snacks in place of junk food by those who have very busy lifestyle with insufficient time for having proper in-between meals. They are easy to manufacture, and contain various ingredients such as cereals, nuts, fruits, chocolate, and sweeteners. Cereal bars can be customized for various target groups such as protein rich, fibre rich, high or low calorie bars or even functional ingredients can be added in the bars such as omega-3 enriched flaxseed bar, sesame seed bars or addition of prebiotics" [1]. Recently, there has been an increase in the consumption of snack bars, as a result of increase changes in the lifestyles of people and the desire for convenient and fast meals [2]. Consumers have come to accept snack bars due to the fact that they are nutritionally balanced high-fibre snacks, and also because they are adequately balanced in vitamins, protein, minerals, fibre, fat and calories and are produced with whole grains which are beneficial to health [3].

Dietary fibre, according to British Nutrition Foundation [4], is defined as "a group of substances present in plant foods that cannot be broken down completely by digestive enzymes of humans. These substances include lignin, waxes and some polysaccharides such as cellulose and pectin. It was originally believed that fibres were completely indigestible and could not provide any amount of energy. It has been made clear today that certain fibres can be fermented in the large intestine by some intestinal bacteria, to produce short chain fatty acids (SCFA) and gases." Dietary fibres are found in cereals, legumes, fruits, vegetables and whole grain breads. Most of the sources of these dietary fibres have a combination of some insoluble fibres and soluble fibres in different proportions.

Soluble dietary fibres are fermentable while Insoluble fibres are less fermentable or non-fermentable fibres, and are found in varying quantities in all plant foods, including: legumes such as cowpeas, peas, groundnut, soybeans, bambara groundnut, African breadfruit seeds and other beans; wheat, oats, barley, rye, millet and chia; fruits such as bananas, plums, pears, berries, and apples; vegetables like onions, broccoli, potatoes, carrots, cucumbers, pumpkins, sweet potatoes, and cabbages. Soluble dietary fibres, firstly, absorbs water to become a viscous gel during digestion, slowing the stomach emptying process and intestinal transit, trapping carbohydrates and shielding them from enzymes, and delaying (slowing) absorption of glucose, thereby lowering variances in the levels of blood sugar [5] [4]. Secondly, soluble dietary fibre lowers the low density lipoproteins (LDL) and total cholesterol and thus may reduce the risk of cardiovascular disease. Thirdly, it regulates blood sugar and reduces the symptoms or onset risk of metabolic syndrome, and also reduces glucose and insulin levels in diabetic patients, and therefore lowers the risk of diabetes [6][4]. On the other hand, insoluble dietary fibre helps to speed up the movement of foods through the digestive system, and facilitates regular defecation. It also makes the stool bulky and alleviates constipation.

"African breadfruit (*T. africana*), commonly known as *Ukwa* in the South East of Nigeria, constitutes a strategic reserve of essential food nutrients that are available at certain critical periods of the year when common sources of these nutrients are short in supply or out of season" [7]. "African breadfruit seed protein has a fairly well balanced amino acid composition with a comparatively higher level of lysine, compared to wheat protein" [8]. An interesting thing about the utilization of *T. africana* is the different methods of preparation and use of the legume in different areas of the country. In the south eastern part of Nigeria, *Ukwa* could be boiled in water, with salt, pepper and other ingredients of interest to add taste. It could be made into a thick porridge, and the liquid portion could be runoff before it thickens and drank as a beverage. The seeds could also be processed to powder and used as soup thickener [9]. In some communities, the porridge of dehulled seeds could be cooked with maize or guinea corn. Roasted/Toasted *Ukwa* serves as a pleasant snack for families, having groundnut flavor, and is usually eaten with maize and coconut. The roasted/toasted *ukwa* is sold in open market and hawked on streets in the East of Nigeria. *T. africana* are now processed into flour and are used to make snacks like cake, cookies and the likes [10]. Expanding the food applications of African breadfruit seed flour would increase its versatility and utility. One of such application could be processing into malted flour for use in the fortification of snacks, as snack bars, for improved health benefit, variety and convenience.

This study was therefore designed to utilize malted African breadfruit seed flour, with maize and coconut flours, to formulate fibre rich snack bars. The study is intended to observe the effect of malted African breadfruit flour on the dietary fibre content, *in vitro* glycemic index, *in vitro* starch and protein digestibility of the snack bars. The products developed from the study could have positive health benefit to consumers and could also serve as a reference material to researchers, nutritionists and food processors.

