

Assessment of the Anti-Nutritional, Functional and Microbiological Properties of Instant Breakfast Cereals from Yellow Maize (*Zea mays*), Sesame (*Sesamum indicum*) and Oyster Mushroom (*Pleurotus ostreatus*) Flour Blends

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Abstract

Composite flours were processed from blends of yellow maize (*Zea mays*), sesame seed (*Sesamum indicum*) and oyster mushroom (*Pleurotus ostreatus*) powder in the ratio of 80:20:0; 75:20:5; 70:20:10; 65:20:15 and 60:20:20, respectively to produce the breakfast cereal coded as YBC, SBC, FBC, GBC and BBC with YBC as the control. The breakfast cereals were produced by hydration and toasting of yellow maize and sesame to 160 °C for 25 minutes and blended together with oven dried and packaged oyster mushroom. The developed products were analyzed for anti-nutrients, phytochemicals, functional and microbial properties. The anti-nutrients and phytochemical revealed the range (mg/g) as: tannin (1.12-1.21), phytate (0.69-0.53), oxalate (1.21-0.43), flavonoid (0.23-1.22%) and phenolic (0.23-1.23%). The bulk density (0.77-0.63g/ml), water absorption capacity (156.5-126.0%), swelling capacity (309.5-249.5%), least gelation (1.10-0.75g/g) and reconstitution index (49.95-39.95%) were recorded. From the total viable count, it ranged from 3.3×10^2 to 4.2×10^2 cfu/g but no mold growth was detected. Oyster mushroom supplementation reduced the anti-nutrients in the formulated products to significant levels ($p < 0.05$) with low microbial load and functional properties that makes it a suitable meal.

Keywords

Yellow maize, Sesame, Oyster mushroom, Instant breakfast cereal

Introduction

Breakfast cereals are grain formulations that could be consumed without further cooking and provide the body with immense nutrients. According to Spencer [1], breakfast consumption should be about 15–25% of daily energy intake (this translates to, 375–625 calories for men and 300–500 calories for women). Failure to consume breakfast has been reported to cause injurious effect on cognitive performance among children [2]. Ready-to-eat breakfast is considered a viable tool to address micronutrient deficiency and malnutrition due to its nutritional quality and ease of preparation. Plant foods, when properly processed and blended, have been shown to provide nutritious diet to adults and children [3].

The term "maize" comes from the Spanish word "maiz," which best describes the plant. In several parts of the world, it is regarded as a staple food and ranks third among crops of the world after rice and wheat [4]. Maize is commonly used as an animal feed. Cornmeal, grits, flakes, starch, pasta, tortillas, cookies, and breakfast cereals are all made from it. Plant proteins are now an important part of human nutrition, especially in the developing world where protein intake on the average falls short of what is needed. Since animal proteins are in short supply, scientists are constantly looking for novel protein sources to use as

beneficial food materials and dietary supplements [5]. Majority of the Nigerian plant foods, which are low-cost protein sources, are underutilized and seldomly diversified in for use as food, especially to supplement nutritional quality. Sesame (*Sesamum Indicum* L.) provides many benefits to health due to its bioactive components such as sesamin, sesaminol and gamma-tocopherol as well as unsaturated fatty acids composition. The search for low cost plant protein for food production to enhance nutritional quality has led researchers to explore use of sesame flour as an alternative source of protein. Sesame seed has more protein(17-40%) compared to meat(18-25%) and cereals(7-14%) which makes it suitable for incorporation with maize and oyster mushroom so as to produce a nutritious diet to reduce malnutrition and micro nutrient deficiency [6]. Sesame seeds contain 5-6% ash, 20-25% protein, 20-25% starch and 40-50% oil [7]. It is rich in vitamins and minerals with nutraceutical compounds that have antioxidant activity, such as phenolic and tocopherols, which have a major effect on lowering lipid profile, blood pressure, and vessel relapse, as well as influence reduction of chronic ailments [8]. Oyster mushroom is the second most commercialized mushroom after *Agaricus bisporus* from Southeast Asia, Europe, India and Africa from the family Basidiomycetes. It has enormous advantages above other edible mushrooms based on the fact the it grows under a wide range of temperature (10-30 C) and pH(6-8) while secreting enzymes that degrade lignocellulosic biomass of substrates) [9]. Oyster mushroom is useful as food due to its flavor, proteinous and fiber content. Mushrooms according to Bano [10], have about twice the protein content of vegetables and four times that of oranges and are high in vitamins such as riboflavin, biotin, and thiamine. Since oyster mushrooms contain no starch, have low sugar content, and are high in fiber, they are the least fattening food [11]. It is rich in flavonoid and phenols which are good sources of nutraceuticals. A formulated breakfast cereal product with maize, sesame and mushroom has the potentials to use utilized local foods to solve nutritional imbalance such as micro-nutrient deficiency and malnutrition. Oyster mushroom supplements the maize-sesame formulated product to enhance the functional and phytochemical properties of the product with reduced microbial load and anti-nutrients that makes nutrients unavailable.

