The influence of Bambara groundnut (Vigna subterranea) flour on the nutritional, physical and antioxidant properties of steamed bread

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ABSTRACT

This study aimed to evaluate the influence of fortifying steamed breads with Bambara groundnut (BGN) flour (5%, 10%, 15% and 20%) on their physicochemical and antioxidant characteristics. The water absorption capacity of the flour increased with increasing BGN flour fortification levels, while oil absorption capacity decreased. The pH and leavening capacity of the BGN flour fortified dough decreased during incubation, whilst the titratable acidity for dough fortified with 15% and 20% BGN flour increased. The inclusion of BGN flour improved the protein, ash, and crude fibre contents of flour and steamed breads. The HunterLab colour values show that BGN flour increased the yellowness (b* value) of flour and steamed bread samples, while the lightness, redness and Chroma decreased. The antioxidant properties of flours and steamed breads increased significantly (p < 0.05) with the BGN flour fortification levels. Moreover, the weight and spread ratio of the breads increased, whilst the texture became soft. The BGN flour can be utilised as a functional ingredient to enhance the nutritional and antioxidant properties of cereal-based foods such as steamed bread.

Influencia de la harina de frijol de bambara (Vigna subterránea) en las propiedades nutricionales, físicas y antioxidantes del pan al vapor

RESUMEN

Este estudio se propuso evaluar la influencia de la fortificación de los panes al vapor elaborados con harina de frijol de bambara (BGN) (5%, 10%, 15% y 20%) sobre sus características fisicoquímicas y antioxidantes. Cuando se incrementaron los niveles de fortificación de la harina de BGN, la capacidad de absorción de agua de la harina aumentó y la capacidad de absorción de aceite disminuyó. El pH y la capacidad de leudar de la masa enriquecida con harina de BGN se redujeron durante la incubación, mientras la mejor valorable de las masas enriquecidas con harina de BGN al 15 y al 20% aumentó. La inclusión de harina de BGN mejoró los contenidos de proteínas, cenizas y fibra bruta de la harina y de los panes cocidos al vapor. Por otra parte, los valores de color de HunterLab muestran que la harina de BGN elevó la amarillez (valor b*) de las muestras de harina y de los panes cocidos al vapor, mientras que la luminosidad, la rojez y el croma disminuyeron. Las propiedades antioxidantes de harinas y panes cocidos al vapor aumentaron significativamente (p < 0.05) conjuntamente con los niveles de fortificación de la harina de BGN. Además, el peso y la ratio de extensión de los panes se incrementaron, en tanto su textura se volvió suave. La harina de BGN puede utilizarse como ingrediente funcional para mejorar las propiedades nutricionales y antioxidantes de los alimentos a base de cereales, como el pan al vapor.

1. Introduction

Steamed bread is made with the typical ingredients of the traditional fermented wheat bread, including the basic ingredients – wheat flour, yeast, salt and water. The steam bread is processed in the same manner as the traditional bread, except that it is steamed instead of being baked (Loong & Wong, 2018; Yue & Rayas-Duarte, 1997; Zhu, 2014). The literature indicates that steamed bread originated from China about 1700 years ago, where it is also known as mantou. In Chinese cuisine, mantou is a common and staple food of the wheat growing areas of Northern China where 70% of the flour produced in the region is utilised in the production of steamed bread (Sha et al., 2007). In South African cuisine, many cultural groups usually prepare steamed bread at home. The steamed bread is known by various names in South Africa, such as ledombolo (the most common name), dombolo, umbhako, dodogoyi and sigenza depending on the region and ethnic group (Manley & Nel, 1999). The steamed bread is usually consumed on the same day. Research on the production of steamed bread on a commercial scale is at advanced stages in South Africa, which is largely influenced by its current mass production in China (Lombard et al., 2000).

Like other cereal-based products, steamed bread is low in protein and other essential nutrients – some minerals, vitamins and essential amino acids, especially lysine (Siddiqi et al., 2020). In addition, some of the essential amino acids are depleted during the processing of wheat flour into...
different products (Yagoub & Abdalla, 2007). Thus, there is a need to fortify steamed bread with other ingredients, including plant sources, such as vegetables and fruits, as well as animal products, such as dairy products (Liu et al., 2016). Further, globally, there has been a sustained trend of consumers demanding foods that not only meet basic nutritional requirements but also possess health-promoting properties, which can be derived from nutraceutical and functional ingredients (Chen et al., 2021; Irakli et al., 2019). Thus, currently, there is an increased interest in developing steamed bread types with enhanced antioxidant properties (health-promoting potential) and a low glycaemic response, by including suitable ingredients in the bread formulations (Zhu & Chan, 2018; Zhu et al., 2016). This creates an opportunity to develop a healthy fortified steamed bread to satisfy the current consumer demands (Lau et al., 2015).

Bambara groundnut (Vigna subterranean L. Verdc.) is a legume crop originating from Africa – it is well adapted to the generally harsh agro-climatic conditions that are prevalent in sub-Saharan Africa (Oyeyinka et al., 2020). Bambara groundnut (BGN) has an attractive nutritional composition—a high carbohydrate (glycaemic and non-glycaemic (fibre) content, ranging from 57% to 67%; and quite a high protein content, up to 27%); good amino acid profile; and a low fat content of less than 10% (Arise et al., 2015; Feldman et al., 2019; Oyeyinka et al., 2017). The high nutritional value of BGN, especially essential amino acid content, including methionine, lysine and leucine as well as its adaptation to harsh environmental conditions makes it a valuable crop for food and nutrition security, especially in sub-Saharan Africa, where nutrient deficiencies affect the majority of the population (Yao et al., 2015). In addition, BGN has been found possessing appreciable antioxidant properties and hence a good health-promoting potential (Oyeyinka et al., 2019).

