Microbiology Research Journal International
19(4): 1-9, 2017; Article no.MRJI.32133
Previously known as British Microbiology Research Journal
ISSN: 2231-0886, NLM ID: 101608140

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www.sciencedomain.org

Proximate and Mineral Composition of Bread Fortified with Mushroom (*Plerotus ostreatus* and *Calocybe indica*)

V. O. Oyetayo[1] and R. R. Oyedeji[1*]

[1]Department of Microbiology, School of Science, Federal University of Technology Akure, P.M.B. 704, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author VOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RRO managed the analyses of the study and also managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/32133
Editor(s):
(1) Joao Lucio Azevedo, Department of Genetics, University of Sao Paulo, Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ / USP), Sao Paulo, Brazil.
Reviewers:
(1) Vijayakhader, Acharya N. G. Ranga Agricultural University, Hyderabad, India.
(2) Eduardo Bernardi, Universidade Federal de Pelotas, Brazil.
(3) Patrícia Matos Scheuer, Federal University of Santa Catarina, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/18675

Original Research Article

Received 9th February 2017
Accepted 23rd March 2017
Published 17th April 2017

ABSTRACT

The proximate and mineral composition of two mushrooms *(Pleurotus ostreatus* and *Calocybe indica*) was firstly determined. The mushrooms was dried and milled into powder. The Mushroom Powder (MP) was used to substitute wheat flour in bread formulation at 0%, 5%, 10%, 15% and 20%. The 0% inclusion served as the positive control while bread obtained from the market served as the negative control. The proximate and mineral properties of the bread were determined before and after storage using standard methods. The study revealed that the ash content, fiber and protein content increased significantly with increase in the addition of the mushroom powder. Protein content increased from 9.12% in the negative control to 18.7% with 5% MP inclusion and 25.1% with 20% MP inclusion. Potassium, sodium, calcium, magnesium, manganese, copper, zinc and iron content of the bread increased significantly (P≤0.05) with increase in the mushroom powder. The results from this study revealed

**Corresponding author: E-mail: rotimioyedeji@yahoo.com;*

mushroom could be used to improve the nutritional value of bread and this may help in the reduction of protein and mineral malnutrition prevalent in Nigeria and other developing countries.

Keywords: Mushroom powder; control; protein.

1. INTRODUCTION

Bread is one of the most widely consumed food product in the world and bread making technology is probably one of the oldest technology known [1]. It is an important staple food for many countries. The product is basically made of hard wheat flour, yeast, fat, sugar, salt and water [2]. It is a cereal product that is naturally low in protein and not a balance diet because it is low in lysine which is an essential amino acid [3].

Evidence from food consumption survey indicated that there is an increase in the consumption of bread in Nigeria [4]. The preference for bread apart from the taste and good eating quality almost certainly reflects the convenience it offers to urban and probably some rural consumers, requiring no preparation [4]. Worldwide bread consumption accounts for one of the largest consumed foodstuffs, with over 20 billion pounds (9 billion kg) of bread being produced annually [5].

Since bread is an important food that is generally accepted, they could be an excellent and convenient food item for protein fortification to improve the nutritional well-being/health of the people. Fortification of wheat flour with high protein materials from plant sources, macro fungal and many more sources to increase the protein and improve the essential amino acid balance of the resultant baked product such as bread has been recognized [3].

Mushrooms are edible fungi that contain high quality digestible protein that varied between (10-40%), Carbohydrate (3-21%) and dietary fiber (3-35%) on dry weight basis depending on species [6]. Mushroom will complement well with wheat flour to produce nutritionally balanced high quality bread. Mushroom is also a good source of B-vitamins (thiamin, riboflavin, niacin, biotin, pyridoxine, panthotenic acid) and vitamin C. It also contain significant amount of mineral elements like Sodium, Calcium, Potassium, Manganese, Copper, Phosphorus, Zinc, Magnesium and Iron [7].

The annual world production of cultivated mushrooms was 6 million metric tons in 1997, increased more than 1 million in 1994 [8]. [9] reported that *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide.