2. MATERIALS AND METHODS

2.1 Source of Materials

African breadfruit seeds were purchased from Ndoro market, Abia State. Maize (white dent variety) was obtained from Uyo main market. Coconuts were obtained from a local farmer in Uyo, Akwa Ibom State, Nigeria

2.2 Processing of Materials

2.2.1 Production of malted African breadfruit seed flour

Production of malted African breadfruit seed flour was carried out according to the method outlined (Fig. 1) by Nwabueze and Uchendu [11]. The seeds were washed with potable water and steeped for 24 h. The liquor was changed every 8 h to reduce microbial load and also prevent suffocation of the respiring embryo due to depletion of oxygen. The liquor was drained off at the end of steeping and the seeds were spread on a previously sterilized jute bag, placed on a laboratory bench. Germination was carried out at room temperature for seven (7) days. The sprouted seeds were kilned in an oven at 45 ^{o}C for 12 h to terminate germination and the temperature was later increased to 60 ^{o}C for 6 h for drying. The dried malted seeds were then toasted and milled to flour using a manual mill (Victoria Grain Mill, Model: 530025, Colombia). The flour was stored at ambient temperature (27±2 ^{o}C) in a clean, dry plastic container with a secured lid.

2.2.2 Production of maize flour

“Maize grains were processed into flour according to the procedures outlined” by Edima-Nyah et al., [12]. The grains were sorted to remove extraneous materials and cleaned by winnowing. The cleaned maize was toasted at 150 ^{o}C for 20 min in a hot air oven, then milled using Victoria Grain Mill (Model Ref: 530025, Colombia) to flour. Maize flour was packaged in a clean dry plastic container, securely covered, labelled and stored at room temperature.

2.2.3 Production of full fat coconut grits

Coconut was processed to grits following the steps described by Edima-Nyah et al., [12]. Mature coconuts were harvested, dehusked, cracked, and the coconut flesh (meat) were manually removed from the hard endocarp with the aid of a sharp pointed stainless steel knife. The flesh was grated manually (with a plastic grater) to shreds. The grated flesh was dried at 60 ^{o}C for 6hrs and toasted at 150 ^{o}C for 20 mins in a Precision Compact Oven (Model: PR305225M). The toasted shreds were then milled with a hand operated colloid mill (Victoria Grain Mill, Model Ref: 530025, Colombia) to yield coconut grits. The grits were stored in a plastic container at room temperature until used.

2.3 Characterization of Flours

The particle sizes of the raw materials were determined according to the AOAC [13] using a shaker sieve mesh with a series of sieves which varied from 20 to 100 mesh. The sieves were vibrated at the speed of 5000 rpm and the quantity of flour retained in each sieve was reported as percentage flour retained.

2.4 Flour Blend Formulation

Six composite flours of African breadfruit seed, maize and coconut were blended in the proportions of 0:95:5 for T_0, 20:75:5 for T_{20}, 25:70:5 for T_{25}, 30:65:5 for T_{30}, 35:60:5 for T_{35}, 95:00:5 for T_{95} respectively. The blend A, which had 95% maize flour and 5% coconut grits, represent the positive control while the blend F, which had 95% African breadfruit seed flour and 5% coconut grits, was the negative control. The flours were mixed in a Kenwood mixer for 3 min to obtain a homogeneous mixture.

2.5 Snack Bar Recipe

Six snack bar samples were prepared, each based on each of the composite flours previously blended. From each flour blend, 100g of flour was weighed out. Other dry ingredients; 15g of margarine, 5g of milk powder, 2g of baking powder, 2g of nutmeg and 0.2g of common salt were blended with 100g of each composite flour. Also, liquid ingredients, 25g of caramel and 10g of coconut oil, and each blend was mixed with 40g of portable water.

2.6 Production of Snack Bars

The snack bars were produced, as shown in Fig. 1, according to the method described by Edima-Nyah et al. [1]. The dry ingredients were manually mixed together in a stainless steel bowl for about 3min to obtain a uniform mixture. The liquid ingredients (caramel and coconut oil) were added and mixed for 3min, water was incorporated slowly and the entire dough was mixed thoroughly for about 2min to obtain a uniform dough. The dough was transferred into greased aluminum pans and compressed in the pans using a spatula to give a uniform mass. The pan covers were placed over them to smoothen the tops and give the bars the desired shape. The dough was baked in an oven at 150^{o}C for 25min. They were cooled to about 60^{o}C, de-panned and cut into bars seizes: 5cm x 3cm x 2cm. The bars were further dried in an air-circulation oven at 60^{o}C for 6h to reduce the moisture content, cooled at ambient temperature (27±2^{o}C) and packaged in a high density polyethylene. The packaged snack bars were labeled, sealed using an electronic sealing machine, Double Leopard (Model: SP 200H, Taiwan) and stored at ambient temperature in the laboratory for various determinations.

