

Full Length Research Paper

Microbiological, nutritional and sensory evaluation of snack bars developed using Bambara groundnut (*Vigna subterranean* L.) and maize (*Zea mays*)

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Over the years, snacking has become a regular habit for majority of the population with increasing need for food manufacturers to meet consumers' demand through product development. In this study, snack bars were prepared using maize and Bambara nuts mixed in the following ratios: A, 100% maize; B, 100% Bambara nuts; C, 50% maize: 50% Bambara nuts; D, 75% maize: 25% Bambara nuts; E, 75% Bambara nuts: 25% maize. The total heterotrophic bacterial count of samples A to E was within the limit stipulated by International Commission on Microbiological Specification for Food. The frequency of occurrence of bacterial isolates from the samples include *Bacillus* species (24%), *Staphylococcus* species (24%), *Lactobacillus* species (24%), *Escherichia coli* (19%) and *Serratia* species (9%), while the fungal isolates include *Aspergillus* (33%), *Penicillium* (27%), *Rhizopus* (20%) and *Saccharomyces* species (20%). The moisture, ash, carbohydrate, crude protein, fat and fiber content of the samples were within the range of 11.47±0.99-17.45±1.01, 1.09±0.07-2.00±0.15, 56.05±0.65-70.37±0.71, 6.32±0.36-15.00±0.22, 4.60±0.50-7.00±0.30 and 2.60±0.25-3.10±0.31%, respectively. There was a significant difference ($p<0.05$) in the proximate composition among the samples except for crude fiber. The calorie value range between 347.20-367.69 kcal and acceptability of the samples compared favourably with a commercialized snack bar.

Key words: Healthy snacking, natural sweetener, underutilized legumes, cereal bar, food product development.

INTRODUCTION

It has been the norm in different societies for people to eat three sizeable meals per day. In the past few decades, majority of the population have formed the habit of consuming smaller amounts of food and/or drink at short intervals between three standard meals (Chaplin

and Smith, 2011). This feeding habit is referred to as snacking (Potter et al., 2018). Large population of inhabitants in cities and semi-urban areas experience daily hectic and busy lifestyle on which among others could be attributed to increasing job demands and

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dwindling economies. Consequently, workers are forced to spend long hours at work which encourages heavy snacking (Sharma et al., 2014). The manner at which an individual feel hungry and thirsty sensation before and after snacking depends on the size of snack consumed and its nutritional content and is usually not the same as with a standard meal (Chaplin and Smith, 2011; Boon et al., 2012). In a bid to lessen hunger by snacking, it is recommended that health-promoting foods containing vital nutrients should be consumed. According to Eke-Ejiofor and Okoye (2018), the habit of snacking irresponsibly could lead to weight gain and nutrition deficiency. Despite these assertions, the relationship between snacking and human health is still debatable (Potter et al., 2018). Ready-to-eat (RTE) foods which include wide varieties of snacks are predisposed to microbial contamination by diverse species of bacteria, fungi and parasites. Some viruses have also been implicated in contaminating RTE foods. Consumption of contaminated snacks could lead to microbial foodborne illness (Makinde et al., 2020).

Healthy snacking is gaining popularity among the people due to higher level of awareness for human health and nutrition (Ishak et al., 2021). Instead of relying heavily on snacks prepared using refined sugar, the use of natural sweeteners such as honey and date (*Phoenix dactylifera* L.) is preferable on health grounds (Nissar et al., 2017; Ibrahim et al., 2021). The use of honey as a sweetener is an age long practice (Ajibola et al., 2012). Honey is a well-known, sweet, aromatic and viscous liquid obtained from the nectars of plants by honeybees which store the product in hives (Hebbar et al., 2008). Honey has a high sugar and water content including vitamins, amino acids, minerals, trace compounds and enzymes. The use of honey as a natural sweetener in food product development contributes to nutritional, therapeutic and health benefits (Saha, 2015; Sharma et al., 2020).

The snack bar also known as the cereal bar is a popular snack prepared by compressing a mixture of cereals, nuts and dried fruits (Rush et al., 2016; Edima-Nyah et al., 2019). The shelf life of snack bars is moderate and does not require refrigeration (Ravindra and Sunil, 2018; Eyiz et al., 2020). A study carried out by Verma et al. (2018) reported that shelf life of sorghum based cereal bars was 60 days. Preparation of cereal bar involves the use of a wide range of ingredients such as walnut, almond nut, oats, dried raisins, coconut, sesame seed, honey and dried fig. These ingredients are combined in different proportions based on individual's choice and baked until it becomes crisp (Eke-Ejiofor and Okoye, 2018; Herawati et al., 2019). Many researchers have developed varieties of cereal bars using a wide range of nutrient dense ingredients (Jethwani et al., 2020; de Melo et al., 2020; Maia et al., 2021).

Fruit-based snack bars, wheat or soy-based bars, cereal snack bars, fruit and vegetable-based snack bars,

vegetable snack bars and high-protein snack bars are various types of snack bars (Constantin and Istrati, 2018).

Initially, snack bars were meant for athletes to provide them energy. Due to high demand of snack bars by non-athletes, sales have been on the increase in many countries such as the US, UK, Germany and Brazil (Sharma et al., 2014; Carvalho and Conti-Silva, 2017; Dahri et al., 2017; Pinto et al., 2017). By 2025, it is expected that the cereal bar market globally will reach \$16.9 billion (Lasta et al., 2021). This could be attributed to high level awareness about convenient, natural, nutritious and healthy food products. The snack bar is a source of nutrients which include fat, protein, fibers, minerals, vitamins, calories and carbohydrates (Ho et al., 2016; dos Prazeres et al., 2017; Constantin and Istrati, 2018). The nutritional composition of each ingredient used in preparing snack bars influences the nutrients and energy value of the product. Oftentimes, the dietary needs of consumers are taken into consideration during the stage of selecting ingredients to be used in preparing snack bars (Maia et al., 2021). According to Pinto et al. (2017), substituting meals with snack bars is effective in achieving weight loss. However, due to the level of available carbohydrates in cereal bars, the product is capable of increasing glycemic indexes (GI) which is not suitable for persons suffering from Type 2 Diabetes mellitus (Farago et al., 2021). The calorific value of Bambara groundnut and cereal grains are such that they can be used as ingredients in the production of snack bars (Igbabul et al., 2013).

Bambara groundnut (*Vigna subterranean* (L.) Verdc or *Voandzeia subterranea* (L.) Thouars) is generally regarded as an underutilized legume that grows abundantly in Africa (Orhevba and Mbamalu, 2017; Udeh et al., 2020; Khan et al., 2021). There has been a considerable increase in the utilization of Bambara groundnut in the past few years (Igbabul et al., 2013; Nwadi et al., 2020). Based on its nutritional composition, Bambara groundnut is a complete food. Surprisingly, it is also inexpensive. According to Anhwange and Atoo (2015), Bambara groundnut contains 6.35-7.78% moisture, 3.53-3.94% ash, 4.58-5.50% crude fiber, 18.25-20.44% protein, 5.82-6.31% lipid and 52.08-56.01% soluble carbohydrate. Accordingly, Bambara groundnut is a rich protein source (Orhevba and Mbamalu, 2017; Tan et al., 2020). It also contains minerals which include calcium, iron, potassium, and sodium in reasonable quantities. In Nigeria, many local dishes, well appreciated by people, are prepared using Bambara groundnut flour (Barimalaa et al., 2005; Ndidi et al., 2014).