There is a need for research to generate scientific knowledge and technology for facilitating the utilisation of BGN in high-value commercial food products. There are several challenges to be addressed to promote utilisation of BGN in commercial foods, including the “hard to mill and hard to cook” phenomenon (Diedericks et al., 2020; Mubaiwa et al., 2019). For key food security, traditional food products would be ideal for incorporation of BGN. If used as an innovative ingredient in traditional foods like bread, BGN could significantly affect critical quality attributes of the food, such as texture and colour, which may affect consumer acceptability of the product (Siro et al., 2008). Previous studies have investigated using BGN to improve the nutritional and health-promoting properties of several food products, for example, tortillas, snacks and yoghurt (Falade et al., 2015; Mashau et al., 2020; Oyeyinka et al., 2018). Fortification of bread is one of the most successful programmes for addressing nutritional problems in developing countries (Uchendu et al., 2012). Blending wheat flour with a legume flour such as BGN flour, which has a higher content of essential nutrients, including total protein and the essential amino acids methionine and lysine would be nutritionally beneficial (Mubaiwa et al., 2018). The reported high health-promoting potential of BGN (Oyeyinka et al., 2019) would be an additional advantage. A desirable white bread incorporated with insoluble dietary fibre extracted from BGN flour was produced by Diedericks and Jideani (2015). Studies in which non-wheat flours and functional ingredients, including black and green tea, pineapple core fibre, wheat germ and chia seeds, were added to steamed bread showed improvement in product variety and nutritional and health-promoting properties (Ananingsih et al., 2013; Shiaw et al., 2015; Sun et al., 2015; Zhu et al., 2016). However, the incorporation of these ingredients resulted in the modification of the properties of the steamed dough, which influenced the quality of the steamed bread. However, from the literature available to us, there are no reports of studies on incorporation of BGN flour in steamed bread. Thus, the influence of BGN flour on the nutritional, physical and antioxidant (health-promoting potential) properties of steamed bread seems not known. Therefore, the aim of this work was to nutritionally fortify steamed bread with BGN flour and determine the influence of BGN flour on the chemical, physical and antioxidant properties of steamed bread.

2. Materials and methods

2.1. Materials and chemicals

Creamy white BGN grains, cake wheat flour, instant dry yeast, baking powder, golden brown sugar and salt were bought from a supermarket in Thohoyandou, Limpopo province, South Africa. Analytical grade chemicals were used for this experiment. Reagents such as Folin–Ciocalteu, catechin and gallic acid were commercially procured from Merck Pty (Centurion, Gauteng province, South Africa).

2.2. Preparation of Bambara groundnut flour

Creamy white BGN grains were sorted, and foreign matters and spoiled groundnuts were removed. Then, BGN grains were measured, approximately 2.5 kg was soaked in a container with tap water for 72 h at 25°C. The BGN grains were further boiled for 10 min and cooled. The grains were dehulled manually and oven dried (Module 278, Labotech Ecotherm, South Africa) at 60°C for 24 h. Afterwards, the dried BGN grains were milled (ZM 200 ultra-centrifugal mill, Retsch, Germany) and sieved through a 250 µm sieve into fine flour.

2.3. Preparation of composite flour and steamed bread

The composite flours were obtained by partially replacing wheat flour with 5%, 10%, 15% and 20% of BGN flour, respectively. The formulation of composite flours was chosen following the previous study by Olaoye et al. (2018). The four resultant BGN composite flour blends were kept at 4°C until used. All the ingredients were weighed according to the formulation of steamed bread fortified with BGN flour as shown in Table 1 using a weighing balance, whereas a measuring cylinder was used to measure the amount of water needed. Bambara groundnut wheat composite flour and baking powder were sieved to avoid dough lumps; salt was added to the sifted flour, and all dry ingredients were mixed together in a bowl. Sugar was added separately into warm water and continuously stirred and yeast was then added into the sugar solution. The solution was left to rest for 1 min at 25°C to allow activation of the yeast, and then, the yeast mixture was slowly added into dry ingredients as well as shortening (margarine).

A hand mixer was used to mix the batter at low speed and kneaded until an elastic and workable dough was
achieved. The dough was put into a bowl covered with a moist cloth and allowed to stand for 45 min in an incubator (Memmert, Germany) at 40°C to induce the fermentation process. Afterwards, the proofed dough was kneaded again to ensure consistency and even shape (round-like). The dough samples were then steamed using the traditional method of placing a metal plate with dough wrapped in a hot water pan for 25 min in a stove. A control steamed bread sample was made of 100% wheat flour. The steamed bread for each formulation was cooled for 30 min at 25°C and packed separately into low-density polyethylene plastic bags and stored at 4°C. When required, the steamed breads were taken from the refrigerator and re-steamed for 10 min for further analyses. The flour and steam bread production and analyses were replicated three times.

2.4. Water and oil absorption capacity

About 2 g of flour sample was added into a test tube and mixed with 40 mL of distilled water or vegetable oil and vigorously shaken for 2 min. The sample was left to stand for 30 min and centrifuged for 15 min at 5000 rpm. Afterwards, the liquid separated from solids and the volume of the water/oil left after centrifuge were measured. Then, the volume of water or oil absorbed was determined by subtracting the original volume from volume after centrifuge (Kumar et al., 2019).

2.5. The pH and total titratable acidity (TTA) of the dough

A standard method of AACC (2000) was used to measure the pH and TTA values of each dough at 0, 30, 60 and 90 min at 25°C. For the pH of the dough, a pH probe was directly placed in the dough sample and analysed. The reading at measured stable pH was recorded. For TTA of the dough, 10 g of each dough was homogenised with 90 mL distilled water. About 10 mL of the mixture was deposited into a beaker with the addition of three drops of phenolphthalein indicator while being vigorously mixed. Afterwards, 0.1 N NaOH was used to titrate the mixture until a pink colour was reached. The amount of acid produced was calculated.

2.6. Leavening capacity of the dough

After kneading, 100 g of the dough was placed in a beaker and leavened at 30°C for 90 min. The leavening behaviour of the dough sample was assessed after 30, 60 and 90 min of leavening periods and compared with the original volume and the volume determined after each leavening period (Cappa et al., 2018). The percentage increase in the dough during the leavening period was measured as the mean value of three replications.

2.7. Proximate composition of composite flour and steamed bread samples

The contents of moisture, ash, protein, fibre and fat were determined using the approved method of Association of Official Analytical Chemists AOAC (2006). An oven dryer at 60°C for 12 h was used to measure the moisture content. Ash content was measured by a muffle furnace. Fibre Tech was used for measuring fibre content. The Kjeldahl method was used to measure protein content, and for fat content, Soxhlet extraction was followed. The carbohydrate percentage was calculated as follows: % Carbohydrates = 100 – moisture (%) + ash (%) + protein (%) + fibre (%) + fat (%).