2.7 Analyses

2.7.1 Determination of proximate composition

Proximate analyses of the materials were carried out using standard methods of AOAC [13] for moisture content, crude fat, crude protein, total ash, crude fiber and carbohydrate.

2.7.2 Determination of energy value

The total energy was determined by the method described by Osborne and Voogt [14]. The total energy or the caloric values was estimated by calculation using the water quantification factors of 4, 9 and 4 kcal/100g respectively for protein, fat and carbohydrate as expressed below. Calorific value (Kcal/100g) = P x 4 + F x 9 + C x 4. Where: P = Protein content (%), F = Fat content (%), C = Carbohydrate content (%).

2.7.3 Determination of Phytochemicals

Tannin, phytate, and trypsin inhibitor activity content were determined using the standard method of Onwuka, [15]. Oxalate and saponin contents were determined using the solvent extraction gravimetric method described by AOAC [13].

2.7.4 Determination of soluble, insoluble and total dietary fibre

“Soluble, insoluble and total dietary fibre in foods was determined using the Enzymatic-Gravimetric method MES-TRIS Buffer” [13]. Samples were extracted with 85% ethanol to remove most of the sugars. Residues were suspended in MES-TRIS buffer and digested sequentially with heat-stable α-amylase at 95–100 ^{o}C, protease at 60 ^{o}C, and amylo-glucosidase at 60 ^{o}C. Enzyme digestates were filtered through trittled crucibles with celite. Crucibles containing the digestates residues (insoluble fibre) were rinsed with dilute alcohol followed by acetone, and dried overnight in hot air oven at 105 ^{o}C. Filtrates plus rinses (Soluble fibre) were mixed with 4-volume of 95% ethanol to precipitate materials that were soluble in the digest. After 1 h, precipitates were filtered through trittled crucibles with celite. The digestates residue (insoluble fibre residue) and the filtrate precipitates (soluble fibre residue) were made in duplicates. One of each set of duplicate insoluble fiber residues and soluble fiber residues were ashed in a muffle furnace at 550 ^{o}C for 3 h. Another set of residues were used to determine protein as Kjeldahl nitrogen multiplied by 6.25. Insoluble or soluble dietary fibre residues (% original sample weight) minus% ash and% crude protein found in the residues were taken to be the values for insoluble (IDF) and soluble (SDF) dietary fibre fractions respectively. Total dietary fibre, TDF**,** was calculated as the sum of insoluble and soluble dietary fibre.

2.7.5 *In vitro* glycemic index analysis

The snack bars' in vitro glycemic index (GI) was evaluated using the method reported by Goi et al*. [16], as modified by Leoro* et al*. [17]. The samples were combined with 10 mL of HCl-KCl buffer in exactly 50 mg increments (pH 1.50). Using a vortex, the liquids were homogenized for 2 minutes (Buck Scientific Limited, LV, USA). Each combination received 0.20 ml of pepsin solution containing 1 mg pepsin in 10 ml of HCl–KCl buffer (pH 1.50). For 60 minutes, the mixtures were incubated at 40°C in a water bath with continual shaking.* The digests were diluted to 25 ml by adding 15 ml Tris-maleate buffer (pH 6.9). Starch hydrolysis was initiated by adding 5 ml tris-maleate buffer containing 2.60 IU porcine pancreatic α-amylase. The mixtures were incubated at 37°C in a water bath maintain at moderate agitation. Exactly 1 ml sample were taken from each flask every 30 min from 0 to 3 h. The α-amylase was inactivated immediately by holding the flask in a boiling water bath for 5 min. Then, 3 ml of 0.40 M sodium acetate buffer (pH 4.75) followed by 60 µl amylo-glucosidase from *Aspergillus niger* was added and the mixture was incubated at 60°C for 45 min.

The glucose concentration was determined using a glucose oxidase-peroxidase kit (Baloworld scientific G3254 – Acap 01). The rate of starch digestion was expressed as a percentage of the total starch hydrolyzed at different times (30, 60, 90, and 120 min). A non-linear model was applied to describe the kinetics of the starch hydrolysis [16]. The first order equation had the form

$$C = C_\infty (1 - e^{-kt}) \tag{1}$$

And the areas under the Hydrolysis Curve (AUC) were calculated using the following equation:

$$AUC = C_\infty (t_f - t_0) - (C_\infty/k)\, [1 - \exp (t_f - t_o)] \tag{2}$$

C = Percentage of starch hydrolyzed at time t, C_∞= Equilibrium percentage of starch hydrolyzed after 120 min, k = Kinetic constant, t = Time, t_f= Final time (120 min) and t_o = Initial time (0 min).