Raw materials procurement

Three kilograms (3 kg) of yellow maize (*Zea mays* L.) was sourced from the National Cereals Research Institute of Nigeria and one kilogram of Sesame (*Sesamum indicum* L.) purchased from Daudu Market in Benue State. One kilogram (1 kg) of oyster mushroom (*Pleurotus ostreatus*) was cultivated and harvested from the National Biotechnology Development Agency, South East Center, University of Nigeria, Nsukka, Enugu State.

Preparation of sample

Yellow maize, sesame and oyster mushroom samples were prepared to formulate a product with desirable quality and nutritional characteristics.

Yellow maize flour processing

Maize was processed to flour using the procedure of Ing-

bian and Akpapunam [12]. Two kilograms (2 kg) of maize grains were winnowed, sorted, and steeped in water for 8 hours. The steeped grains were dewatered twice for extraction of the steep water and drained for 10 to 15 minutes. It was roasted at temperature of 160 °C and milled with a hammer mill to obtain the flour which was passed through a sieve with a 60-inch mesh (British Standard Screen) and stored in high density polyethylene bag as shown in figure 1.

Sesame flour processing

Sesame seeds were processed using the procedure described by Makinde and Akinoso [13]. Two kilograms of sesame seeds were sorted, drained, and washed with water. The seeds were dehulled and roasted for 25 minutes at 160°C and milled into sesame flour using a hammer mill. It was packed in a high-density polyethylene bag at room temperature until required for formulation and analysis as shown in figure 1.

Oyster mushroom powder processing

The oyster mushroom (*Pleurotus ostreatus*) powder was processed as described by Okeke et al. [14] in the laboratory from fresh oyster mushroom. The dirt and weakened portion of the fresh oyster mushroom were removed. The fresh mushroom was blanched for three minutes in hot portable water containing 3% salt and 0.01% citric acid at 32 °C. Thereafter, the water was drained with the mushroom dried in an oven at 105 °C for 3 hours. Hammer mill was used in the laboratory to mill the dried mushroom and sieved using a 60-inch mesh sieve (British Standard Screen). It was packed in a low-density polyethylene bag and held in the refrigerator (4 °C) until required for use in formulation of product as shown in figure 1.

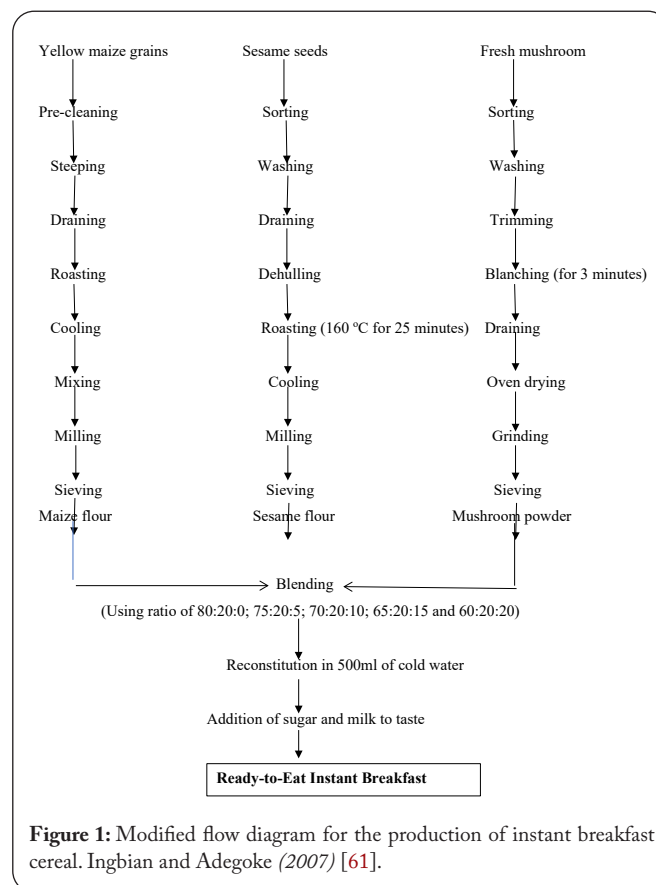


Figure 1: Modified flow diagram for the production of instant breakfast cereal. Ingbian and Adegoke (2007) [61].

Formulation of product

The formulation of the composite flour was done using the method described by Igbabul et al. [15]. The composite flour were formulated from a blend of yellow maize, sesame seed and oyster mushroom flours in the ratio of 80:20:0; 75:20:5; 70:20:10; 65:20:15 and 60:20:20 to produce the breakfast cereal. The best ratio was determined using preliminary studies as described by Igbabul et al. [15] formulated to breakfast cereal as shown in figure 1.