2.8. Colour analysis of flour and steamed bread samples

The colour of the flour samples and crust or outer layer of the steamed breads were determined by Hunter Lab with D65 light source (Hunter Lab, Mini Scan XE Plus and Reston, VA, USA). Each sample was individually measured in different areas in triplicate. Data were reported as $L^*$, $a^*$, and $b^*$ values. $L^*$ value signifies lightness, and positive and negative $a^*$ and $b^*$ values represent redness and greenness, yellowness and blueness, respectively. Chroma, Hue angle and total colour difference ($\Delta E$) were calculated as follows:

$$ c = \sqrt{a^2 + b^2} $$

where $C = \text{chroma;}$ $a^* = \text{redness;}$ and $b^* = \text{yellowness}$

$$ E = \sqrt{\left(L - L_c\right)^2 + (a - a_c)^2 + (b - b_c)^2} $$

Where $\Delta E = \text{colour difference,} \; L = \text{Lightness,} \; L_c = \text{lightness on control sample,} \; a = \text{redness,} \; b = \text{yellowness and} \; bc = \text{yellowness of control sample}$
2.9. Antioxidant properties of flour and steamed bread samples

2.9.1. Measurement of total phenolic content (TPC)
The method of Wang et al. (2011) was used to measure the TPC of flour and steamed bread samples. Briefly, 1.60 mL of distilled water was added to 0.2 g of flour and steam bread extract in a test tube. Afterwards, about 0.1 mL Folin – Ciocalteu reagent was added, followed by 0.3 mL of 7.5% sodium carbonate. An aluminium foil was used to cover the mixture and allowed to rest at 25°C for 30 min before its absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Zenyth 200rt Biochrom, Cambridge, UK). The same model number of spectrophotometer was used to measure the TFC and antioxidant activity. The TPC was determined as milligrams GAE per gram of the dry sample.

2.9.2. Analysis of total flavonoids content (TFC)
The TFC of the flour and steamed bread samples was measured according to the method of Kalita et al. (2013). Briefly, 0.5 mL of flour and steamed bread sample was deposited in a test tube and 1.5 mL of ethanol was added followed by 1 mL of 1% aluminum chloride. The mixture was incubated for 10 min at 25°C before measuring its absorbance at 415 nm using a UV–Vis spectrophotometer. Standard curve for calibration was prepared with a catechin solution, and results were measured as milligram of catechin equivalent (CE) per gram of dry sample.

2.9.3. Determination of DPPH radical scavenging activity
The DPPH assay is used to determine the antioxidant property of the sample through scavenging of a free radical (DPPH) by the antioxidative chemical components of the sample. Thus, the assay measures the reducing ability of antioxidants toward the DPPH radical. The method of Nsabimana et al. (2018) was used to measure the DPPH assay of flour and steamed bread samples. Briefly, 2 mL of the extract was deposited into a test tube and 3.8 mL DPPH solution (0.1 mM DPPH in ethanol) was added. The mixture was continuously shaken and left to stand in the dark at 25°C for 30 min before measuring its absorbance at 520 nm using a UV–Vis spectrophotometer. Standard curve was prepared with a gallic acid solution, and results were measured as a percentage inhibition of the DPPH radical.

2.9.4. Measurement of ferric reducing power (FRAP)
The FRAP assay of flour and steamed bread samples was examined as described by Langley-Evans (2000). Briefly, 300 mL of distilled water was added to a volume of 100 μl of flour and steamed bread samples in a test tube. Afterwards, FRAP reagent (3 mL) was added and mixed with 250 μl of the extract. The mixture was vigorously shaken and kept in a water bath for 10 min at 37°C. The absorbance of the mixture was measured using a UV–Vis spectrophotometer at 593 nm. Results were measured as milligrams GAE per gram of the dry sample.

2.10. Determination of steamed bread characteristics
Steamed bread characteristics were determined by measuring weight, volume, specific volume, diameter, height and spread ratio. After cooling the steamed bread at 25°C, the weight was obtained by weighing the steamed bread using a weighing balance, whereas the rapeseed displacement method was used to measure the volume with some modifications (AACC, 2000). The steamed bread was placed into a fixed dimensional container with a known volume and filled up with BGN grains instead of rapeseed. The steamed bread was removed, and the BGN grain volume was measured. The specific volume was measured by dividing the steamed bread volume by its corresponding steamed bread weight. Vernier caliper was used to determine the spread ratio, which was calculated from diameter and height.

2.11. Texture profile of steamed bread crumb and crust
Texture parameters of steamed bread were measured by the TA.XTplus (Stable Microsystems Ltd., Surrey, UK). The slice of each steamed bread was put in a plastic plate and compressed to 40% of its initial thickness at 3.0 mm s⁻¹ speed. A 35 mm diameter aluminum cylindrical probe was used to compress the slices of steamed bread. Afterwards, the compression was paused for 5 s, and the probe was taken back to its original position. Hardness, cohesiveness, springiness and adhesiveness were determined independently for three times.

2.12. Statistical analysis
The data were analysed using a completely randomised design using a one-way analysis of variance (ANOVA) for different treatments of flour and steamed bread samples. The statistical significance difference at p<0.05 was determined using Duncan’s multiple range test. The SPSS software 26.0 (SPSS Chicago, IL) was used to analyse the data, and the results were expressed as the mean ± standard deviation. All analyses were determined as the mean of three replicates.

3. Results and discussion
3.1. Water and oil absorption capacity of flour samples fortified with Bambara groundnut flour
Fortification of wheat flour with BGN flour significantly increased (p<0.05) the WAC of flour samples (Figure 1). The higher WAC of fortified flour samples could be due to the increase in the residues of polar amino acid of BGN protein which have a connection with water molecules (Yusuf et al., 2008). The high WAC observed in all fortified flour samples suggested that they are desirable for baking since more water can be added to the dough, thereby keeping the freshness of steamed bread. However, low WAC for control sample is suitable for thinner consistency because gelatinisation is influenced by WAC via the water available in the flour (Khoozami et al., 2020).