The Hydrolysis Index (HI) was obtained by dividing the area under the hydrolysis curve of each sample by the corresponding area of a reference sample (glucose).

$$HI = \text{AUC of sample} \backslash \text{ AUC of glucose} \tag{3}$$

The Glycemic Index (GI) was calculated using this equation:

$$GI = 39.71 + (0.549 \times HI) \tag{4}$$

2.7.6 Determination of *in vitro* starch digestibility

The technique of Singh et al*. [18] was used to determine in vitro starch digestibility. Each snack bar was weighed exactly 50 mg and combined with 1 ml of 0.2 M phosphate buffer in test tubes (pH 6.9). The sample mixtures were incubated at 37^{0}C for 2 hours with pancreatic α-amylase (0.5 ml; 20 mg enzyme dissolved in 50 ml of the same buffer).* After incubation, 2 ml of 3,5-DNS reagent (prepared by dissolving 200 mg crystalline phenol, 1 g of 3,5-dinitrosalycyclic acid and 50 mg sodium sulphite in 1% NaOH solution) was added immediately. The mixture was heated for 5-15 min in a boiling water bath. Exactly 1 ml of K-Na Tartarate solution was added to the mixture test tubes and allowed to cool at 25 ^{o}C. The solution was therefore made up to 25 ml with distilled water and filtered prior to reading of the absorbance at 550 nm. A blank was run simultaneously. A standard curve was prepared using maltose and values obtained were expressed as mg maltose equivalent per 100 mg of sample.

2.7.7 Determination of *in vitro* protein digestibility

The enzymatic approach described by Kanu et al. [19] was used to measure the in vitro protein digestibility of each sample. Each of the formed samples was weighed into 5 ml centrifuge tubes, and 15 ml of 0.1 M HCl with 1.5 mg pepsin-pancreatin was added. The tubes were incubated for 3 hours at 37˚c. A phosphate buffer (pH 8.0) containing 0.005 M sodium azide was used to neutralize the solution. To prevent microbial development, 1 mL of toluene was added, and the mixture was gently mixed and incubated at 37°C for 24 hours. Following incubation, samples were treated with 10 mL of 10% trichloroacetic acid (TCA) and centrifuged at 5000 rpm at room temperature for 20 minutes. The nitrogen content of the TCA soluble fraction in the supernatant liquid was determined using the micro-Kjedahl method. The percentage of protein digestibility was calculated using the formula;

Protein digestibility (%) = [(N in the supernatant – N in the blank)/ N in the sample] x 100

2.8 Analysis of Data

Data obtained from the analyses conducted were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS version 20 software. Significant differences at p<0.05were determined. Mean separation were carried out using the New Duncan Multiple Range Test (NDMRT).

3. RESULTS AND DISCUSSION

3.1 Particle Size Distribution of Flours

The results of the particle size analysis of the flours are shown in Table 1. All raw materials showed heterogeneous particle size distribution. Significant (P<0.05) differences existed between all the particle sizes the flour samples retained at 2.00 mm, 1.18 mm, 425 µm, and 75 µm sieve opening. At 2.36 mm opening, the weight of malted African breadfruit flour (MA) and defatted African breadfruit flour (FA) retained were statistically the same at p>0.05. The coconut grits showed a different particle size distribution (a reverse) from the other raw materials. Sieve no. 0.8 (2.36mm opening) showed 70% particle retention, while sieve no. 0.16 and 0.40 showed 22% and 4% particle size retention respectively. This is probably the reason it is referred to as grits.

Flours of malted African breadfruit seeds and maize (MA and MF respectively) were not fine, but coarse in nature. The particles of coconut were larger and gritty in nature. Leoro et al. [17] also reported heterogeneous particle size distribution in passion fruit fibre with 29%, 32% and 22.5% retention between 20-32 mesh, 32-60 mesh and <100 mesh, respectively. These particle size distributions could have been the reason for the unique chewiness characteristic of the snack bars.