Material and Methods

Analysis of functional properties

Determination of bulk density

To determine the bulk density of the samples, Okaka and Potter [16] procedure was used. A 100 ml measuring cylinder was filled with five grams (5 g) of flour sample. On a laboratory bench, the cylinder was tapped multiple times to achieve a steady volume. The sample volume was measured. The cylinder was tapped repeatedly against a laboratory table until no further volume was lost.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample}}{\text{Volume of sample after tapping}}$$

Determination of water absorption capacity

Water absorption capacity was determined by Sathe and Salunkhe [17]. One gram (1.0 g) of samples was blended for 30 seconds at high speed with 20 milliliters (20 ml) of distilled water. For 30 minutes, the mixture was permitted to stand at 30 °C. The supernatant was measured using a 10 ml graduated cylinder. Water absorption capacity was determined in %.

Determination of swelling capacity

The method of Takashi and Sieb [18] was used with slight modifications. One gram (1.0 g) of flour blend sample was weighed to a 50 ml centrifuge tube. A total of 50 milliliters of distilled water was applied and gently mixed. For 15 minutes, the slurry was heated in a water bath at temperatures of 70, 80, 90, and 100 degrees Celsius. The slurry was gently mixed during heating to prevent the flour from clumping. After 15 minutes, the tube containing the paste was centrifuged for 10 minutes at 3000 rpm using a centrifuge. The supernatant was immediately decanted after centrifugation. The sediment's weight was measured and registered. The moisture content of the sediments gel was measured using the dry matter content of the gel.

$$\text{Swelling power} = \frac{\text{Weight of wet mass sediment}}{\text{Weight of dry matter in the gel}} \times \frac{100}{1}$$

$$\text{Solubility index (\%)} = \text{Weight of dry solid after drying} \times 100$$

Determination of least gelation capacity

The method described by Sathe and Salunkhe [17] was used to determine the least gelation capacity. Sufficient sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 percent (w/v) were prepared in 5 ml distilled water. The suspensions were

then heated in test tubes in a boiling water bath for one hour before being quickly cooled under cold tap water. At 4 °C, the test tubes were cooled for another 2 hours. To determine the concentration of the least gelation potential, the sample from the inverted test tube which did not fall down or slip visually was measured.

Determination of reconstitution index

Reconstitution index was determined by Onwuka [19] method. To 50 ml of boiling water, five grams (5 g) of flour sample were dissolved. After 90 seconds of agitation, the mixture was moved into a 50 ml graduated cylinder, and the volume of the sediment was measured after 30 minutes of settling.

$$\text{Reconstitution index} = \frac{\text{Weight of sediment}}{\text{Weight of sample}}$$

Determination of viscosity

The viscosity of the product was measured with a Rapid Visco-Analyzer (Model RVA-4; Newport Scientific Pty. Ltd, Warriewood, Australia), as defined by Sathe and Salunkhe (1981) [17]. In a dried empty canister, three grams of flour samples were weighed. Afterwards, 25 mL of distilled water was poured into the sample canister. The slurry was heated from 50 to 95 °C for 2 minutes, and then cooled to 50 °C for 2 minutes. The rate of heating and cooling was kept constant at 11.25 °C for 1 minute. The Windows Software ThermoLine which was connected to a device was used to determine the viscosity.

Determination of pH

The pH of the sample was read after a 10 percent (w/v) flour-water suspension was prepared in a 200 mL clean beaker and allowed to settle at 302 °C for 15 minutes [20].

Determination of anti-nutrient of formulated products

Determination of tannin

The procedure of Pearson [21] was used to measure tannin content. In a conical flask, one gram (1 g) of the sample was measured, and 10 ml of distilled water was added. The mixture was permitted to sit at room temperature for 30 minutes, with gentle shaking every five minutes. The mixture was centrifuged after 30 minutes with 2.5 mL of the supernatant weighed into a separate tannin solution. In a separate 50 ml volumetric flask, the tannin solution was measured. Another 2.5 ml of standard tannin solution was also measured into separate 50 ml flask (1 ml) of Folin-Den reagent followed by 2.5ml of saturated Na₂CO₃ solution into the each of it. The solution was made up to the mark and incubated for 90 minutes at room temperature and absorbance read at 250 nm wavelength. Percentage tannin was calculated thus:

$$\% \text{Tannin} = \frac{A_n \times 100 \times W \times V_f}{A_s \times V_a}$$

Where: A_n = Absorbance of test sample; A_s = Absorbance of the standard; C = Concentration of standard; V_f = Total volume of extract; V_a = Volume of extract analyzed.

Determination of phytate

Oberlas [22] method was used to determine phytate. Then, 0.2N HCl was used to remove one gram. One milliliter of the extract was pipetted into a test tube and 2 milliliters of solution (2) added with the test tube sealed. The test tube was heated for 30 minutes in boiling water before being cooled at room temperature. In 100 ml of 2N HCl, four milliliters (4 ml) of iron III sulphate solution with 0.2 g was added and made up to mark with 100 ml distilled water. In solutions 1, 2, and 3, 10 g of bipyridine and 10 mL thioglycollic acid were combined with 1000 mL distilled water.