In terms of oil absorption capacity (OAC), the control and fortified flour samples significantly differed at p<0.05 (Figure 1). Incorporation of BGN flour did not affect the AOC of flour samples since the control sample had the highest significant OAC. The low OAC of fortified flour samples could be attributed to limited amounts of hydrophobic proteins in BGN flour which exhibit better binding ability of lipids. High OAC in control sample might be attributed to hydrophobic protein content and fats, which play a part in the absorption of oil. The mechanism of OAC absorption involves associations of capillary in the matrix of food which retains the oil absorbed (Bhinder et al., 2020).
Figure 1. Water and oil absorption capacity of the flour. Means ± SD with different superscripts are significantly different (p < 0.05). Control = flour without Bambara groundnut flour; BGN5–BGN20 = flour with 5, 10, 15 and 20% Bambara groundnut flour.

Figura 1. Capacidad de absorción de agua y aceite de la harina. Las medias ± DE con superíndices diferentes son significativamente diferentes (p < 0.05). Control = harina sin harina de frijol de bambara; BGN5–BGN20 = harina con 5, 10, 15 y 20% de harina de frijol de bambara.

Table 2. The pH and total titratable acidity of dough samples during storage.

| Sample    | pH 0 min | Acidity % | pH 30 min | Acidity % | pH 60 min | Acidity % | pH 90 min | Acidity % |
|-----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Control   | 5.71 ± 0.08a | 5.58 ± 0.12a | 5.57 ± 0.15a | 5.28 ± 0.24a | 5.48 ± 0.09a | 4.80 ± 0.26a | 5.45 ± 0.11a | 3.98 ± 0.52a |
| BGN5      | 5.68 ± 0.03a | 6.95 ± 0.52a | 5.51 ± 0.07a | 6.32 ± 0.41a | 5.46 ± 0.03a | 5.99 ± 0.27a | 5.40 ± 0.04a | 5.50 ± 0.50a |
| BGN10     | 5.60 ± 0.05a | 8.25 ± 0.70a | 5.40 ± 0.08a | 7.75 ± 0.50a | 5.36 ± 0.04a | 7.48 ± 0.32a | 5.32 ± 0.04a | 7.05 ± 0.42a |
| BGN15     | 5.58 ± 0.05a | 8.50 ± 0.93a | 5.37 ± 0.02a | 8.65 ± 0.10a | 5.20 ± 0.02a | 8.73 ± 0.66a | 5.10 ± 0.03a | 8.90 ± 0.57a |
| BGN20     | 5.40 ± 0.05a | 9.56 ± 0.78a | 5.22 ± 0.02a | 9.80 ± 0.32a | 5.16 ± 0.07a | 10.22 ± 0.78a | 5.06 ± 0.26a | 10.33 ± 1.04a |

Means ± SD with different letters within same column are significantly different (p < 0.05). Control = dough without Bambara groundnut flour; BGS–BGN20 = dough with 5%, 10%, 15% and 20% Bambara groundnut flour.

Las medias ± DE con letras diferentes dentro de la misma columna son significativamente diferentes (p < 0.05). Control = masa sin harina de frijol de bambara; BGS–BGN20 = masa con 5, 10, 15 y 20% de harina de frijol de bambara.

Protein content and type and starch and particle size influence the OAC of flour samples (Du et al., 2014).

3.2. The pH and total titratable acidity of the composite dough

The fortification of steamed bread with BGN flour decreased the pH of the dough during storage (Table 2). The ideal pH of the doughs is normally in the range of 5.5 which corresponds to the results of this study. The decrease in pH values of the dough is attributed to the generation of lactic and acetic acids by microbial growth associated with the yeast in the dough (Wehrle & Arendt, 1998). Moreover, lactic and acetic bacteria ferment carbohydrate, fat and protein and generate lactic acid in the dough (Rayner, 2009). The decrease in pH value because of these organic acids influences the viscoelastic behaviour of the dough (Wehrle & Arendt, 1998). At low pH, the lactic and acetic acids find the undissociate form and go through the cellular membrane and prevent cellular metabolite (Oginean, 2015). Factors such as buffering potential of the flour, acid species and quantity of acids available in the flour influence the pH of the dough.

Bakery industry uses total titratable acidity (TTA) measurement to monitor the fermentation rate of the dough. There were variations in terms of the TTA of the dough during storage with control and dough fortified with 5%, 10% and 15% BGN flour experiencing a decrease after 30, 60 and 90 min (Table 2). However, the TTA of the dough fortified with 15% and 20% BGN flour significantly increased at p < 0.05. The rise in TTA of fortified dough samples during incubation might be attributed to the carbon dioxide gas which dissolved into bicarbonate ions and this resulted in the acidification of the dough (Borsuk et al., 2021). Moreover, the initiation of spontaneous fermentation and generation of acids might have contributed to high TTA values since the dough has a great number of natural microorganisms (Doerry, 1998). Similar results of low pH and rise in TTA of the dough were observed by Palacios et al. (2008).

3.3. Leavening capacity of the dough fortified with Bambara groundnut flour

The control (100% wheat flour) sample had a significantly higher dough increase percentage (DIP) value, and dough fortified with 5–15% BGN flour did not
differ significantly at \(p < 0.05\) and had acceptable \(DI\%\) after 30 min of storage (Figure 2). After 60 min of storage, control sample and the dough fortified with 5% BGN flour had the highest \(DI\%\) values, while the control sample continued to have significantly higher values at 5% BGN flour after 90 min of storage. The fortification of 20% BGN flour significantly \(p < 0.05\) decreased the \(DI\%\) since this sample recorded lower values after 30, 60 and 90 min of storage. The low values of \(DI\%\) in fortified dough samples might be due to the poor gluten network since gluten plays a major role in retaining the gases generated by yeast and bacteria during the fermentation of the dough. Starch granules are usually submerged tightly in the protein matrix (Zhang et al., 2018). However, the destruction of gluten network causes the starch granules to swell (Tester, 1997). Aboaba and Obapkolor (2010) reported similar data whereby the incorporation of cassava flour at 20% significantly decreased the dough volume during the leavening process. Therefore, a decrease in gluten content, which is attributable to carbon dioxide entrapment, continuously caused a decrease in the \(DI\%\) of fortified dough samples.