3.2 Proximate Composition and Energy Values of Malted African Breadfruit Seed Flours, Maize Flour and Coconut Grits

Proximate composition of materials for production of snack bars are shown in Table 2. Moisture content of samples were all below 10%, which suggests reduced chances of spoilage by microorganisms and consequent increase in shelf life [20]. Coconut grits had the highest (6.28, 42.12, 27.11) while maize flour had the least (1.83, 4.79, 9.65) content of ash, crude fat and protein respectively. The protein content of maize flour was higher while carbohydrate was lower than that reported (6.9% and 73.58% respectively) by Gwirtz and Garcia-Casal [21]. The difference could be due to varietal difference in the maize used. The coconut grits showed the highest energy value, while the malted African breadfruit flour showed the least value. The ash, crude protein, crude fibre and crude fat content of the malted African breadfruit flour could qualify it as valuable source of nutrients. These results suggest that African breadfruit seed could be important for a developing country like Nigeria [22].

Crude fibre content of these materials were desirable since high fibre foods are said to benefit the heart, lowers the risk of blocked arteries, heart attack and stroke, as well as reduces appetite, thus protect against obesity [23]. Whereas, diets low in fibre are undesirable as they could cause constipation and are implicated with disease of colon like pile, hemorrhoids, appendicitis and even

Table 1. Particle size distribution of malted African breadfruit seed flour, maize flour and coconut grits

Samples Weight Retained (g/100g)	Sieve No. (openings)				
	0.8 (2.36mm, 0.0937inches)	**0.10 (2.00mm, 0.0787inches)**	**0.16 (1.18mm, 0.0469inches)**	**0.40 (425µm, 0.0165inches)**	**0.200 (75µm, 0.0029inches)**
MA	0.32±1.02^b	0.43±0.21^b	7.21±0.10^b	67.20±0.13^a	24.84±0.08^a
MF	0.33±0.32^b	0.30±0.12^c	36.74±0.04^a	56.80±0.06^b	5.26±0.02^b
CG	70.12±0.22^a	22.95±0.11^a	4.14±0.11^c	2.60±0.10^c	0.06±0.04^c

Means in the same column with different superscript are significantly different at P<0.05.
MA = Malted African breadfruit seed flour, MF = maize flour, and CG = coconut grits

Table 2. Proximate composition and energy values of malted African breadfruit seed flour, maize flour and coconut grits

Sample	Moisture content %	Ash content %	Crude Fat %	Crude Fibre %	Crude Protein %	Carbohydrate %	Energy value Kcal/100g
MA	3.76±0.12^{c}	3.52±0.02^{b}	11.52±0.10^{b}	20.18±0.10^{a}	23.32±0.12^{b}	37.70±0.14^{b}	305.76±0.01^{c}
MF	3.82±0.01^{b}	1.83±0.12^{c}	4.79±0.03^{c}	7.73±0.14^{c}	9.65±0.04^{c}	72.18±0.01^{a}	370.43±0.02^{b}
CG	4.86±0.04^{a}	6.28±0.01^{a}	42.12±0.01^{a}	10.67±0.03^{b}	27.11±0.01^{a}	10.95±0.02^{c}	531.41±0.04^{a}

Means along the same column with different letters are significantly different at P<0.05
MA = Malted whole African breadfruit seed flour, MF = Maize flour, CG = Coconut grits

Table 3. Phytochemical composition of malted African breadfruit seed, maize and coconut flours

Flour Sample	Tannin (%)	Oxalate (mg/100g)	Phytate (mg/100g)	Saponin (%)	Trypsin Inhibitor (TIU /mg)
MA	0.33^{b}±0.03	0.21^{c}±0.02	0.37^{b}±0.01	3.43^{c}±0.02	10.01^{a}±0.04
MF	0.27^{c}±0.01	0.31^{a}±0.11	7.42^{a}±0.01	10.21^{a}±0.01	1.03^{b}±0.01
CG	0.50^{a}±0.04	0.27^{b}±0.01	6.25^{a}±0.03	7.21^{b}±0.04	0.95^{c}±0.00

Values are means ± standard deviation of triplicate determinations
Means on the same column with different superscript are significantly different at P<0.05
MA = malted African breadfruit seed flour, MF = maize flour, CG = coconut grits

cancer [24]. “Coconut fibre stands out more importantly than other fibre sources. Coconut fiber slows down the rate of emptying food from the stomach. This allows food more time in the stomach to release minerals, leading to higher levels of minerals available for the body to absorb” [25].