Determination of oxalate content

The titration method defined by Oladele et al. [23] was adopted to determine the oxalate content. In a 100 mL conical flask, one gram of the sample was weighed, 75 mL 3N H₂SO₄ was added, and a magnetic stirrer was used to stir the mixture for one hour. It was filtered with a Whatman No. 1 filter paper with 25 mL of the filtrate taken and titrated against 0.1N KMnO₄ solution when hot (80 - 90°C) until a slight pink color remained for at least 30 seconds.

Determination of flavonoids content

Bohm and Kocipal [24] process was adopted to measure the flavonoids content. Five grams (5 g) of formulated breakfast sample were boiled under reflux for 30 minutes and after cooling, it was filtered with a Whatman No. 42 grade filter paper. Starting with a drop, a measured volume of the extract was treated with an equal volume of ethyl acetate. Filtration with weighted filter paper was used to recover the flavonoid precipitate. The weight difference that resulted was used to calculate flavonoid.

$$\% \text{ flavonoid content} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of phenolic content

The procedure of Pearson [21] was adopted. In a conical flask, one gram (1 g) of the sample was measured with 10 ml of distilled water added. The mixture was permitted to sit at room temperature for 30 minutes, with gentle shaking every five minutes. Centrifugation of the mixture was done after 30 minutes, and 2.5 mL of the supernatant weighed into a separate phenol solution. In a separate 50 ml volumetric flask, phenol solution was measured. Thereafter, 2.5 ml of standard phenol solution was measured into separate 50 ml flask (1 ml) with Folin-Den reagent added into each flask followed by 2.5 ml of saturated Na₂CO₃ solution. The solution was made up to the mark and incubated for 90 minutes at room temperature. The absorbance was read at 250 nm wavelength. Percentage phenol was calculated thus:

$$\% \text{ Phenol} = \frac{A_n \times 100 \times W \times V_f}{A_s \times V_a}$$

Where: A_n = Absorbance of test sample; A_s = Absorbance of the standard; C = Concentration of standard; V_f = Total volume of extract; V_a = Volume of extract analyzed.

Microbiological analysis

The pour plate method by Harrigan and McCance [25] was used to determine the total viable and mold count. One gram (1 g) of the test sample was immersed in 9 milliliters of sterile water. For 10 minutes, the suspension was continually agitated. Then, using a 10-fold serial dilution method, 1 ml of the test sample suspensions were diluted in 9 ml of sterile water. The dilution was carried out until the seventh test tube was reached. With the aid of a permanent marker and a Pasteur's pipette, the oven-dried agar plate was divided into eight equal parts, and 0.02 ml of the 10⁻¹ dilution was transferred to each segment of the plate. From 10⁻² to the corresponding agar plate, the procedure was repeated (Nutrient agar for total viable count and Sabouraud dextrose agar for mold). The process was repeated until the dilution reached 10⁻⁷. Before incubation, the plates were allowed to stand for 15 minutes before being inverted and incubated for 24 hours at 37 °C for bacteria growth and 48 hours at 25 °C for mold growth. The plates were examined and the colonies counted after the incubation time.

$$\text{Original cell population} = \frac{\text{Mean colony per drop} \times \frac{1}{\text{Dilution factor}}}{\text{Estimated volume per drop}}$$

Data analysis and experimental design

The data was analyzed using a one-way analysis of variance (ANOVA) with a completely randomized design and Duncan's New Multiple Range Test for mean separation [26]. The software Statistical Package for Service Solution (SPSS) version 21 was used, with significance set at p<0.05.

Results and Discussion

Anti-nutrient composition of instant breakfast cereal product

Instant breakfast cereals' anti-nutrient content are presented in table 1.

Tannin

Tannin content amplified with mushroom powder supplementation from 1.12 mg/100g in sample YBC to 1.21 mg/100g in sample BBC which can be attributed to mushroom tannin content. Sample FBC and GBC showed no difference significantly (p>0.05) to each other. Sample GBC and BBC had no significant (p>0.05) differences to each other. Sample YBC and SBC had some differences significantly (p<0.05) to each other and with all the other instant breakfast cereal products. The result for tannin content in the developed instant breakfast cereal products fell within the range of 0.61 to 1.72% reported by Usman et al. [27] for local rice, soybeans and defatted coconut flours breakfast cereal. Tannins bind proteins in foods but in the instant breakfast cereal product were within the safe limits of 4 to 9 mg per day reported by Siddhuru and Becker [28].