Maize (*Zea mays* L.) is in the second position behind sorghum among all the cereals grown and consumed in Nigeria. Globally, maize is ranked third behind rice and wheat. Maize is referred as the 'queen of cereals' considering its maximum yield when compared with other cereals (Adeniyi and Ariwoola, 2019). Maize is the

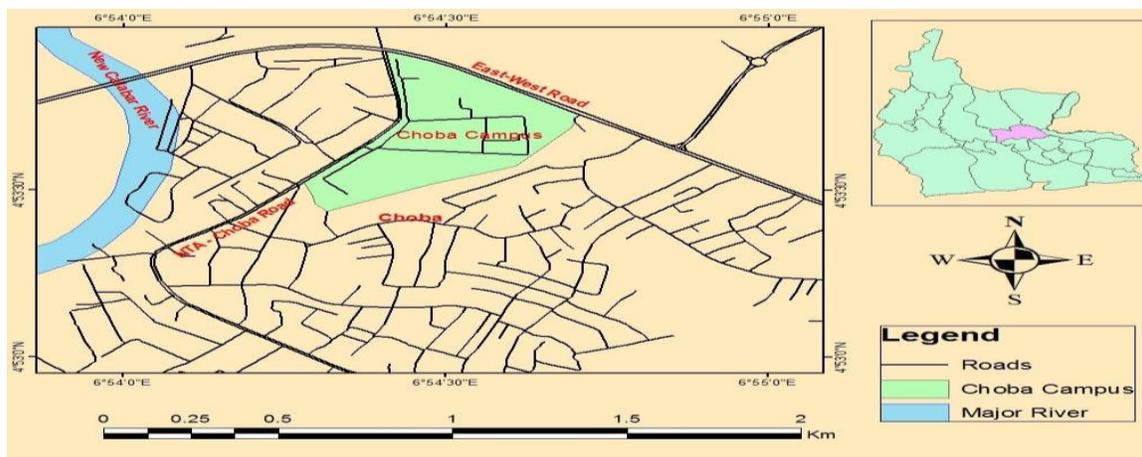


Figure 1. Choba Town, Obio-Akpor LGA, Rivers state, Nigeria.

Source: Geographic Information System Laboratory, Geography and Environmental Management, University of PortHarcourt, Choba, Nigeria.

highest source of energy in the national diets of 22 countries across the world which includes 16 African countries (Ahaotu et al., 2021). Cereals such as maize are rich sources of energy in diets. The quality of protein in cereals such as maize is poor because it contains little amount of amino acids known as lysine and tryptophan (N'Guessan et al., 2014; Moses and Makanjuola 2018). Maize is a staple food for inhabitants in sub-Saharan Africa. It is estimated that 30% of the total calorie intake of the people comes from maize. In different parts of Africa, many local foods are prepared using maize (Ndukwe et al., 2015; Ekpa et al., 2019). Food industries make use of maize flour to produce different products (Gwirtz and Garcia-Casal, 2013; Oladapo et al., 2017). A recent study carried out by Edima-Nyah et al. (2019) involved the use of African breadfruit (*Treculia africana*), maize (*Z. mays*), and coconut grits (*Cocos nucifera*) to prepare snack bars. Interestingly, these snack bars were nutritive and well acceptable by the sensory panelist. In a related study, Eke-Ejiofor and Okoye (2018) developed cereal bars using locally available cereals which include millet, guinea corn, yellow and white maize. The study revealed that these cereal bars possess better nutritional and sensory qualities than oat bar (control).

Introduction of new food products, such as energy or cereal bars, into the market takes into consideration the nutritional and sensory quality of the products (Srebernich et al., 2016). Food safety of such products is equally important. However, microbiological assessment of cereal bars developed by many researchers were not carried out nor reported. Munhoz et al. (2014) detected the presence of *Bacillus cereus* in snack bars containing bocaiuva. Snack bars are not popular in this part of the world. In Nigeria, most of the commercially available snack bars are imported and expensive. They usually contain high amount of refined sugar which might not be

suitable for diabetic patients and the elderly. In the light of the aforementioned, this study is aimed at carrying out microbiological analysis, nutritional and sensory evaluation of a healthy snack bar developed using honey, Bambara groundnuts and maize.

MATERIALS AND METHODS

Yellow variety of maize grain, Bambara groundnut, fresh eggs from fowl and refined palm olein (branded) were purchased from traders at Choba market. A bottle of honey (local brand; sourced from hive), oat, iodized table salt, and liquid milk (branded; sourced from cow) were obtained from superstores along Choba-NTA road. Coconut (*C. nucifera*) was harvested from a coconut tree planted in Choba. Figure 1 shows the map of Choba town where all the materials were purchased. They were put inside a big sterile polythene bag and transported to Food and Industrial Microbiology Laboratory, University of Port Harcourt, Choba, Rivers State.

Preparation of snack bar

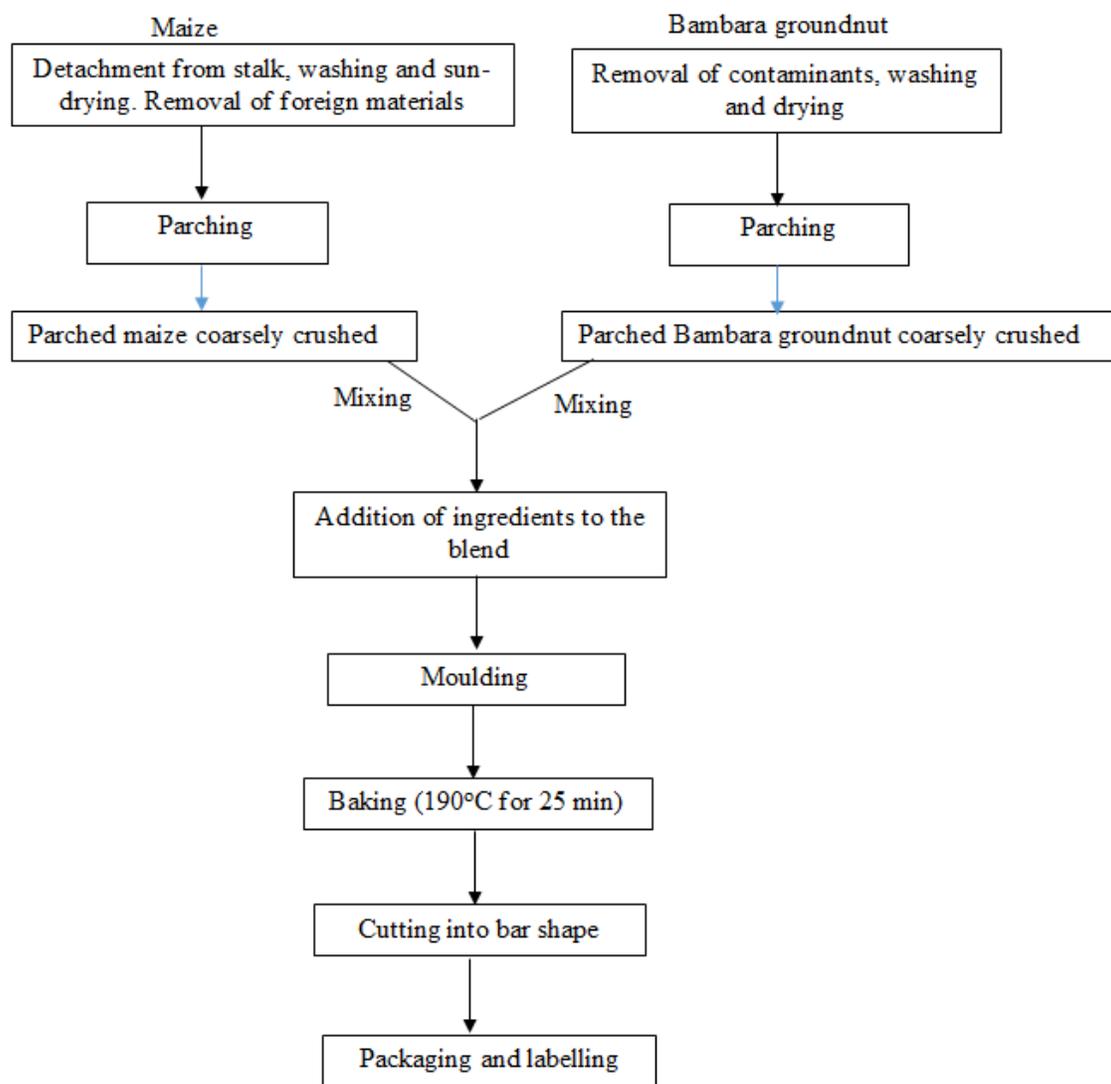
A composite of crushed maize and Bambara groundnut weighing 200 g was prepared in five different proportions (Table 1). The particle sizes vary from 1.2 to 3.8 mm. Each portion was mixed thoroughly in separate bowls. Dehusked coconut was grated manually. For each bowl, 5 tablespoons of coconut, 5 tablespoons of refined palm olein, 5 tablespoons of oat, 50 ml of liquid milk, 6 tablespoons of honey, one fresh egg and a pinch of salt were added and mixed thoroughly. After kneading, each of the portions was moulded into a flat thin shape and then baked in the oven for 25 min at 190°C. After baking, the samples were cut into bar shape and packaged. In Figure 2, the flow chart for the production of the snack bars is shown.