3.3 Phytochemical Composition

“Results of tannin, phytate, oxalate, saponin and trypsin inhibitor activity content of flours from malted African breadfruit seed, maize and coconut for snack bars formulation are presented in Table 3. Tannin content ranged from 0.27 to 0.50%. The concentrations of tannin in the flours posed no health risk, since the reported safe level is 90 mg/100g” [26];[27]. “Tannins are the oligometric higher molecular of polyphenols compound occurring naturally in plants” [28]. “Due to their binding ability with protein and carbohydrate, tannin can inhibit digestive enzymes and reduces the bioavailability of proteins” [29]. The amount of oxalate in the processed flour (0.21 – 0.31 mg/100g), equally, could not be toxic under meal portion since they were lower than the safe level (15-30 g/100g food consumed) reported in literature for man [30]. Concentrations of phytate in the flours was 0.37 – 7.42 mg/100g, and were lower than 250 mg/100g, the amount considered safe level to health [31];[27]. This indicated that the concentration of phytate in the flour samples were of acceptable safe levels. According to Kumar et al. [32], high levels (< 350 mg/100g) of phytates in human foods limit the bioavailability, consequently, utilization of minerals, especially calcium, magnesium, iron, manganese, by forming insoluble compounds that are indigestible. Saponin content was between 3.43 and 10.21%. Adeoti et al*.* [28] reported “saponin content of 9.54 – 18.50 mg/100g for akee apple seed and ariel flour. Saponin has both beneficial and adverse effects on human health.” “Apart from their hypocholesterolemic properties [33], and also shows hemolytic activity by reacting with the sterols of erythrocyte membrane” [34]. Trypsin inhibitor activity in the flours ranged from 0.95 to 10.01 TIU/mg. Trypsin inhibitor activity has a safe level of 200mg/100g in human [35], therefore, the materials were safe for use for snack bars formulation.

3.4 Soluble (SDF), Insoluble (IDF) and Total Dietary Fibre (TDF) Content of Snack Bars

Soluble, insoluble and total dietary fibre content of snack bars produced with different levels of malted African breadfruit seed flour, maize flour and coconut grit blends are shown in Table 4. TDF of the bars ranged from 12.33 to 22.39% and significantly ($p<0.05$) increased with increasing addition of malted African breadfruit seed flour in the formulation. Insoluble dietary fibre (IDF), also increased significantly ($p<0.05$) with increasing addition of malted African breadfruit flour and its content ranged from 7.23 to 19.23%. Soluble dietary fibre (SDF) content of the snack bars were between 3.15 and 5.15%, and significantly ($p<0.05$) decreased with increasing level of malted African breadfruit seed flour in the snack bars. An increase of TDF (12.43 – 17.63%) and IDF (7.25 – 14.76%), with decrease in SDF (5.18 – 2.87%) was also reported by Edima-Nyah et al*.* [12] for snack bars with whole African breadfruit, maize and coconut blends. Silva de Paula et al. [36] reported lower results of 4.7-12.8 g/100g TDF, 2.9 – 7.9 g/100g IDF, 1.8 – 4.9 g/100g SDF in cereal bars enriched with dietary fibre and omega3. Wadikar et al. [37] reported SDF (3.58%), IDF (12.58%) and TDF (16.168%) for multi-millet extruded snacks.

Snack bars produced with malted African breadfruit seed flour could be referred to as high fibre snack bars since they contain more than 3g/100kcal or 6g/100g [38] and their consumption could benefit the heart; lower the risk of blocked arteries, heart attack and stroke, and fill the stomach, reduce appetite and thus protect against obesity [23]. “Also, the consumption of fibre plays an important role in the prevention of diseases such as colon cancer, diabetes and gastro-intestinal disorders” [17]. This is important because fibre acts like a broom, sweeping through the intestinal contents and causing timely expulsion of parasites, toxins and carcinogens from the human system [39].

3.5 *In Vitro* Glycemic Index (GI) of Snack Bars

Results of *in vitro* glycemic index (GI) of snack bars formulated with malted African breadfruit seed, maize and coconut grits are presented in Table 5. Values of% GI ranged from 45.65 to 57.30, decreasing significantly ($p<0.05$) with increasing substitution of malted African breadfruit seed flour in the formulation. This implies that the process of malting reduced the GI of the snack bars.

A negative correlation was observed (Fig. 1) between the *in vitro* glycemic index and the total dietary fibre of the snack bars, with a linear equation:

Table 4. Soluble, insoluble and total dietary fibre content of snack bars produced with different levels of malted African breadfruit seed flour

Snack bar	SDF %	IDF %	TDF %
T_0 (control)	5.15 ± 0.00^a	7.23 ± 0.01^f	12.33 ± 0.02^f
T_{20}	4.44 ± 0.01^b	13.46 ± 0.00^e	17.90 ± 0.02^e
T_{25}	4.14 ± 0.02^c	14.53 ± 0.02^d	18.68 ± 0.04^d
T_{30}	4.04 ± 0.01^d	15.95 ± 0.00^c	19.99 ± 0.02^c
T_{35}	3.89 ± 0.02^e	17.84 ± 0.00^b	21.71 ± 0.03^b
T_{95}	3.15 ± 0.00^f	19.23 ± 0.02^a	22.39 ± 0.02^a

Means in the same column with different superscript are significantly different at p<0.05
SDF = Soluble Dietary fibre, IDF = Insoluble Dietary Fibre, TDF = Total Dietary Fibre

Table 5. *In vitro* glycemic index of snack bars produced from malted African breadfruit, maize and coconut blends.