Phytate (phytic acid)

Phytic acid decreased as more mushroom powder was supplemented to the products from 0.69mg/100g in sample

YBC to 0.53mg/100g in sample BBC. Sample BBC and GBC were not different ($p>0.05$) significantly to each other but differed significantly ($p<0.05$) with the other products. Sample FBC with 0.61mg/100g significantly differed ($p<0.05$) to all the other products while as sample YBC and SBC had no differences significantly ($p>0.05$) to each other. Oyster mushroom are much lower in phytate, its supplementation in the traditional cereal further decreased the level of phytic acid thus making more nutrients available in the developed product. Filipiak-Szok et al. [29] reported that supplements could improve and complement the human diet with ingredients that can be of immense contribution to health rather than being sources of anti-nutritional elements. From the research, oyster mushroom decreased phytate significantly ($p<0.05$) and hence, could be deduced that it had a positive effect on diet by making nutrients more available. Phytic acid was less than the safe limit of 25 mg per meal recommended by the American Academy of Nutrition and Dietetics [30].

Oxalate

Oxalate content in the instant breakfast cereal product depleted with increased mushroom powder supplementation from sample YBC with 1.21 mg/g to 0.43 mg/g in sample BBC. Sample BBC and GBC showed no significant differences ($p>0.05$) to each other just as sample SBC and FBC. The control (sample YBC) had the highest content of oxalate with 1.21 mg/100g and differed significantly ($p<0.05$) to the other instant breakfast cereal. The oxalate content for the instant breakfast cereal products in this study was in the range of 0.077 to 1.47% recorded by Usman et al. [27] for local rice, soybeans and defatted coconut flour blend breakfast cereal. The instant breakfast cereal products upon supplementation with oyster mushroom powder had decreased oxalate content from 1.21 to 0.43% which is less than 0.51% reported by Ekanah and Obueh [31] for yellow maize gruel (pap). The instant breakfast cereal developed fell below the lowest safe limit of 5 to 9 mg per meal serving and thus safe diet for those suffering from kidney stone formation or labile to it.

Phytochemical composition of instant breakfast cereal product

Instant breakfast cereals phytochemical content are presented in table 1.

Flavonoid

As shown in table 1, the flavonoid content amplified with mushroom powder supplementation from 0.23% in sample YBC to 1.22% in sample BBC. Mushroom is rich in flavonoid, which accounts for the effect on the product. Sample BBC and GBC had 1.22% respectively which did not differ significantly ($p>0.05$) to each other but significantly differed ($p<0.05$) to the other instant breakfast cereal products. Sample SBC and FBC had 0.50 and 0.52 % which were not different significantly ($p>0.05$) to each other though had differences significantly ($p<0.05$) to the other formulated instant breakfast cereal products. The control (sample YBC) however had notable differences significantly ($p<0.05$) with all the other instant breakfast cereal products. Ayo et al. [32] reported flavonoid content of 0.54 to 1.44% for Acha-mushroom blend flour and biscuit with higher values of 1.22% which was evident in the developed instant breakfast cereal products. Flavonoids are compounds with $C_6-C_3-C_6$ skeletons that consist of two aromatic rings joined by a three carbon link and found in the pericarp of cereals in minor quantities. Flavonoids are indicated to have anti-inflammatory, anti-oxidant, anti-cancer, and anti-allergic properties [33].

Phenolic compound

As shown in table 1, phenols increased on addition or supplementation of traditional cereal with oyster mushroom powder from 0.23% in the control (sample YBC) to 1.23% in sample BBC. Sample YBC and SBC showed no significant differences ($p>0.05$) to each other just as sample FBC and GBC also did not differ significantly ($p<0.05$) from each other. Sample BBC with the highest content of phenols showed distinct significant ($p<0.05$) difference to the other breakfast cereal products. The results for phenolic content in this study was similar to 0.33 to 1.11% reported by Annuciacao et al. [34] for whole grain breakfast cereals but less than 1.81% reported by Agbor et al. [35] for cornflakes, 3.19% for cerelac and 8.41% for golden morn. The result in this study was in range of 1.23 to 2.67% based on findings by Ayo et al. [32] for phenolic content in acha-mushroom blend flour and biscuit. The products of secondary metabolism in plants are phenolic compounds which provide vital function in the reproduction and growth of plants with defence mechanism against pathogens and parasites while contributing to plant colour. In diets, phenolics contribute value to health issues such as lesser risk of chronic illnesses. The anti-oxidant properties act against degenerative diseases like hearts disease and cancer [33].

Table 1: Anti-nutrient and phytochemical composition of instant breakfast cereal product.