Serial dilution

A portion of each sample was ground into powder using laboratory blender (Usha Mixer Grinder, India) sterilized with 70 % ethanol.

Table 1. The proportion of maize and Bambara groundnut in the snack bar samples.

Trials	Maize (%)	Bambara groundnut (%)
A	100	0
B	0	100
C	50	50
D	75	25
E	25	75

**Figure 2.** Flow chart for the production of snack bar.

Aseptically, 10 g of the sample was weighed using electronic balance (Mettler MT-2000) and dispensed into 90 ml sterile peptone water. It was mixed properly to form a suspension which serves as the stock solution of the samples. Ten-fold dilution was carried out by stepwise transfers to achieve higher dilutions with a sterile pipette for each transfer until dilution 10^{-4} was achieved.

Microbiological analysis

Total heterotrophic bacterial count

Aseptically, 1 ml of dilutions 10^{-1} and 10^{-2} was inoculated into Petri dishes containing sterile molten nutrient agar (NA) and Sabouraud

dextrose agar (SDA) prepared following manufacturer's instruction. The NA and SDA were autoclaved at 121°C for 15 min at 15 psi. The inoculated plates (NA) were incubated at 37°C for 24 h. The method described by Ekpakpale et al. (2021) with slight modification was used to enumerate the fungal population after incubating the SDA plates at 25°C for 96 h. All the culture plates were examined for microbial growth and the colonies which appeared were enumerated and recorded as colony forming units (CFU/ml).

The bacterial and fungal population of the samples was calculated using the formula:

$$\text{CFU/mL} = \text{No. of colonies} \times \frac{1}{\text{serial dilution}} \times \frac{1}{\text{dilution plated}}$$

Obtaining pure culture

Representative colonies from the NA and SDA culture plates were subcultured by repeated streaking in freshly prepared NA and SDA plates, respectively to obtain pure cultures. The NA plates were incubated at 37°C for 48 h. Similarly, the SDA plates were incubated at 25°C for 7 days as described by Ekpakpale et al. (2021). Further identification of the bacterial and fungal cultures was carried out. The pure culture of bacteria and fungi were inoculated into NA and SDA slants, respectively and stored in the refrigerator at 4°C until analyses is completed.

Identification of the bacterial isolates

Cultural characteristics of the isolates on the culture plates were examined. Gram reactions and cell morphology were examined from heat-fixed smears. Biochemical tests on the isolates which include indole, methyl red, citrate, catalase, oxidase, sugar fermentation, motility and triple sugar iron agar (TSIA) test for H₂S were carried out using the procedure described by Shoaib et al. (2020). Identification of all cultures was done using the procedure described by Holt et al. (1994) and Buchanan and Gibbons (1974).

Identification of the fungal isolates

Microscopically, the colonial characteristics and cell morphology of the fungal isolates were ascertained after staining with lactophenol cotton blue. A portion of fungal mycelium was teased out in a drop of lactophenol cotton blue on grease-free microscope slide and was examined under the microscope with low power high dry objective. The cultural and morphological characteristics of each fungal isolate were compared with earlier descriptions (Barnett and Hunter, 1972).

Proximate composition

The moisture, ash, fat, crude protein and fiber contents of the snack bars were determined using the AOAC (1995) methods. The difference method was used to determine the carbohydrate content.

Calorie value

The method described by Ho et al. (2016) was adopted in determining the calorie value of the snack bar samples. It involves multiplying the total crude protein, crude fat and carbohydrate content of each sample by the factor value (for each gram of carbohydrate and protein, what is obtained is 4 kcal and 1 g of crude fat provides 9 kcal of energy).

$$\text{Energy} = (\text{crude protein} \times 4) + (\text{carbohydrate} \times 4) + (\text{crude fat} \times 9).$$

Sensory evaluation

Sensory evaluation of the snack bar samples was carried out by ten semi-trained panelists familiar with good quality snacks. The panelists were undergraduate students in the Department of Microbiology, University of Port Harcourt between the ages of 18 and 26 years. All the samples presented to them were coded with alphabets A - E. The panelists used 9-point Hedonic scale which range from 1 (dislike extremely) to 9 (like extremely) as a guide to evaluate the sensory attributes of each sample which include taste, color, aroma, appearance, mouthfeel and overall acceptability. Self-explanatory questionnaires were given to the sensory panelists to enter scores for the sensory parameters evaluated for each sample. Potable bottled water was provided for the panelists to rinse their mouth before and after evaluating each sample.

Statistical analysis

Data generated were subjected to one-way Analysis of Variance (ANOVA) with the aid of IBM SPSS Statistics version 23 software. It also determined significant differences at $p < 0.05$. Duncan Multiple Range Test (DMRT) was used in separating the means.

RESULTS

Figure 3 shows the total heterotrophic bacterial count (THBC) of the snack bar samples. Among all the samples, the THBC of snack bar was prepared using 75% maize: 25% Bambara groundnut (4.43 log₁₀ CFU/ml) and 100% maize (4.10 log₁₀CFU/ml) were the highest and least values, respectively. Figure 4 depicts the total fungal count (TFC) of the snack bar samples. The TFC of snack bar was prepared using 100% maize (3.53 log₁₀ CFU/ml) and 100% Bambara groundnut (3.68 log₁₀ CFU/ml) had the least and highest values, respectively.

Plates 1 and 2 show the samples of snack bar developed in this study using maize and Bambara groundnut in different proportions. Table 2 shows the result of biochemical tests carried out on the bacterial isolates from snack bar samples. The isolates identified were *Bacillus* species, *Staphylococcus* species, *Lactobacillus* species, *Serratia* species and *Escherichia coli*. Table 3 shows the macroscopic and microscopic characteristics of the fungal isolates from snack bar samples. The fungal isolates were *Aspergillus*, *Rhizopus*, *Penicillium* and *Saccharomyces* species.