This present study is meant to assess the proximate and mineral properties of bread fortified with mushroom *(Pleurotus ostreatus and Calocybe indica).*

2. MATERIALS AND METHODS

2.1 Source for Samples

The Mushroom (*Pleurotus ostreatus* and *Calocybe indica*) was obtained from Afe Babalola University Ado-Ekiti and was identified at the Department of Crop and Pest Management, Federal University of Technology Akure. Other ingredients used for the bread production includes Flour and sugar which was supplied by Golden Penny Nigeria, Salt supplied from Dangote salt, Shortening supplied from STK Margarine, Skim milk powder supplied from Dano milk, and Yeast supplied by STK Royal instant yeast.

2.2 Preparation of Mushroom Powder

The mushroom powder was prepared using the method described by [10]. The mushroom was washed and dried at 60℃ in a Chinese Binatone mini oven (MO 4500). The dried mushroom was milled using an electronic table top milling machine (Hanzhong) made in china and sieved by passing through a 60 mesh sieve (SS304 Grade). The powder was packaged in a low density polyethylene bag, sealed, stored in a made in china Haier Thermocool refrigerator (4℃) until required.

2.3 Production of Wheat Bread Fortified with Mushrooms

The preparation of the bread involves the replacement of part of the Wheat Flour with 0%, 5%, 10%, 15% and 20% Mushroom Powder (MP). The 0% MP bread and conventional bread obtained from the bread market served as control.

2.4 Baking Process

The blend formulations were baked using the method of [11]. The bread constituent were first mixed in a Kenwood Mixer (DSM5) for 5 minutes with baking formula of 56% wheat flour blend, 36% water, 3.4% sugar, 1.6% shortening, 1% skim milk powder, 1% salt and 1% yeast [12]. The dough was fermented in bowls, covered with wet clean muslin cloth for 55 minutes at room temperature (29℃), punched, scaled to 250 g dough pieces, proofed in a proofing cabinet (Doyon E236R 2401) for 90 min at 30℃, 85% relative humidity and baking was done at 250℃ for 30 min following the method of [13].

2.5 Moisture Content Determination

Clean Petri dishes were labelled, oven dried and weighed (W1). Two grams of each sample were weighed into respective dishes (W2) and were evenly spread and then transferred into desiccators immediately to prevent absorption of moisture from the atmosphere. The dishes containing the samples were transferred into the oven at 105^{o}C and dried for three hours. After three hours, they were cooled in the desiccators for 30 minutes and re-weighed. This process was continued until a constant (W3) was obtained. The percentage moisture content was then calculated [14]

% Moisture content = (loss in weight during drying / weight of sample taken) X 100

= ((W1-W3)/(W2-W1)) X 100

2.6 Ash Content

Clean dried crucibles were weighed (W1). About 1g of each sample was put into the clean, dried pre-weighed crucibles and re-weighed (W2). The crucibles were then heated in the muffle furnace (Gallenkamp) set at 550^{o}C for three hours. Heating was continued until a light grey or white ash was obtained. The crucibles were removed from the furnace, cooled in desiccators to room temperature and weighed (W3). Cooling and weighing were continued until a constant weight was obtained (AOAC, 2012).

% Ash = ((W3-W1)/(W2-W1)) X 100

2.7 Crude Fat Content

Filter paper was weighed (W1) and 1g of each sample was weighed into the filter paper, wrapped neatly with thread and weighed (W2). Round bottom flask was filled with petroleum ether (b.pt 40-600C) up to ¾ of the flask. Soxhlet extractor was fixed with a reflux condenser and the heat source was adjusted so that the solvent boils gently. The filter paper with the sample was inserted into soxhlet apparatus and extraction under reflux was carried out using petroleum ether (40 -60% boiling range) for 6 hours. At the end of the extraction, the filter paper and their contents were dried in the oven for one hour at 100℃ and later cooled in the desiccator and weighed again (W3) [14].