Sample	Tannin mg/100g	Phytate mg/100g	Oxalate mg/100g	Flavonoid %	Phenolic %
YBC	1.12a ± 0.00	0.69c ± 0.01	1.21c ± 0.02	0.23a ± 0.00	0.23a ± 0.01
SBC	1.16b ± 0.00	0.67c ± 0.01	1.09b ± 0.01	0.50b ± 0.02	0.23a ± 0.02
FBC	1.19c ± 0.00	0.61b ± 0.02	1.05b ± 0.01	0.52b ± 0.01	0.99b ± 0.01
GBC	1.20c ± 0.00	0.55a ± 0.01	0.44a ± 0.02	1.22c ± 0.00	1.03b ± 0.01
BBC	1.21cd ± 0.02	0.53a ± 0.02	0.43a ± 0.01	1.22c ± 0.02	1.23c ± 0.01

*Values are in mean ± SD. Means with the same superscript in the same column are not significantly ($p > 0.05$) different

Key: YBC= 80% yellow maize flour+20% sesame flour + 0% oyster mushroom powder; SBC = 75% yellow maize flour+20% sesame flour + 5% oyster mushroom powder; FBC = 70% yellow maize flour+20% sesame flour +10% oyster mushroom powder; GBC = 65% yellow maize flour+20% sesame flour+15% oyster mushroom powder; BBC = 60% yellow maize flour+20% sesame flour + 20% oyster mushroom powder.

Table 2: Functional properties of instant breakfast cereal product.

Sample	BD (g/ml)	WAC (%)	SC (%)	LG (g/g)	RI (%)	VC(cps)	pH
YBC	0.77d ± 0.00	156.5d ± 0.70	249.5a ± 0.70	1.10b ± 0.00	49.95e ± 0.21	119.70e ± 0.42	6.70cd ± 0.00
SBC	0.72c ± 0.00	146.5c ± 0.00	270.0b ± 0.00	1.10b ± 0.00	48.95d ± 0.07	85.00d ± 0.00	6.60c ± 0.00
FBC	0.70b ± 0.00	135.5b ± 0.70	309.5c ± 0.70	0.90ab ± 0.00	45.20c ± 0.14	70.65c ± 0.63	6.65c ± 0.07
GBC	0.64a ± 0.00	134.5b ± 0.70	295.0d ± 0.00	0.85a ± 0.07	41.45b ± 0.21	55.20b ± 0.28	6.40b ± 0.00
BFC	0.63a ± 0.00	126.0a ± 0.00	291.5c ± 0.70	0.75a ± 0.07	39.95a ± 0.21	52.05a ± 0.02	6.30a ± 0.00

*Values are in mean ± sSD. Means with the same superscript in the same column are not significantly ($p > 0.05$) different

Key: YBC= 80% yellow maize flour+20% sesame flour + 0% oyster mushroom powder; SBC = 75% yellow maize flour+20% sesame flour + 5% oyster mushroom powder; FBC = 70% yellow maize flour+20% sesame flour +10% oyster mushroom powder; GBC = 65% yellow maize flour+20% sesame flour+15% oyster mushroom powder; BFC = 60% yellow maize flour+20% sesame flour + 20% oyster mushroom powder.

Functional properties of instant breakfast cereal product

The functional properties inclusive of bulk density, water absorption capacity, swelling capacity, least gelation, reconstitution index and viscosity for the developed instant breakfast cereal products are presented in table 2. Functional properties help to determine the intermolecular and chemical interaction in a new developed food product to determine its physical characteristics.

Bulk density

Bulk density in the instant breakfast cereal products decreased with oyster mushroom powder supplementation from 0.77 g/ml in the control (YBC) to 0.63 g/ml in the sample BBC. GBC and BBC samples were not different significantly ($p > 0.05$). Sample FBC, SBC and YBC differed significantly ($p < 0.05$) to each other and to GBC and BBC. The range of the bulk density in the instant breakfast cereal products were lower than the range of 0.89–0.80 g/ml reported in a similar study on water melon rind fortified sorghum based mumu [36] and 0.76 to 0.81 g/ml recorded by Shakpo and Osundahunsi [37]. It was within the range of 0.64 to 0.76 g/ml based on findings by Otunola et al. [38] and 0.59–0.97 g/ml [39]. According to Oppong et al. [40] bulk density could be used to determine the heaviness of flour, as well as handling requirements, packaging material type appropriate for storage of food and transportation. The more the bulk density, the greater packaging space needed [41].

Water absorption capacity

Water absorption capacity of the products decreased with increase in supplementation of oyster mushroom from 156.50% in the control (sample YBC) to 126% in sample BBC. There was no significant difference ($p > 0.05$) between sample GBC and FBC but all other product differed significantly ($p < 0.05$) for water absorption in the instant breakfast cereal products Water absorption capacity in the products was within the range of 314.17 to 119.36% [39] though significantly ($p < 0.05$) less than reported in this study for some of the products. It was lesser than the range of 171 to 195% reported by Tersoo-Abiem et al. [42] and 93.42 to 100.23% reported by Azeez et al. [43] for fortified bread from cassava and mushroom flours. The result was, however, higher than 123.6 to 133.1% reported by Gbaa et al. [36].