A total of twenty-one bacterial isolates were encountered in all the snack bars prepared using maize and Bambara groundnut in different proportions. The bacterial isolates encountered in the snack bar prepared using 50% maize: 50% Bambara groundnut include *Bacillus*, *Staphylococcus*, and *Lactobacillus* spp. The snack bar prepared using 100% maize and the sample prepared using 75% maize: 25% Bambara groundnut were contaminated with *Lactobacillus* spp., *Bacillus* spp., *Staphylococcus* spp. and *E. coli*. The bacterial genera isolated from snack bar prepared using 25% maize: 75%

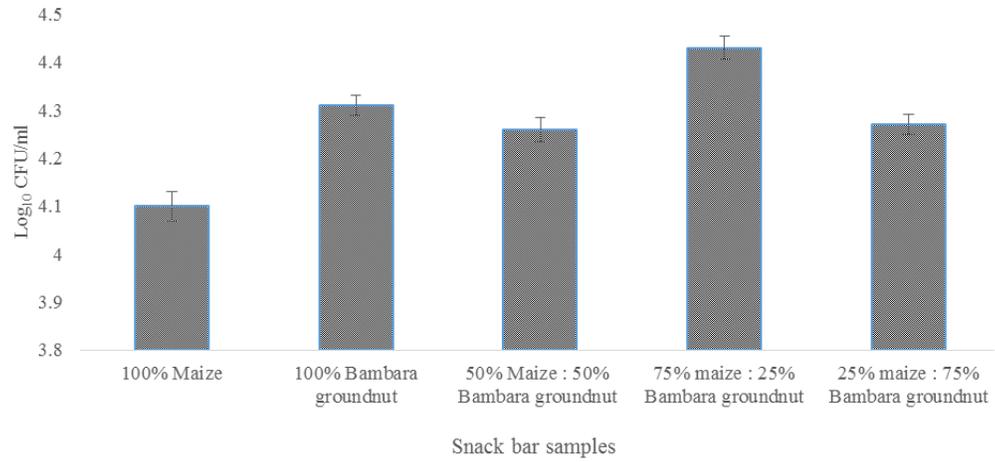


Figure 3. Total heterotrophic bacterial count of the snack bar samples.

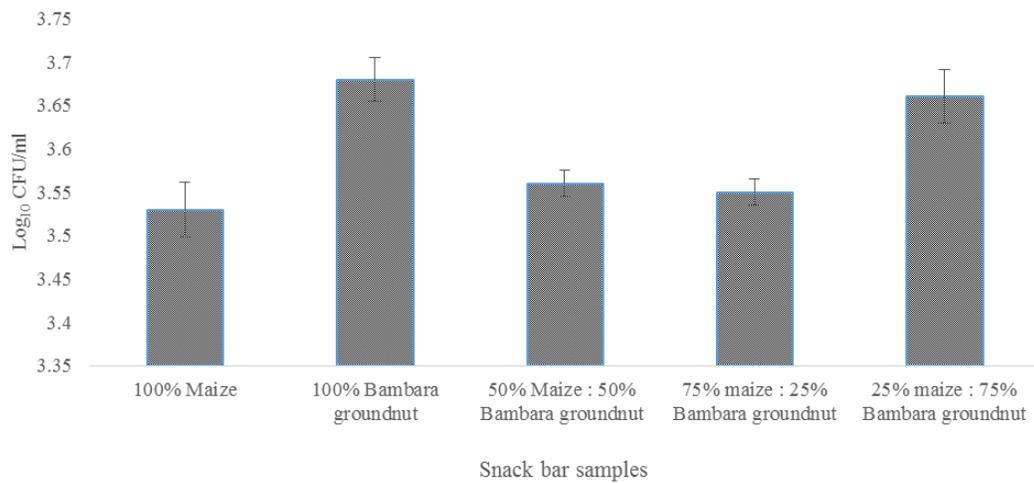


Figure 4. Total fungal count of the snack bar samples.



Plate 1. Snack bar samples before packaging.



Plate 2. Snack bar samples after packaging.

Table 2. Biochemical characteristics of bacteria isolated from the snack bar samples.

Isolate code	Gram reaction	Catalase	Oxidase	Citrate	Indole	Motility	Methyl Red	Voges Proskauer	Slant	Butt	Gas	H ₂ S	Glucose	Lactose	Probable organism
A1	+	-	-	+	-	-	+	-	A	A	+	+	A ⁺	+	<i>Lactobacillus</i> spp.
A2	+	+	-	+	-	+	-	+	B	A	-	-	+	-	<i>Bacillus</i> spp.
A3	+	+	-	+	-	-	-	+	A	A	-	-	+	+	<i>Staphylococcus</i> spp.
A4	-	+	-	-	+	+	+	-	A	A	+	-	+	+	<i>Escherichia coli</i>
B1	+	-	-	+	-	-	+	-	A	A	+	+	A ⁺	+	<i>Lactobacillus</i> spp.
B2	-	+	-	-	+	+	+	-	A	A	+	-	+	+	<i>Escherichia coli</i>
B3	+	+	-	+	-	+	-	+	B	A	-	-	+	-	<i>Bacillus</i> spp.
B4	+	+	-	+	-	-	-	+	A	A	-	-	+	+	<i>Staphylococcus</i> spp.
B5	-	-	-	+	-	+	-	+	B	A	-	-	+	-	<i>Serratia</i> spp.
C1	+	+	-	+	-	+	-	+	B	A	-	-	+	-	<i>Bacillus</i> spp.
C2	+	+	-	+	-	-	-	+	A	A	-	-	+	-	<i>Staphylococcus</i> spp.
C3	+	-	-	+	-	-	+	-	A	A	+	+	A ⁺	+	<i>Lactobacillus</i> spp.
D1	-	+	-	-	+	+	+	-	A	A	+	-	+	+	<i>Escherichia coli</i>
D2	+	-	-	+	-	-	+	-	A	A	+	+	+	+	<i>Lactobacillus</i> spp.
D3	+	+	-	+	-	+	-	+	B	A	-	-	+	-	<i>Bacillus</i> sp.
D4	+	+	-	+	-	-	-	+	A	A	-	-	+	+	<i>Staphylococcus</i> spp.
E1	-	-	-	+	-	+	-	+	B	A	-	-	+	-	<i>Serratia</i> spp.
E2	+	-	-	+	-	-	+	-	A	A	+	+	A ⁺	+	<i>Lactobacillus</i> spp.
E3	+	+	-	+	-	+	-	+	B	A	-	-	+	-	<i>Bacillus</i> spp.
E4	+	+	-	+	-	-	-	+	A	A	-	-	+	+	<i>Staphylococcus</i> spp.
E5	-	+	-	-	+	+	+	-	A	A	+	-	+	+	<i>Escherichia coli</i>

+, Positive; -, negative; A, acid production; B, alkaline production.

Bambara groundnut and the sample prepared using 100% Bambara groundnut include *Lactobacillus* spp., *Bacillus* spp., *Staphylococcus* spp., *E. coli* and *Serratia* spp. Among the bacterial isolates encountered in all the samples of snack bars prepared using maize and Bambara groundnut in different proportions, *Staphylococcus* (24%), *Lactobacillus* (24%) and *Bacillus* spp. (24%) had the highest frequency of occurrence, followed by *E. coli* (19%) and *Serratia* spp. (9%) had the least frequency of occurrence.

A total of fifteen fungal isolates were

encountered in all the snack bars prepared using maize and Bambara groundnut in different proportions. *Aspergillus*, *Penicillium* and *Rhizopus* spp. were isolated from snack bars prepared using 100% maize and 25% maize: 75% Bambara groundnut. The fungal genera isolated from snack bars prepared using 50% maize: 50% Bambara groundnut and 75% maize: 25% Bambara groundnut include *Penicillium*, *Saccharomyces* and *Aspergillus* spp. The snack bar prepared using 100% Bambara groundnut was contaminated with *Aspergillus*, *Rhizopus* and *Saccharomyces*

spp. Among the fungal isolates encountered in the snack bars prepared using maize and Bambara groundnut in different proportions, *Aspergillus* spp. (33%) recorded the highest frequency of occurrence, followed by *Penicillium* (27%), *Saccharomyces* and *Rhizopus* spp. each had the lowest frequency of occurrence (20%).