### 3.4. Proximate composition of Bambara groundnut fortified flour and steamed bread samples

The proximate composition of flours and steamed breads is presented in Table 3. The moisture content of composite flours increased significantly \(p < 0.05\) varying from 12.48% to 20.01%. The control flour sample had the lowest moisture content which is good for the prevention of microbial growth during storage. The high moisture content of fortified flour samples might be due to the fact that dry food usually absorbs moisture from the atmosphere of the storage until equilibrium is reached. The moisture content of steamed breads was higher than that of flour blends ranging from 19.69% to 34.24%. The high moisture content might be as a result of adding water during the development of the dough as well as BGN flour which increased the WAC of the steamed bread (Mashau et al., 2020). This corresponds to the WAC result in Figure 1. The results of this study were lower than those reported by Sha et al. (2007) where the skin of steamed bread had higher moisture content (41.5%) than the center crust (39.3%) after cooking and this is unlike the moisture of baked bread.

### Table 3. Proximate compositions of fortified flour blends and steamed breads (dry basis).

| Sample       | Moisture (%) | Ash (%)  | Protein (%) | Fat (%)  | Fat (%)  | Carbohydrate |
|--------------|--------------|----------|-------------|----------|----------|--------------|
| **Flours**   |              |          |             |          |          |              |
| Control      | 12.48 ± 2.58a| 1.01 ± 0.01a| 3.98 ± 0.13a| 0.97 ± 0.09a| 1.77 ± 0.02a| 79.77 ± 2.41d |
| BGN5         | 14.11 ± 0.99b| 1.13 ± 0.03b| 4.18 ± 0.29b| 1.52 ± 0.01b| 1.85 ± 0.04b| 77.20 ± 0.75d |
| BGN10        | 14.68 ± 1.50c| 1.93 ± 0.33c| 5.27 ± 0.82c| 1.99 ± 0.01c| 2.13 ± 0.07c| 73.99 ± 1.38d |
| BGN15        | 15.56 ± 1.63d| 2.73 ± 0.5d | 6.82 ± 0.55d| 2.10 ± 0.01d| 2.28 ± 0.02d| 70.50 ± 1.99d |
| BGN20        | 20.01 ± 1.50e| 4.24 ± 0.7e | 7.37 ± 0.26e| 2.34 ± 0.01e| 2.48 ± 0.08e| 63.56 ± 1.37e |
| **Steamed bread** |              |          |             |          |          |              |
| Control      | 19.69 ± 1.99f| 1.16 ± 0.10f| 3.53 ± 0.09f| 1.15 ± 0.05f| 1.75 ± 0.04f| 72.72 ± 2.21f |
| BGN5         | 24.28 ± 2.99g| 1.69 ± 0.18g| 3.93 ± 0.40g| 2.03 ± 0.01g| 1.85 ± 0.04g| 66.20 ± 3.49d |
| BGN10        | 27.89 ± 1.73h| 1.86 ± 0.21h| 4.33 ± 0.27h| 1.99 ± 0.73h| 2.17 ± 0.05h| 61.75 ± 1.32d |
| BGN15        | 31.92 ± 1.45i| 2.05 ± 0.12i| 4.94 ± 0.05i| 2.51 ± 0.01i| 2.27 ± 0.03i| 56.29 ± 1.39i |
| BGN20        | 34.24 ± 0.79j| 2.11 ± 0.15j| 5.67 ± 0.24j| 2.99 ± 0.02j| 2.47 ± 0.08j| 52.51 ± 1.13j |

Means ± SD with different superscripts within the same column are significantly different \(p < 0.05\). Control = flour and steamed bread without Bambara groundnut flour; BGN5–BGN20 = flour and steamed bread with 5%, 10%, 15% and 20% Bambara groundnut flour.

Las medias ± DE con superíndices diferentes dentro de la misma columna son significativamente diferentes \(p < 0.05\). Control = harina y pan al vapor sin harina de frijol de bambara; BGN5–BGN20 = harina y pan al vapor con 5, 10, 15 y 20% de harina de frijol de bambara.
The ash content of flour samples and steamed breads significantly increased (p < 0.05) ranging from 1.01% to 4.24% and 1.16% to 2.11%. However, the ash content of steamed breads was low compared to flour samples, and this is likely due to the leaching out of minerals during the steaming process. The protein content of flour samples increased with the BGN flour fortification levels. The increase in protein content is likely because BGN is a completely balanced food since it contains protein varying between 18% and 24%, whereas wheat contains protein between 11% and 12% (Basman et al., 2003). The protein content of steamed breads was low compared to flour samples. This was probably caused by the breakage of protein links or amino acid chains due to heat generated from the steam which led to thermal decomposition of the protein. The fibre content of flour blends and steamed breads significantly increased ranging from 0.97% to 2.34% and from 1.15% to 2.99%, respectively. The high fibre content may probably be ascribed to the development of fibre protein networks that are resistant to heat. The variation in the fibre content of the samples might be related to the type of variety, processing and method of analysis (Elleuch et al., 2011; Kamal-Eldin et al., 2009).

Fortified flour and steamed bread samples had higher fat content ranging from 1.77% to 2.48% and from 1.75% to 2.47%. The fat content of steamed bread was slightly lower compared to flour samples, and this is attributed to heat treatment during steaming which affected the quality of steamed breads. Abdualrahman et al. (2012) reported similar data of increase in fat content when evaluating wheat bread fortified with BGN flour. The carbohydrate content of flours and steamed bread significantly decreased (p < 0.05) with the BGN flour fortification levels. The decrease in carbohydrate content might be due to the fact that legumes contain lower carbohydrate content compared to cereal grains (Akpapunam & Darbe, 1994). The dilution of wheat flour with BGN flour resulted in the decrease in carbohydrates of flours and steamed bread samples. In addition, a decrease in carbohydrate content of steamed breads compared to control and fortified flours is attributed to heat destruction of the protein and fibre during steaming (Kavitha & Parimalavalli, 2014). Moreover, exposure of carbohydrate-containing foods to heat tends to degrade carbohydrates into simple sugars.