Snack bars	(%) *In vitro* glycemic index
T_0	57.30 ± 0.00^a
T_{20}	53.42 ± 0.01^b
T_{25}	51.92 ± 0.00^c
T_{30}	50.35 ± 0.00^d
T_{35}	48.87 ± 0.01^e
T_{95}	45.65 ± 0.01^f

Means in the same column with different superscript are significantly different at p<0.05
T_0 = 0:95:5, T_{20} = 20:75:5, T_{25} = 25:70:5, T_{30} = 30:65:5, T_{35} = 35:60:5, T_{95} = 95:0:5 for malted African breadfruit seed flour: Maize flour: Coconut grit blends

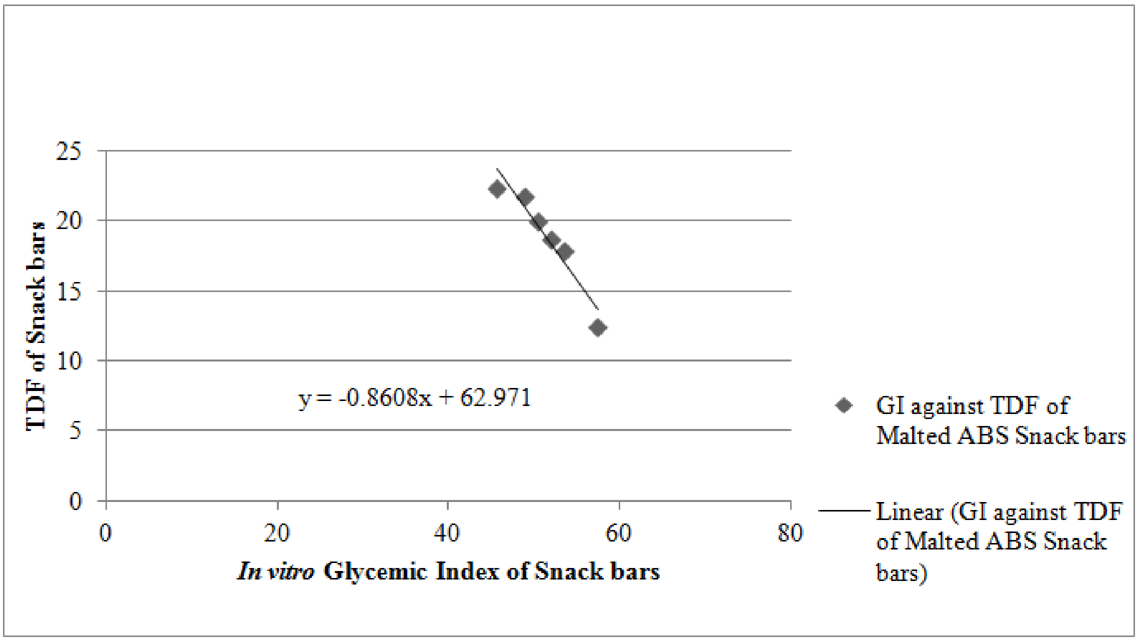

Fig. 1. A graphical plot showing the correlation between *in vitro* Glycemic index (GI) and Total Dietary Fibre (TDF) of Snack bars formulated with blends of malted African breadfruit seed (ABS) flour, maize flour and coconut grits

$$y = -0.8608x + 62.971 \ldots\ldots \text{ eqn. 1}$$

Being a property of starchy foods, glycemic index describes the rate of blood glucose absorption in the blood after consumption. According to standard classification [40], snack bars formulated with malted African breadfruit flour ($T_{20} - T_{95}$ = 53.42 – 45.65) could be considered "low glycemic index foods", while the Control (T_0) could be "medium glycemic index food". These snacks may be considered as possible "health food" alternatives for people on weight control diets, diabetics, or those seeking for healthier eating habits. They could also be used as glycemia control diets, where slower release of glucose is desired [17].