Table 4 shows the proximate composition of the snack bar samples. Considering each of the proximate parameters analyzed, protein and carbohydrate content showed significant difference ($p < 0.05$) among all the samples of

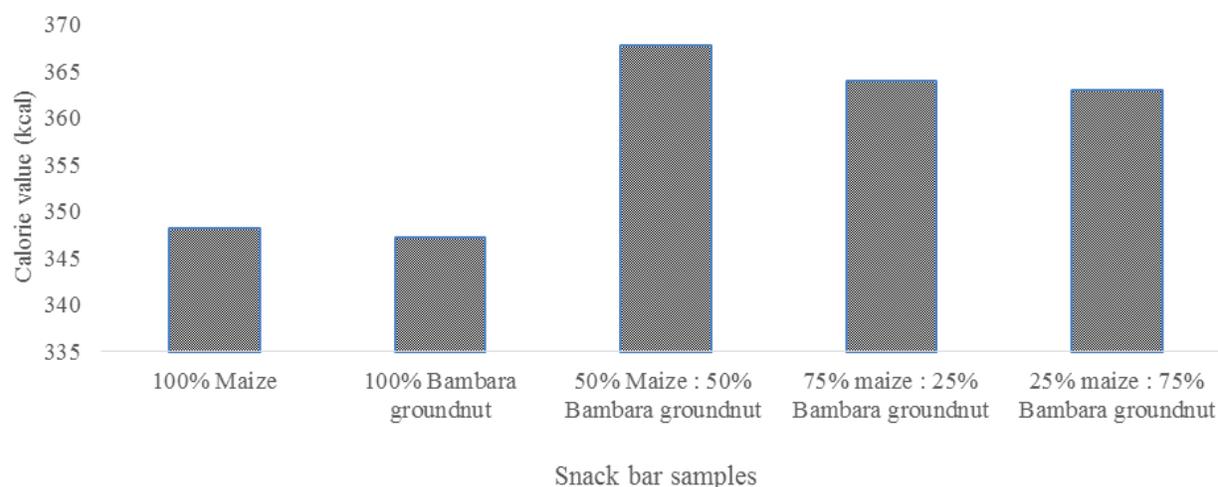
Table 3. Macroscopic and microscopic characteristics of the fungal isolates

Organism	Macroscopic	Microscopic
<i>Aspergillus</i> spp.	Fastidious growth within 3-7 days, green colonies which appear velvety with flat rough-walled stripes and has cracked surface behind.	The phalides produced chains of round, rough conidia that appears pale green when viewed under the microscope.
<i>Penicillium</i> spp.	Between 3-7 days, there was a moderate growth. Flat surface, velvet-like texture with gray-green brush like clusters	The conidiophores appear branched with round to ovoid rough-walled chains.
<i>Saccharomyces</i> spp.	Colonies were white to cream in color, smooth, usually large, yeast-like cells that are roughly spherical in shape ranging from 3-10 mm in size.	They occur in clusters; round/ovoid in shape.
<i>Rhizopus</i> spp.	Fast growing mould with white mycelia and black sporangia which turns black with age.	Non-septate or sparsely septate broad hyphae, sporangiophores, and rhizoids

Table 4. Proximate composition of the snack bar samples.

Sample code	Moisture (%)	Ash (%)	CHO (%)	Crude Protein (%)	Crude fat (%)	Crude Fiber (%)
A	14.56±0.72 ^{ab}	1.09±0.07 ^a	70.37±0.71 ^e	6.32±0.36 ^a	4.60±0.50 ^a	3.06±0.21 ^a
B	17.45±1.01 ^b	1.90±0.17 ^{cd}	56.05±0.65 ^a	15.00±0.22 ^e	7.00±0.30 ^c	2.60±0.25 ^a
C	11.47±0.99 ^a	1.67±0.21 ^{bc}	64.94±0.51 ^c	12.29±0.43 ^c	6.53±0.55 ^{bc}	3.10±0.31 ^a
D	11.69±1.21 ^a	1.46±0.14 ^b	67.55±0.48 ^d	10.62±0.51 ^b	5.70±0.52 ^b	2.98±0.26 ^a
E	12.50±0.82 ^a	2.00±0.15 ^d	62.55±0.47 ^b	13.80±0.61 ^d	6.40±0.44 ^{bc}	2.75±0.37 ^a

Values show the means of triplicate analysis ±SD. Means with different superscript along the column are significantly different ($p < 0.05$). The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100% Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut.

**Figure 5.** Calorie value of the snack bar samples.

snack bars. In contrast, there is no significant difference ($p > 0.05$) in fiber content among all the snack bar samples.

The calorie value of the snack bar samples are

presented in Figure 5. The snack bar prepared using 50% maize and 50% Bambara groundnut had the highest calorie value (367.69 kcal) whereas the sample which had the lowest calorie value (347.2 kcal) was prepared

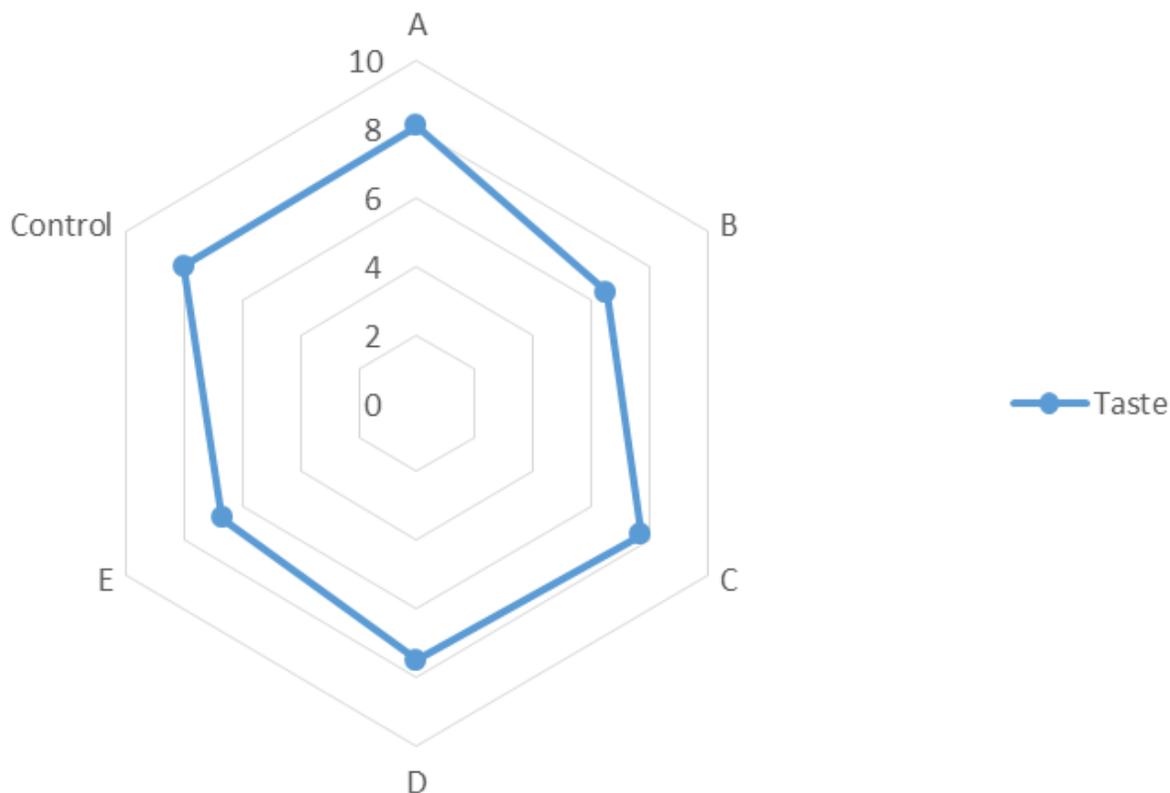


Figure 6. Taste of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100% Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

using 100% Bambara groundnut. Worthy to note is that the snack bar prepared using 100% maize and the sample prepared using 100% Bambara groundnut had a little difference in their calorie content. Similarly, the calorie content of snack bar prepared using 75% maize: 25% Bambara groundnut is slightly different from the value recorded for snack bar prepared using 25% maize: 75% Bambara groundnut.

Figures 6 to 11 show the mean panelist score assigned to the snack bars evaluated based on their sensory attributes which ranged from 6.5-8.1 for taste, 7.1-8.0 for color, 6.4-8.0 for aroma, 7.0-8.0 for appearance, 7.1-8.0 for mouthfeel, and 7.4-8.2 for overall acceptability, respectively.