### 3.5. Colour measurement of composite flours and steamed breads

The colour profile of flour blends, crust and crumb of steamed breads, is presented in Table 4. A significant decrease (p < 0.05) of lightness (L*) in all flour and steamed bread samples with the BGN flour fortification levels was noted. The brightness or lightness (L* values) of samples decreased ranging from 91.39 to 90.50 (flour blends), 74.88–71.21 (crust) and 76.27–74.36 (crumb). The decrease in brighter colour of flours is similar to a study of fortifying Chinese steamed bread with Jerusalem artichoke flour (Laohaprasit & Sricharoenpong, 2018). The L* of the crust was lower to the crumb, and this is attributed to the heat from the steam that reacts with the sugar in the dough and caused a caramelisation reaction which resulted in a darker crust than the crumb. The results obtained show a significant reduction (p < 0.05) of a* values within all the samples, which changed to a darker colour with the increased level of BGN flour. The a* values decreased ranging from 0.68 to 0.54 (flours), 4.33–3.58 (crust) and 3.15–2.44 (crumb). Laohaprasit and Sricharoenpong (2018) obtained similar results on steamed bread incorporated with Jerusalem artichoke flour and Zhu and Sun (2019) found similar results on steamed bread fortified with purple sweet potato flour. The results of a* value obtained show a significant increase in all samples fortified with BGN flour, making the yellowness colour to be dominant on the flour and steamed bread samples. The yellowness values of crust and crumb were higher than that of flours likely because of caramelisation reaction that takes place during the steaming process as sugar added on dough reacts with heat (Çelik & Gökmen, 2020).

The Chroma refers to the colour intensity; the higher the Chroma, the more the product is desirable to consumers. Ouazib et al. (2016) produced the same results after

| Table 4. Colour of flours, crust and crumb of steamed breads fortified with BGN flour. |
|---|
| **Sample** | **L*** | **a*** | **b*** | **Chroma** | **ΔE** |
| **Flours** | | | | | |
| Control | 91.39 ± 0.01<sup>a</sup> | 0.68 ± 0.02<sup>d</sup> | 10.29 ± 0.04<sup>d</sup> | 10.32 ± 0.04<sup>d</sup> | 0.01 ± 0.01<sup>a</sup> |
| BGN5 | 91.00 ± 0.01<sup>c</sup> | 0.67 ± 0.01<sup>c</sup> | 10.41 ± 0.03<sup>c</sup> | 10.44 ± 0.03<sup>c</sup> | 0.14 ± 0.03<sup>a</sup> |
| BGN10 | 90.98 ± 0.01<sup>c</sup> | 0.64 ± 0.02<sup>c</sup> | 10.42 ± 0.01<sup>c</sup> | 10.43 ± 0.01<sup>c</sup> | 0.85 ± 0.03<sup>d</sup> |
| BGN15 | 90.76 ± 0.01<sup>c</sup> | 0.55 ± 0.01<sup>c</sup> | 10.68 ± 0.01<sup>c</sup> | 10.70 ± 0.01<sup>c</sup> | 1.11 ± 0.01<sup>c</sup> |
| BGN20 | 90.50 ± 0.00<sup>c</sup> | 0.54 ± 0.01<sup>c</sup> | 11.11 ± 0.01<sup>c</sup> | 11.13 ± 0.01<sup>c</sup> | 0.52 ± 0.02<sup>c</sup> |
| **Steamed bread** | | | | | |
| **Crust** | | | | | |
| Control | 74.88 ± 0.04<sup>c</sup> | 4.33 ± 0.06<sup>d</sup> | 28.25 ± 0.82<sup>c</sup> | 28.58 ± 0.09<sup>c</sup> | 0.70 ± 0.02<sup>a</sup> |
| BGN5 | 74.61 ± 0.04<sup>c</sup> | 4.19 ± 0.02<sup>c</sup> | 28.89 ± 0.01<sup>c</sup> | 29.12 ± 0.13<sup>c</sup> | 0.80 ± 0.25<sup>b</sup> |
| BGN10 | 74.11 ± 0.00<sup>c</sup> | 3.88 ± 0.01<sup>c</sup> | 29.35 ± 0.45<sup>c</sup> | 29.60 ± 0.44<sup>c</sup> | 0.93 ± 0.08<sup>c</sup> |
| BGN15 | 72.73 ± 0.03<sup>c</sup> | 3.59 ± 0.01<sup>c</sup> | 29.93 ± 0.03<sup>c</sup> | 30.14 ± 0.03<sup>c</sup> | 1.56 ± 0.10<sup>c</sup> |
| BGN20 | 71.21 ± 0.05<sup>c</sup> | 3.58 ± 0.02<sup>c</sup> | 31.60 ± 0.06<sup>c</sup> | 30.80 ± 0.05<sup>c</sup> | 2.26 ± 0.05<sup>c</sup> |
| **Crumb** | | | | | |
| Control | 76.27 ± 0.09<sup>c</sup> | 3.15 ± 0.01<sup>c</sup> | 23.19 ± 0.02<sup>c</sup> | 23.40 ± 0.02<sup>c</sup> | 0.70 ± 0.01<sup>a</sup> |
| BGN5 | 76.18 ± 0.03<sup>c</sup> | 2.64 ± 0.01<sup>c</sup> | 23.72 ± 0.05<sup>c</sup> | 23.87 ± 0.05<sup>c</sup> | 1.33 ± 0.06<sup>b</sup> |
| BGN10 | 75.02 ± 0.03<sup>c</sup> | 2.64 ± 0.02<sup>c</sup> | 24.40 ± 0.03<sup>c</sup> | 24.54 ± 0.03<sup>c</sup> | 1.72 ± 0.06<sup>c</sup> |
| BGN15 | 74.56 ± 0.07<sup>c</sup> | 2.44 ± 0.01<sup>c</sup> | 24.65 ± 0.01<sup>c</sup> | 24.77 ± 0.01<sup>c</sup> | 0.58 ± 0.28<sup>c</sup> |
| BGN20 | 74.21 ± 0.05<sup>c</sup> | 2.35 ± 0.01<sup>c</sup> | 25.15 ± 0.01<sup>c</sup> | 25.25 ± 0.01<sup>c</sup> | 0.61 ± 0.01<sup>c</sup> |

Means ± SD with different letters in the same column are significantly different (p < 0.05). Control = flour and steamed bread without Bambara groundnut flour; BGN5–BGN20 = flour and steamed bread with 5%, 10%, 15% and 20% Bambara groundnut flour.