According to Okafor and Usman [44], the water absorption potential of breakfast cereals is related to the composition of starch granules after toasting. Thin meals are produced by composite flours with low water absorption, which is ideal for infant formulas [45]. The instant breakfast cereal product had decreased in water absorption on addition of oyster mushroom powder which could make it a suitable meal for children.

Swelling capacity

Swelling capacity in the instant breakfast cereal products increased upon supplementation with oyster mushroom powder from 249.50% in the control (sample YBC) to 309.50% in sample FBC. It then declined consistently to 291.50% in sample BBC on further supplementation up to 20% oyster mushroom powder. All the products differed significantly ($p < 0.05$) for swelling capacity. Mbaeyi-Nwaoha and Uchendu [39] reported swelling capacity in the range of 399.51 to 131.32% in breakfast cereal from acha and fermented soybean paste which is different from 309.50 to 249.50% in this study. It was, however, higher than 128.00 to 105.14% recorded by Edima-Nyah et al. [46] for yellow maize, soybeans and unripe banana flour blends breakfast cereals. Tersoo-Abiem et al. [42] also recorded a lower swelling capacity of 217–254% in a similar study on millet fortified with mushroom (*Coprinellus micaceus*). Decrease in swelling capacity could be attributed to report by Wang and Seib [47] that starch granules are inhibited from swelling by the amount of protein present in the food sample. This could be noted in the products as swelling decreases on addition of oyster mushroom powder which is rich in protein. According to Adebowale et al. [48], swelling ability is linked to the starch's amylose-amylopectin ratio, with a low amylose content resulting in a high swelling strength. Swelling variations could be caused by differences in molecular organization within the starch granules.

Least gelation capacity

Least gelation in the instant breakfast cereal products decreased with increased supplementation of oyster mushroom powder from 1.10 g/g in the control (sample YBC) and sample SBC. It decreased to 0.90 g/g in sample FBC and on further supplementation up to 20% to 0.75 g/g in sample BBC. Sample BBC and GBC had no differences significantly ($p > 0.05$) to each other same as the control (sample YBC) to sample SBC. Sample FBC did not differ significantly ($p > 0.05$) from sample

GBC for least gelation capacity. The result in this study varied with the range of 2.10 to 4.190 g/g reported by Shakpo and Osundahunsi [37]. The result agreed with Mbaeyi [49] that the formation of intermolecular hydrogen bonds between amylase molecules in a cooled gel can explain the low level of least gelation concentration. The high fibre content and high water absorption ability of the oyster mushroom powder, which did not gel when heated with high water, could also be responsible for the low levels and steady decrease in gelation. According to Sridaran et al. [50], ionic ability, pH, and the existence of non-protein components, could all affect gelation properties.

Reconstitution index

The instant breakfast cereal products reconstitution index decreased with increase in oyster mushroom powder supplementation from 49.95% in the control (sample YBC) to 39.95% in sample BBC. The samples were different significantly ($p < 0.05$) for reconstitution index. Reconstitution index in this study was similar to 49.9 to 48.7% reported by Gbaa et al. [36] though it decreased lower to 39.95% on continuous oyster mushroom supplementation. Oyster mushroom addition improved the reconstitution ability of the instant breakfast cereal products.

Viscosity

The instant breakfast cereal viscosity decreased with increased oyster mushroom concentration from 119.70 cps in the control (sample YBC) to 52.05 cps in sample BBC. The samples differed significantly ($p < 0.05$) for viscosity. The trend is similar to that reported by Mbaeyi [49] though lower than the range of 132.6 to 113.0 reported in the study but higher than 31.08 to 19.73 cps reported by Okafor and Usman [44]. According to Ihekoronye and Ngoddy [51], low viscosity in the product as observed might be attributed to lesser disruption of intermolecular hydrogen bonds, which resulted in noticeable granule swelling and gelation.

pH

The pH of the instant breakfast cereal decreased with increased supplementation of oyster mushroom n from 6.70 in the control (sample YBC) to 6.30 in samples BBC. The products differed significantly ($p < 0.05$) for pH except for sample YBC and SBC which were not significantly ($p > 0.05$) different. The result in this study was slightly higher than 4.70 to 6.56 reported for African yam bean, maize and defatted coconut fortified breakfast cereal by Usman [52]. pH defines the shelf stability of foods and susceptibility to microbial attack. The product had low acidity and must be stored properly to avoid spoilage. The range of the pH observed could be as result of partial hydrolysis of maize during soaking.

Microbiological quality of raw materials and products

Microbiological evaluation is of utmost importance in food products starting from the basic raw materials to determine its quality and safety for consumption. Results for microbiological analysis of raw materials and product are shown in table 3.