DISCUSSION

In this study, snack bars were prepared using maize and Bambara groundnut in different proportions. The total heterotrophic bacterial count (THBC) and total fungal count (TFC) of the snack bar samples is within the range of 4.10-4.43 \log_{10} CFU/ml and 3.53-3.68 \log_{10} CFU/ml,

respectively. The snack bars were considered to be microbiologically safe for human consumption because the THBC of the samples were lower than 6 \log_{10} CFU/ml which is the limit stipulated by International Commission on Microbiological Specification for Food (ICMSF) (Maduka et al., 2021). The total plate count of energy bars prepared by Bhavani et al. (2018) is within the range of 1.2-1.8 $\times 10^2$ CFU/g whereas fungi was not detected in their product. In a related study that involved the production of snack bar using a blend of African breadfruit seed flour, maize flour and coconut grits, Edima-Nyah et al. (2019) also reported that the samples met the ICMSF specification.

Bacteria isolated from all the samples of snack bars include *Staphylococcus* spp., *Bacillus* spp., *Lactobacillus* spp., *Serratia* spp. and *E. coli*. Different cereals used as ingredients to prepare ready-to-eat (RTE) snack bars are possible sources of microbial contamination of the product (Los et al., 2018). At different stages of preparing snacks bars which include crushing of cereals and few other ingredients, mixing the ingredients, moulding, and cutting the snacks into bar shape and packaging, the products are predisposed to microbial contamination. In a

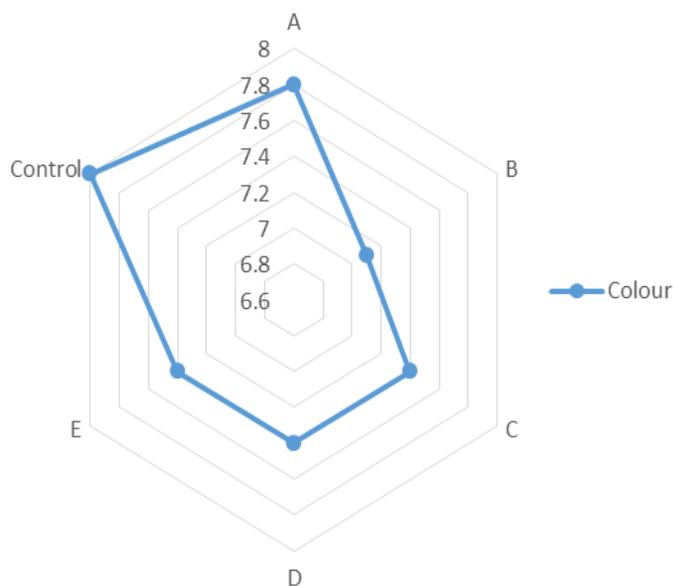


Figure 7. Colour of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100 % Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize: 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

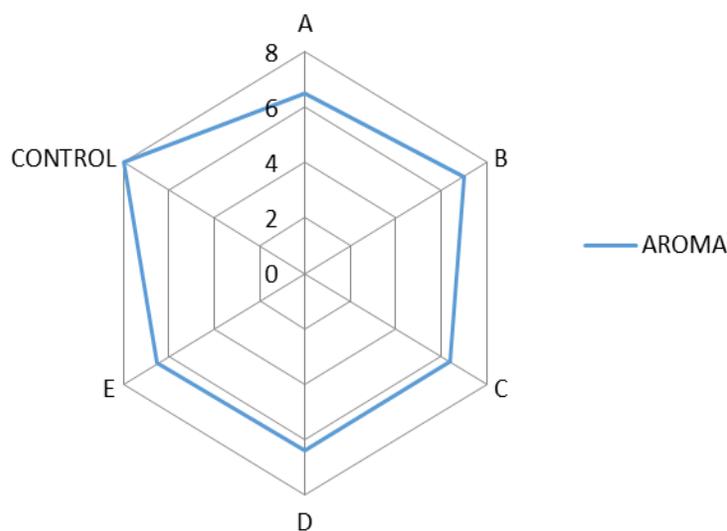


Figure 8. Aroma of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100 % bambara groundnut; C, 50% maize : 50% bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

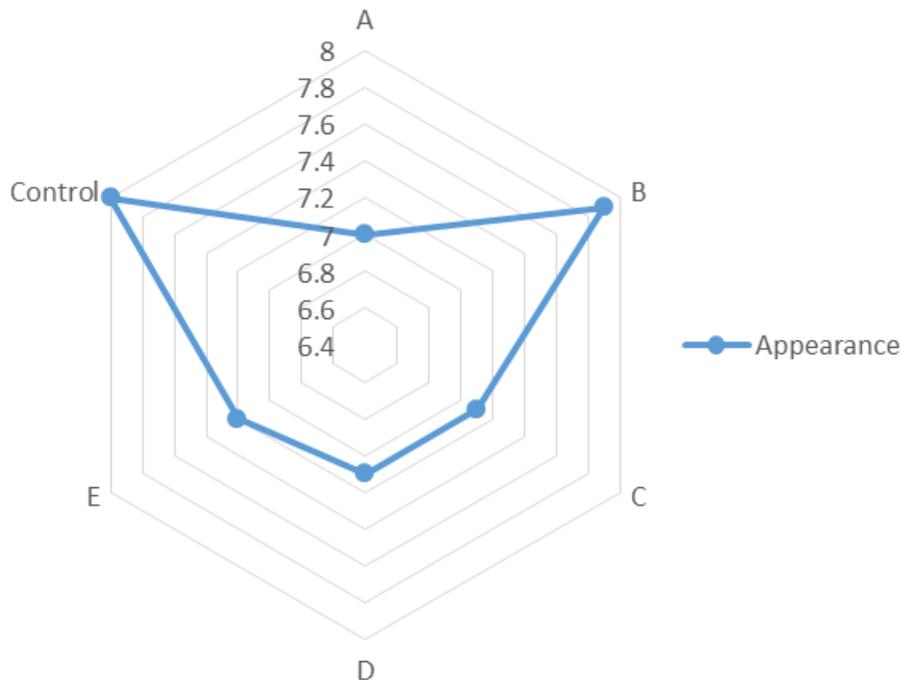


Figure 9. Appearance of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100 % Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

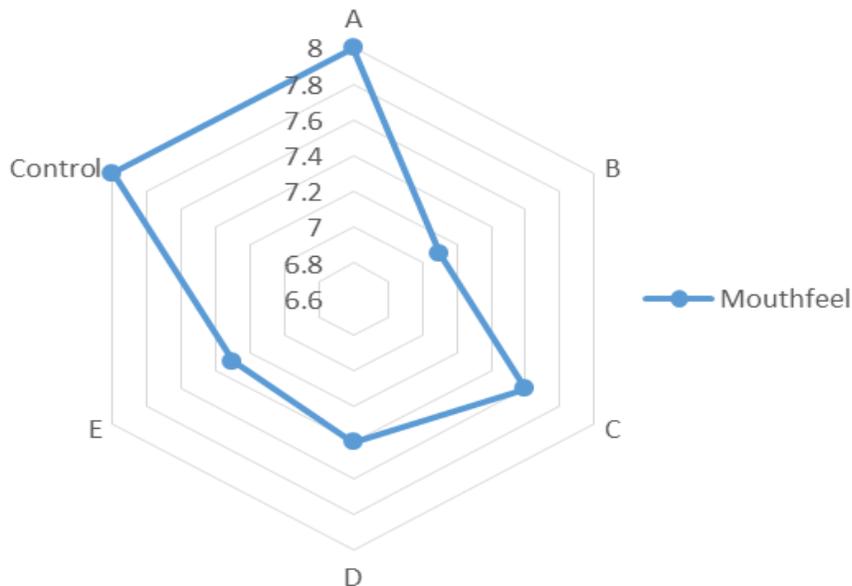


Figure 10. Mouthfeel of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100 % Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