Las medias ± DE con letras diferentes en la misma columna son significativamente diferentes (p < 0.05). Control = harina y pan al vapor sin harina de frijol de bambara; BGN5-BGN20 = harina y pan al vapor con 5, 10, 15 y 20% de harina de frijol de bambara.
fortifying bread with chickpea flour. The colour difference (ΔE) of control sample and composite flours significantly differed (p < 0.05) and the ΔE values of the flour blends were less than 1, and this means that the colour was not obvious to the human eye. Li et al. (2020) produced similar results in steamed bread with millet flour. The ΔE values of the crust and crumb showed that the samples were distinct with the values <3. Moreover, the colour difference of the crust and crumb could be detected by human eyes. Laothaprasit and Srircharoenpong (2018) observed similar data on steamed bread fortified with Jerusalem artichoke flour.

### 3.6. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of Bambara groundnut fortified flour and steamed bread samples

The control samples for both flour and steam bread had some TPC, TFC and antioxidant activity as measured by FRAP and DPPH assays (Table 5). This shows that wheat contains various natural bioactive compounds such as phenolic acids, flavonoids, carotenoid, tocopherol and others (Yu, 2007). The TPC values of the flour samples significantly increased (p < 0.05) ranging from 32.72 to 71.79 mg GAE/g and 37.18 to 79.88 mg GAE/g for steamed bread samples, respectively. Steamed breads had higher TPC compared to flour samples. The high TPC of steamed breads was likely due to thermal degradation during steaming, other fractions dissolved in boiling water, and the redox interactions between BGN phenolic acid and gluten in wheat. The binding between BGN flour polyphenols and gluten in wheat and polysaccharide has also contributed to the increase in TPC (Swieca et al., 2013; Wang & Zhou, 2004). Moreover, the covalent and non-covalent bond associations probably made the phenolic in steamed breads less extractable. Zhu et al. (2016) reported similar data where the incorporation of black tea enhanced the TPC of steamed bread. The data obtained in this study indicate that BGN contains high amounts of TPC and may be incorporated to value added products to improve their polyphenolic compounds thereby improving the well-being of consumers. The TFC of composite flour samples and steamed breads were significantly different (p < 0.05) to the control sample. The TFC values ranged from 9.57 to 22.25 mg CE/g and 11.45 to 23.39 mg CE/g for flour and steamed bread samples, respectively. The result shows that the TFC of steamed bread was higher compared to that of flour samples. The high TFC of steamed bread is associated with the improved accessibility to extract and to a more systematic liberation of polyphenolic and flavonoid compounds from intracellular proteins (Wachtel-Galor et al., 2008). Similarly, an increased yield of flavonoids of steamed bread added with black tea was previously reported by Zhu et al. (2016). However, low TFC of the control sample (steamed bread) could be due to flavonoid being sensitive to heat. Heat breaks down flavonoid and glycoside extraction during steaming or cooking (Cholpocha et al., 2012).

The fortification of wheat flour and steamed bread samples with BGN flour significantly increased (p < 0.05) the FRAP activity with values ranging from 1.95 to 2.38 mg GAE/g and 1.91 to 2.35 mg GAE/g for flour and steamed bread samples. The control of both flour and steamed breads had the least FRAP activity. Similarly, Benzie and Strain (1999) indicated that FRAP assay results correlate with TPC. However, there was a decrease in FRAP activity of steamed breads compared to flour. These results indicate that the heat produced during the steaming process caused the reduction of FRAP activity in steamed breads. However, BGN flour can be utilised as a source of antioxidants of both flours and steamed breads. Like the FRAP activity, the DPPH activity of flours and steamed breads increased ranging from 17.25% to 37.92% and 41.03% to 80.96%, respectively. The high DPPH activity values of both composite flours and steamed breads might be attributed to heat development during steaming which contributes to the interconnection between the increased liberation of polyphenolic compounds such as fenolic acid from food matrix with few anti-oxidant components that are soluble during dough formation (Dewanto et al., 2002). These results demonstrate that the control flour and steamed bread have low radical scavenging capacity compared to fortified samples. Similarly, an increase in DPPH activity of steamed bread with black tea was previously reported by Zhu et al. (2016).

### 3.7. Physical characteristics of steamed breads fortified with Bambara groundnut flour

The physical properties of steamed breads are represented in Table 6. The values of steamed bread weight varied from 468.63 to 474.30 g with the increased fortification of BGN.
Table 6. Physical characteristics of steamed breads fortified with BGN flour.

| Sample | Weight (g)     | Volume (ml)    | Specific volume (cm³) | Diameter (cm) | Height (cm) | Spread ratio |
|--------|----------------|----------------|-----------------------|---------------|-------------|--------------|
| Control | 468.63 ± 0.25a | 636.67 ± 3.51b | 1.36 ± 0.07c          | 14.03 ± 0.15d | 6.70 ± 0.20e | 2.09 ± 0.07f |
| BGN5   | 469.27 ± 0.25b | 622.67 ± 5.69c | 1.33 ± 0.03d          | 14.67 ± 0.15f | 6.43 ± 0.11g | 2.28 ± 0.02h |
| BGN10  | 469.70 ± 0.02ab| 582.33 ± 14.57d| 1.24 ± 0.09g          | 15.37 ± 0.25h | 5.90 ± 0.10i | 2.60 ± 0.01j |
| BGN15  | 471.60 ± 1.67b | 477.33 ± 44.09d| 1.01 ± 0.03b          | 15.83 ± 0.12d | 5.83 ± 0.06e | 2.71 ± 0.02d |
| BGN20  | 474.30 ± 1.61c | 414.67 ± 14.50d| 0.87 ± 0.19c          | 16.03 ± 0.15e | 5.40 ± 0.30f | 2.97 ± 0.15g |

Means ± SD with different letters in the same column are significantly different (p < 0.05). Control = steamed bread without Bambara groundnut flour; BGN5–BGN20 = steamed bread with 5%, 10%, 15% and 20% Bambara groundnut flour.