3.6 *In Vitro* Starch and Protein Digestibility of Snack bars

Results of *in vitro* starch digestibility (IVSD) of snack bars is shown in Table 6, with values ranging from 31.44 to 57.48%. IVSD decreased significantly with increase in malted African breadfruit seed flour substitution in the formulation. Azzollini et al. [41] reported "a range of 34 to 57% for IVSD of extruded insect-enriched snacks." Flores-silva et al. [42] reported "lower values (11.6 – 13.4%) for snacks from unripe plantain, chickpea and maize flour blends, while Wadikar et al. [37] recorded 4.65mg/g digestibility for multi-millet extruded snacks. The degree of starch digestibility is linked to its gelatinization" [43]. "The reduction in *in vitro* starch digestibility due to increase in malted African breadfruit flour could probably be due to corresponding increase in fat (lipid) content (Table 2) and consequent limited starch transformation." Xiaoli [44] reported that "the presence of 5% fat reduced the mechanical energy and the melt temperature, causing the decrease of starch gelatinization and thus starch digestibility. Fat was also said to prevent absorption of moisture and gelatinization by forming a hydrophobic layer outside starch granules."

The higher the percentage of ABS in the formulation, the higher the protein content (Table 2) and, as a result, the lower the in vitro Starch Digestibility. This observation was in line with the report of Singh et al. [45], that "the presence of protein in the food matrix may influence the rate of starch digestion. They concluded that the digestibility of starches and proteins in various cereal products is significantly affected by their interaction with each other." Choi et al. [46] reported similar trend; by treatment of flour with pepsin, they observed that the lower the protein in sorghum flour, the higher the starch digestibility of the flour.

Particle size of food product also affect the digestibility of the food. The formulated snack bars were made of coarse flour and grits (Table 1), not fine flour. These larger particle sizes may have had a contributing effect to the percentage starch digestibility of the products. According to early studies, decreasing particle size improves starch and protein digestibility [47]; [44]. Decrease in digestibility of large particle sizes may be as a result of reduced surface area for enzymatic activity.

In vitro protein digestibility (IVPD) of the snack bars ranged from 68.19 to 87.45% (Table 6). Chima et al. [48] reported 25.43 – 71.57% IVPD of tigernut-pigeon pea biscuits, while James and Nwabueze [22] recorded 70.43 – 72.86% protein digestibility in extruded soy based snacks. Azzollini et al. [41] reported higher values (76 – 92%) of IVPD for extruded insect-enriched snacks.

Edima-Nyah et al. [12] in their earlier research reported lower IVPD in snack bars developed with whole African breadfruit seed flour, with maize and coconut mixes. The higher digestibility observed in the present study could be due to malting treatment given to the African breadfruit seeds before use for formulation of the snack bars. Improvement could also be attributed to the reduction in phytochemicals. Rahim [49] reported improvement from 69.78 to 89.98%

Table 6. *In vitro* Starch Digestibility (IVSD) and *In Vitro* PROTEIN Digestibility (IVPD) of snack bars produced from malted African breadfruit seed flour, maize flour and coconut grits

Snack Bars	*In Vitro* Starch Digestibility (%)	*In Vitro* Protein Digestibility (%)
T_0	57.48 ± 0.00^a	68.19 ± 0.01^e
T_{20}	51.70 ± 0.00^b	87.45 ± 0.02^a
T_{25}	48.77 ± 0.01^c	76.33 ± 0.01^b
T_{30}	46.54 ± 0.01^d	74.37 ± 0.02^c
T_{35}	40.63 ± 0.02^e	72.31 ± 0.01^d
T_{95}	31.44 ± 0.01^f	69.23 ± 0.01^e

Values are Means ± standard deviation of duplicate determinations. Means on the same column with different superscript are significantly different. T_0 = 0:95:5, T_{20} = 20:75:5, T_{25} = 25:70:5, T_{30} = 30:65:5, T_{35} = 35:60:5, T_{95} = 95:0:5 for malted African breadfruit seed flour: Maize flour: Coconut grit blends in faba beans due to germination. According to Alonso et al. [50], germination is the most effective process in reducing phytic acid and improving the IVPD of foods. Protein quality of food is defined by its amino-acid composition and digestibility; and protein digestibility determines the availability of its amino-acids [49];[50]. This therefore implies that there could be increased availability of the amino acids from the snack bars when consumed.

4. CONCLUSION

The study showed that malted African breadfruit seeds could be used for snack bars production. All snack bars recorded high fibre content, and all the malted African breadfruit inclusion had low glycemic index. *In-vitro* starch and protein digestibility of snack bars were within recommended range for assimilation and use in the body. The snack bars could be considered as ‘high fibre foods’, and may be useful for consumers managing diabetes and body weight, or those seeking alternatives and healthier eating habits.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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