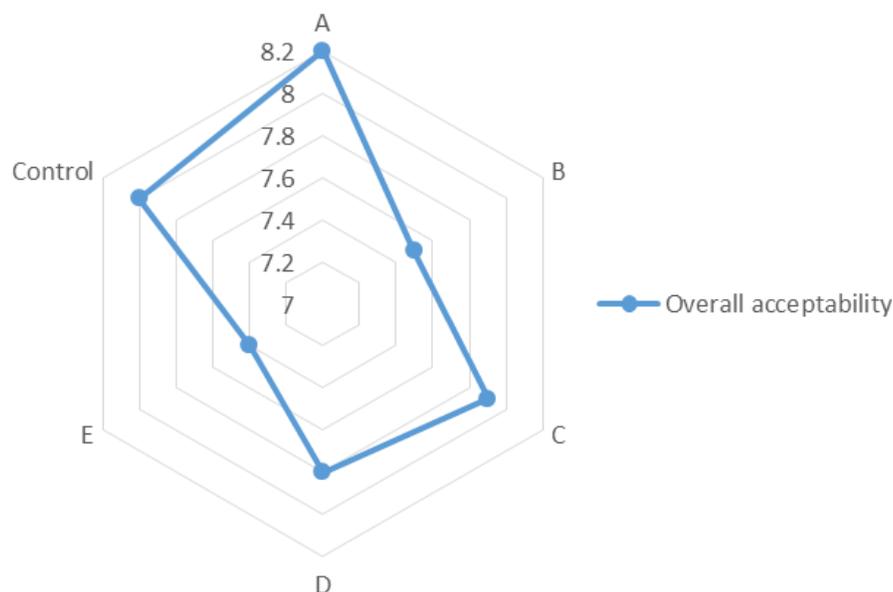


Figure 11. Overall acceptability of the snack bar samples. The samples are composed of maize and Bambara groundnut in the following ratio: A, 100% maize; B, 100 % Bambara groundnut; C, 50% maize : 50% Bambara groundnut; D, 75% maize : 25% Bambara groundnut; E, 25% maize : 75% Bambara groundnut. Interpretation of the 9-point Hedonic scale: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither liked nor disliked; 4-Disliked slightly; 3-Disliked moderately; 2-Disliked very much; 1-Disliked extremely.

related study, Munhoz et al. (2014) reported that *Bacillus cereus* ($< 10 \text{ MPN g}^{-1}$) was detected in cereal bars, but *Salmonella* spp. and coliforms were absent.

Excessive handling of ingredients used in preparing the snack bars with unwashed hands could be one of the sources of *E. coli* and *Staphylococcus* spp. in the samples. *Staphylococcus* spp. is commonly isolated from humans and animals especially their skin and mucus where the organism is part of the normal flora (Møretrø and Langsrud, 2017). It has been established that some cases of foodborne illness are caused by enterotoxigenic *Staphylococcus* strains and *E. coli* strains (Clarence et al., 2009). The presence of *E. coli* in food products is an indication that human and animal fecal contamination has occurred. The source of *Serratia* spp. in the snack bars could be from the environment. According to Møretrø and Langsrud (2017), *Serratia* spp. is commonly found in food processing plants, insects, vertebrate, water and soil. Due to spore forming ability of *Bacillus* spp., the microorganism which is ubiquitous in nature can survive harsh environmental condition. This could explain why *Bacillus* spp. is among the bacterial species which recorded the highest frequency of occurrence in the snack bar samples.

Antimicrobial activity of Bambara groundnut extract against *Klebsiella pneumonia* subsp. *pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* subsp. *aureus* ATCC 33591, *E.*

coli, *B. cereus*, yeast (*Candida albicans*) and mold (*Aspergillus niger*) was reported by Klompong and Benjakul (2015). Given that some of these bacterial isolates were also isolated from the snack bars is an indication that antimicrobial properties of Bambara groundnut was less effective when it was used in preparing the snack bars. Since the steps involved in preparing the snack bars include baking at high temperature capable of destroying the antimicrobial properties of Bambara groundnut and killing all the microorganisms present in the snack bars, the bacteria and fungi found in and on the snack bars could be attributed to handling of the products after their preparation. It is likely that recontamination of the snack bars after baking occurred during cutting the snack into bar shape and packaging the product.

Fungi isolated from all the samples of snack bar include *Aspergillus*, *Saccharomyces*, *Penicillium* and *Rhizopus* spp. The sources of the fungal genera could be from soil and plants in the environment. The existence of some species of *Saccharomyces* only in nature, others as wild and domesticated strains have been described (Boynton and Greig, 2014). The yeast population in the snack bars could have a little influence in the sensory attributes of the samples. *Rhizopus* sp. is ubiquitous in the soil, excreta from animals and rotting vegetable. Both *Rhizopus* spp. and *Saccharomyces* spp. had the least frequency of occurrence (20%) among the fungal genera

isolated from the samples of snack bar. Some fermented products are produced using *Rhizopus* spp. (Gryganskyi et al., 2018). *Aspergillus* spp. is widely distributed in nature. This could have contributed to *Aspergillus* spp. (33%) being the fungal genera with the highest frequency of occurrence in the samples of snack bar. The presence of *Aspergillus* spp. in the snack bar samples is a concern to public health due to possibility of producing mycotoxins harmful to human health. Odetunde et al. (2021) reported that some species of *Aspergillus* isolated from Bambara nuts produced different amounts of mycotoxins which include Aflatoxins B₁, B₂, G₁ and G₂. Some species of *Aspergillus* and *Penicillium* are capable of causing food spoilage. The frequency of occurrence of *Penicillium* spp. in the samples of snack bar is 27%.

Moisture content is associated with the shelf life of food products. Ordinarily, food products with a low moisture content (low water activity) have an extended shelf life. The moisture content of the samples of the snack bars was between 11.47±0.99-17.45±1.01%. The moisture content of snack bars prepared using 100% maize and 100% Bambara groundnut are significantly different (p<0.05). In contrast, snack bars prepared using blends of maize and Bambara groundnuts were not significantly different (p>0.05). In a related study, Edima-Nyah et al. (2019) reported a lower moisture content in snack bars which range from 3.76 to 4.8% which is considerably lower than the results found in this study. Similarly, Eke-Ejiofor and Okoye (2018) reported that moisture content of cereal bars is within the range of 5.09-6.78%. Both products are expected to have a longer shelf life than the samples of snack bar developed in this study. According to Ire et al. (2020), digestion of food materials and some other physiological processes are enhanced by moisture which also helps in nutrient absorption from food. In the human body, water performs many important roles which include being a carrier for nutrients and waste products, among others (Jéquier and Constant, 2010).

The ash content of the snack bars was in the range of 1.09±0.07-2.00±0.15%. These results are in agreement with the ash content of cereal bars prepared by Eke-Ejiofor and Okoye (2018) which was within the range of 1.54 to 1.90%. There are significant differences (p<0.05) in ash content of the snack bars developed in this study. The values were lower than what was reported by Edima-Nyah et al. (2019) in a related study. According to these authors, the ash content of the snack bars are within the range of 2.83 to 4.57%. In order to estimate the amount of minerals in a food sample, the ash content of the sample is taken into consideration. Therefore, any food product reported to have with high ash content is expected to be rich in mineral elements. In humans, the consumption of diets that contain moderate amounts of mineral elements increases the speed at which metabolic processes occur. This brings about improvement in growth and development.