Flour. The high steamed bread weight might be ascribed to BGN flour having a greater WAC which increased moisture absorption. This leads to a decrease in wheat gluten and a reduction in carbon dioxide gas retention within the dough leading to thick dough and steamed bread that is heavy (Olayoye et al., 2006). The volume and the specific volume decreased significantly (p < 0.05) as the fortification of BGN flour increased in the steamed breads. The decrease is ascribable to the dilution effect of gluten since BGN flour is deficient in gluten protein and this results in steam bread cells with low gas-holding capacity and dough mechanical strength during proofing and steaming (Nquimbou et al., 2013). Changes because of dilution of gluten were previously reported in Chinese steamed bread added with quinoa flour and high-amylose maize starch (Wang & Zhou, 2004).

The diameter of the steamed breads varied from 14.03 control to 16.03 cm with the increased fortification levels of BGN flour. The control sample had a lower diameter (p < 0.05) which was significantly different from the composite steamed breads. The increase in diameter of composite steamed breads could be due to the decrease of both steamed bread volume and height which had negative correlation with diameter mainly because of reduced gluten content on composite flours and steamed breads. A similar increase in diameter of steamed bread was reported by Kim et al. (2019) in evaluating the association between physicochemical attributes of Korean wheat flour and quality characteristics of steamed breads. The height of the composite steamed breads decreased (p < 0.05) significantly with fortification levels of BGN flour varying from 6.70 cm for control to 5.40 cm. This is attributed to reduced gluten content within composite bread samples which resulted in low carbon dioxide gas retention and heavy texture since BGN flour has low gluten content. Similarly, Kamaljit et al. (2010) found that the inclusion of unripe plantain flour and BGN protein concentrate decreased the proofing rate of composite breads and decreased the height. The spread ratio of composite steamed bread samples increased with the BGN flour fortification levels ranging from 2.09 to 2.97. The spread ratio is a principal factor in baked products and it correlates with the viscosity of the dough (Parey & Delcour, 2008). The relatively high spread ratio of fortified steamed breads could be due to the availability of high BGN flour which weakens the gluten protein and retards the ideal gluten matrix during fermentation and steaming (Su et al., 2005).

3.8. Texture profile of steamed breads fortified with Bambara groundnut flour

The hardness of steamed breads significantly decreased (p < 0.05) ranging from 49.27 to 25.09 N, springiness from 0.13 to 0.09, cohesiveness from 0.91 to 0.88 whereas adhesiveness did not show an association with BGN flour fortification (Table 7). The control steamed bread had the highest value among all texture profile parameters. These results could be due to the composting of cereal grains and legumes since both are good sources of starch content. Moreover, starch retrogradation might have resulted in excessive hardness, springiness, and cohesiveness of control steamed bread (Mashau et al., 2020). The hardness is associated with starch retrogradation that takes place when the amylose and amylopectin chains are thermally treated (Fernández Muñoz et al., 2002). Moreover, the highest hardness value (49.27 N) of control sample could be attributed to a quicker retrogradation process because of the high amyllose present in the control with linear tremendous polar generation which decreased its capacity to keep water (Kong & Singh, 2016). The lower hardness value of composite steamed breads may be due to fortification of BGN flour which forms the branch bonds of dough that resist this process since it contains high protein and its greater WAC prevents the steamed breads from becoming hard (Yusuf et al., 2008). The decrease in

Table 7. Textural parameters of fortified steamed breads.

| Sample | Hardness (N)   | Springiness (mm) | Cohesiveness (N.s) | Adhesiveness (N.s) |
|--------|----------------|------------------|-------------------|-------------------|
| Control| 49.27 ± 6.36c  | 0.13 ± 0.00a     | 0.91 ± 0.01a      | −8.35 ± 5.77a     |
| BGN5   | 45.98 ± 3.83bc | 0.13 ± 0.01ab    | 0.90 ± 0.01ab     | −12.43 ± 2.300c   |
| BGN10  | 37.10 ± 0.50c  | 0.10 ± 0.03c     | 0.89 ± 0.01bc     | −11.31 ± 167.51c  |
| BGN15  | 36.34 ± 10.73c | 0.09 ± 0.03bc    | 0.89 ± 0.01bc     | −7.28 ± 6.49g     |
| BGN20  | 25.09 ± 0.65c  | 0.09 ± 0.02bc    | 0.88 ± 0.01c      | −6.28 ± 0.95s     |

Means ± SD with different superscripts in the same column are significantly different (p < 0.05). Control = steamed bread without Bambara groundnut flour; BGN5–BGN20 = steamed bread with 5%, 10%, 15% and 20% Bambara groundnut flour.

Las medias ± DE con diferentes superíndices en la misma columna son significativamente diferentes (p < 0.05). Control = pan al vapor sin harina de frijol de bambara; BGN5–BGN20 = pan al vapor con 5, 10, 15 y 20% de harina de frijol de bambara.
hardness value of composite steamed breads was also recorded by Liang et al. (2019) in steamed bread fortified with potato flour. The adhesiveness values of control and composite steamed breads were very low, both showing negative values. The low adhesiveness of steamed breads could be due to the inclusion levels of BGN which formed branched starches in the dough and resulted in longer fermentation. Su et al. (2005) reported similar data after determining the impact of endoxylanases on dough characteristics and steamed bread.

4. Conclusions

In this work, functional steamed bread was produced by fortifying wheat flour with BGN flour. In light of the available results, it can be concluded that fortification of wheat flour with BGN flour improved the water absorption capacity of the flour and titratable acidity of the dough. The flour and steamed bread samples fortified with BGN flour had higher moisture, ash, protein, fat and crude fibre compared to the control steamed bread. Another desirable benefit of BGN flour fortification was the improvement of antioxidant properties of flour and steamed bread samples. The height, diameter and spread ratio of BGN flour fortified steamed breads were also high compared to the control sample. Fortification of wheat flour by up to 10% of BGN flour results in a good compromise to obtain good physical characteristics of steamed bread with an increase in ash, protein contents and antioxidant properties. Therefore, fortification of wheat flour with BGN flour is a successful way of providing consumers with nutritious, healthier and antioxidant rich steamed bread.

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