Total viable count

Total viable count is one of the microbial test carried out to ensure safety of food products. It is used to determine hygienic conditions during food processing for both raw materials and equipment's. It was used to evaluate effectiveness of processing methods, heat treatment and storage conditions for products. As presented in table 3, the instant breakfast cereal products had total viable count range from the lowest of 2.3×10^2 cfu/g in product SBC to the highest of 4.2×10^2 cfu/g in product GBC. This was within the range of 1.0 to 5.00×10^2 cfu/g reported by Gbaa et al. [36]. The result in the study however, differed with the range of 1.0×10^2 to 3.6×10^4 cfu/g based on findings by Ibeanu et al. [53] for seed flour mix made from *Digitaria exilis*, *Sesamum indicum* and *Glycine max*. However, it was lower than 3.80 to 4.7×10^3 cfu/ml reported for maize and cowpea flour blends [37]. The microbial count obtained in this study was within the recommended safe limit of 10^2 - 10^5 cfu/ml in the microbial guidelines for ready-to-eat foods adopted by the international commission of microbiological specification of food [54]. According to Mbata et al. [55], differences in incubation time and temperature, type of cereals used, and mixture recipe, among other factors, could account for variations in the composition of micro-flora of flours. Ukegbu and Anyika [56] reported that food stability is predicted by water behavior in terms of deteriorative reaction rate, microbial growth rate and physical properties of foods such as texture and shelf life. High values promote microbial development with moist food spoiling faster than dry food. Therefore, processing methods such as roasting and drying could be attributed for the low microbial load in the raw materials and products.

Total mold count

Total mold count is a microbial test to evaluate presence of spoilage fungi such as mold in foods which makes it unfit for consumption since it could led to severe health complications. As shown in table 3, total mold count was not detected in all the instant breakfast cereal products from the control (sample YBC) to sample BBC. This might be due to the raw materials being roasted and proper storage which reduces enzymatic activity, microbial load and keeping quality of cereals.

Table 3: Microbiological count of products.

Sample	Total Viable Count (cfu/g)	Total Mold Count (cfu/g)
YBC	3.3×10^2	NG
SBC	2.3×10^2	NG
FBC	3.6×10^2	NG
GBC	4.2×10^2	NG
BFC	3.9×10^2	NG

Key: YBC= 80% yellow maize flour+20% sesame flour + 0% oyster mushroom powder; SBC = 75% yellow maize flour+20% sesame flour + 5% oyster mushroom powder; FBC = 70% yellow maize flour+20% sesame flour +10% oyster mushroom powder; GBC = 65% yellow maize flour+20% sesame flour+15% oyster mushroom powder; BFC = 60% yellow maize flour+20% sesame flour + 20% oyster mushroom powder; NG= No Growth

The mold load of the instant breakfast cereal was within the optimal mold level of breakfast cereals which is 10 cfu/g. The lot would be refused if the safe limit 10^3 cfu/g in one or more samples is exceeded, as this indicates a possible health threat or probable spoilage [57]. The result in this study was less than the range of 6.0×10^1 cfu/g to 2.4×10^4 reported by Ibeanu et al. [53] and 2.2 to 3.17×10^3 cfu/ml [37]. The instant breakfast cereal products are hence fit for consumption. According to Jonathan et al. [58], legumes and cereals such as sesame seed, maize and maize products are not kept properly, it is an excellent substrate for fungi development. Fungi are common food contaminants that affect grain viability and nutritional quality during development, harvest, storage, and processing. *Fusarium spp.* and other fungi as well as *Penicillium spp.*, *Aspergillus spp.* contaminates maize grains in the field by their spores, while *Aspergillus spp.* contaminates them when they are being stored [59]. Mycotoxins are toxic metabolites produced by fungi and released into various foods. Aflatoxin is a mycotoxin of two species of *Aspergillus*, *Aspergillus flavus* and *Aspergillus parasiticus*, which can be found in a variety of foods. Aflatoxins are carcinogenic and mutagenic secondary metabolites that cause cancer in humans [59]. In mushroom, Ezekiel et al. [60] found *Aspergillus (A. flavus, A. niger-clade, A. parvisclerotigenus, A. tamarii, and other Aspergilli)*; *Fusarium spp.*; *Penicillium spp.*; *Mucor spp.*, *Trichoderma spp.*, and other *Mucorales*.

Conclusion

Processing techniques (roasting and drying) reduced anti-nutrients in the formulated products to insignificant level. It also resulted to low moisture content and limited microbial activity, thereby making the products shelf stable and safe for consumption. The study proved that oyster mushroom supplementation to yellow maize and sesame seed flour could serve as an inexpensive source of nutrients, which might only be available in expensive diets and could play a role to reduce nutritional challenges especially in developing countries.

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None.

Conflict of Interest

Authors have no conflict of interest.

Author's Contributions

This work was carried out in synergy with the two authors. Author OAO designed the study, performed the statistical analysis, managed the analyses of the study, wrote the protocol and wrote the first draft of the manuscript while Author IEM supervised the work. The authors read and approved the final manuscript.

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