The protein content of all the snack bars in this study is

within the range of 6.32±0.36 to 15.00±0.22%. With regards to protein content, all the snack bar samples except for Sample A is within the dietary reference intake's (DRI) acceptable macronutrient distribution range (AMDR) between 10 and 35% specifically for adults (Ire et al., 2020). In a related study, Edima-Nyah et al. (2019) reported that protein content of snack bars is within the range of 16.16 to 22.43%. The protein content of the snack bars is higher than the values reported in this study. This could be attributed to differences in the quantity and quality of the ingredients used in preparing the snack bars. The protein content of all the samples of the snack bars was significantly different (p<0.05). According to Kumar et al. (2017), proteins present in foods are required for body building and repair. Proteins are needed for the maintenance of body tissues. They also play a vital role in the synthesis of plasma proteins, hemoglobin, hormones, enzymes, coagulation factors and antibodies.

The crude fiber content of the snack bars in this study is within the range of 2.60±0.25 to 3.10±0.31%. The crude fiber content of the samples of snack bar is not significantly different (p>0.05). The values are lower than the result reported by Edima-Nyah et al. (2019) in a related study. The authors reported that crude fiber content of snack bars produced using blends of African seed flour, maize flour and coconut grits is within the range of 10.12 to 17.76%. Snack bar produced from a blend of 50% maize + 50% Bambara groundnut had the highest crude fiber content. To prevent constipation and other health maladies associated with inefficient waste removal from the body, regular consumption of diets rich in crude fiber is recommended. There are indications that consumption of vegetable fiber reduces the level of cholesterol in the body (Soliman, 2019). It could also reduce the risk of coronary heart diseases. The risk of developing hypertension, colon and breast cancer is also reduced by eating diets rich in vegetable fiber (Jenkins et al., 2001). Glucose tolerance is enhanced by vegetable fiber consumption which also increases insulin sensitivity (Edima-Nyah et al., 2019).

The fat content of all the samples of snack bars was within the range of 4.60±0.50 to 7.00±0.30% significantly different (p<0.05). Given the DRI's AMDR recommendation of 25 to 35% total fats for adults, all the samples of the snack bars did not meet the requirement. In a related study, Edima-Nyah et al. (2019) reported that crude fat content of snack bars range between 7.31 and 8.46%. Although the values are higher than what was reported in this study, it did not meet DRI's AMDR recommendations either. Fat is an energy source for humans necessary for growth and development. It also enables Vitamins A, D, E and K to be absorbed into the body. The amount of fat in food influences the taste and consistency (Ire et al., 2020). According to Prentice (2005), human beings obtain bulk of the food energy it requires from fat and carbohydrate. Relatively low crude

fat content of snack bars reported in this study is an indication that the bulk of the energy in the product comes from carbohydrate.

The carbohydrate content of the snack bars which was within the range of 56.05 ± 0.65 to $70.37 \pm 0.71\%$ and higher than the results reported by Edima-Nyah et al. (2019) in a related study. In contrast, the carbohydrate content of five samples of cereal bars prepared by Eke-Ejiofor and Okoye (2018) using rolled oat, yellow maize, white maize, millet and Guinea corn had a range of 36.6 to 41.4% which is lower than the result reported in this study. This could be attributed to the ingredients used in different proportions to prepare the snack bars. The carbohydrate content of all the snack bars are significantly different ($p < 0.05$). Worthy to note is that carbohydrate content of some of the snack bar samples are within the recommended dietary reference intake's (DRI) acceptable macronutrient distribution range (AMDR) of 45 to 65% of energy obtained from carbohydrate for adults (Ire et al., 2020). According to Ho et al. (2016), post meal and diurnal glucose profiles in patients suffering from insulin resistance and type-2 diabetes might improve as a result of consuming snack bars characterized by high ratio of protein/carbohydrate.

All the samples of snack bar developed in this study had a calorie value within the range of 347.20 to 367.69 kcal. The snack bar prepared using 50% maize and 50% Bambara groundnut had the highest calorie value (367.69 kcal). In contrast, the snack bar (Sample B) prepared using 100% Bambara groundnut had the least calorie value (347.20 kcal). This could be because of high amount of Bambara groundnut which is rich in proteins used in preparing the snack bar (Sample B). Although protein is listed among the four principal classes of energy yielding macronutrients, it is not considered as a key supplier of dietary energy (Prentice, 2005). The calorie value of the snack bars is comparable with the result reported by Edima-Nyah et al. (2019). Their report stated that energy value of snack bars prepared using blends of African breadfruit seeds flour, maize flour and coconut grits is within the range of 336.12 to 369.71 Kcal/100 g.

According to Kim et al. (2009), the sensory characteristics of cereal snack bars go a long way to influence the acceptability of the product by consumers. The sensory report revealed that taste, mouthfeel and overall acceptability of snack bar prepared using 100% maize was liked very much by the panelist while other sensory attributes for all the samples of snack bar were either liked moderately or liked slightly. The panelist reported that appearance and color of all the samples of snack bar prepared using maize and Bambara groundnut in different proportion were liked moderately. All the sensory parameters of snack bars prepared using 100% Bambara groundnut were liked moderately by the panelist except taste which they liked slightly. Taking into consideration the sensory scores assigned to sensory

attributes of Samples C and D, the panelist liked moderately all the sensory parameters of both samples with the exception of aroma which they liked slightly. All the sensory parameters of snack bar prepared using 25% maize and 75% Bambara groundnut were liked moderately by the panelist except aroma and taste which they liked slightly. Overall, the snack bar prepared using 100% maize was assigned the highest score for the sensory parameters. Therefore, it is the most preferred sample of snack bar developed in this study.

The sensory panelist very much liked all the sensory parameters of the commercialized snack bar which serve as control. The preference given to the control sample by the sensory panelist compared with snack bars prepared using maize and Bambara groundnut in different proportions could be as a result of the panelist being familiar with the commercialized snack bar. In a related study, Edima-Nyah et al. (2019) reported a slightly lower sensory score for appearance (5.00-8.33), aroma (5.73-7.76), taste (5.36-7.56) and overall acceptability (5.63-7.80) of snack bars compared with the sensory scores reported in this study. Eke-Ejiofor and Okoye (2018) also reported a slightly lower sensory scores for color (5.20-7.10), taste (5.60-7.10), aroma (5.60-6.10) and overall acceptability (5.63-7.80) of cereal bars compared with the result reported in this study. Differences in ingredients used in preparing the snack bars and preferences of the sensory panelists could have influenced the sensory scores reported by Eke-Ejiofor and Okoye (2018) and Edima-Nyah et al. (2019) which were lower than the result reported in this study.

Conclusion

The snack bars developed in this study are microbiologically safe for human consumption since the total heterotrophic bacterial count of the samples were below the limit stipulated by the ICMSF. Bacteria isolated from the samples were *Bacillus* spp., *Lactobacillus* spp., *Staphylococcus* spp., *Serratia* spp. and *Escherichia coli* while the fungal isolates were *Aspergillus*, *Penicillium*, *Rhizopus* and *Saccharomyces* spp. The moisture, ash, carbohydrate, crude protein, fat and fiber content of the samples were within the range of 11.47 ± 0.99 - 17.45 ± 1.01 , 1.09 ± 0.07 - 2.00 ± 0.15 , 56.05 ± 0.65 - 70.37 ± 0.71 , 6.32 ± 0.36 - 15.00 ± 0.22 , 4.60 ± 0.50 - 7.00 ± 0.30 and 2.60 ± 0.25 - $3.10 \pm 0.31\%$, respectively. The taste, mouthfeel and overall acceptability of snack bar prepared using 100% maize was liked very much by the panelist while other sensory attributes for all the samples of snack bar prepared using maize and Bambara groundnuts in different proportions were either liked moderately or liked slightly. All the sensory parameters of the commercialized snack bar which serve as the control was liked very much by the panelist. Among the samples of snack bar developed in this study, the snack bar prepared using

100% maize was the most preferred sample based on sensorial characteristics. Nutritionally, the snack bar prepared using 50% maize and 50% Bambara groundnut had the highest calorie value; 100% Bambara groundnut had the highest protein content.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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