Crude Fat % (W/w) = ((weight loss by sample (Extracted fat))/Weight sample) X 100 ((W2-W3)/(W2-W1)) X 100

2.8 Protein Content

About 1 g of the sample was weighed into a 50 ml micro kjehldal bottle digestion flask and 15 ml of the concentrated H_2SO_4 was added into the flask and a tablet of selenium catalyst was also added. The mixture was heated at 105℃ in the block digester in a fume cupboard until a clear solution was formed. The flask was rotated at intervals until the digest was clear. The digest was allowed to cool after which the solution was diluted with distilled water to 50ml and 5ml of this was transferred into the distillation apparatus. 5 ml of 2% boric acids was pipetted into a 100 ml conical flask (the receiver flask) and 5 drops of mixed indicator (0.016g methyl red + 0.083 g bromocresol green in 100ml alcohol) was added. 1.5 ml of 4% NaOH was added to the digest in the reaction vessel via the funnel and ensuring it was alkaline by forming a cloudy solution. Steam from steam generator was passed into the reaction vessel with all outlets closed to prevent suck back. The distillation was carried out into the acid solution in the receiver flask with the delivery tube below the acid level. As distillation was going on, the pink color solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until 50ml

of distillate has been collected into receiving flask. The distillate was then titrated against 0.1M HCl to a pink end point. The total nitrogen content was calculated as:

% of Nitrogen = ((Titre value 0.1MHClx 0.014 x 100 x 50/5) / Original weight of sample)

% Crude protein = % Nitrogen x 6.25 (protein conversion factor)

2.9 Crude Fibre

One gram of the sample was put inside clean, dried, and well labeled 50 ml conical flask and weighed (W1). Two hundred milliliters of 1.25% H_2SO_4 was added to the sample in the conical flask and boiled for 30 minutes. The solutions were filtered (to remove fat and sugar) and rinsed well with hot distilled water. The residue was scrapped back into the conical flask with spatula and about 1.25% of NaOH (200 ml) solution was added in each samples and heated to boil for 30 minutes. The boiled sample was filtered through muslin cloth and the residue was washed thoroughly with hot distilled water and then rinsed once with 10% HCl, twice with industrial methylated spirit, then the residue of each sample was scrapped into a crucible, dried in the oven at 1050C, cooled in a dessicator and weighed (W2). The sample was then ashed in the muffled furnace (Gallenkamp) for 3 hours at 500℃. The samples were removed and cooled in the desiccators and weigh again (W3) [14].

% Crude fiber = ((W2-W3)/W1) X 100

2.10 Carbohydrate Content Determination

The Nitrogen-Free Extractive (N.F.E) referred to as soluble carbohydrate is not determined directly but obtained by difference.

% Carbohydrate = 100 – (% Ash + % Crude protein + % Crude fat + % Crude fibre + % moisture)

2.11 Determination of Mineral Elements

The mineral composition of each sample was determined by wet ashing method followed by spectrophometric reading of the level of mineral. Triplicate samples (1 g) of each sample were ashed in muffle furnace at 450℃ for 5-6 hours. The ashed samples and silica dishes were removed and transferred into the desiccators to cool after which the samples were dissolved with 1 ml of 0.5% HNO_3. Little distilled water was added and filtered into a clean small plastic bottle using number 43 Whattman filter. Distilled water was later used to dilute the solution up to 50 ml. Atomic absorption spectrophotometer (Buck 201, VGP) was used in determining the mineral content [14].

The mineral content was calculated using the formula below:

Mineral (mg/100 g) = (RxVxD/Wt)

When R = Solution concentration, V = Volume of sample digested, D = Dilution factor, and Wt = Weight of sample.

2.12 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and means were compared by Duncan's New Multiple Range test (SPSS 16.0 version). Differences were considered significant at $P<0.05$.

3. RESULTS

The proximate composition of the mushrooms (*Pleurotus ostreatus* and *Calocybe indica)* is presented in Fig. 1. Both mushrooms had same moisture content. *Pleurotus ostreatus* reviewed relatively higher protein levels of 26.12% compared to *Calocybe indica* which has 25.3% protein content. *Pleurotus ostreatus* was observed to contain a higher fiber content of 37.23% while *Calocybe indica* had 14.82%. *Calocybe indica* had relatively high carbohydrate content of 46.5%, while that of *Pleurotus ostreatus* had 38%. The mineral composition of *Pleurotus ostreatus* and *Calocybe indica* is shown in Table 1. *Calocybe indica* reviews relatively higher mineral composition than *Pleurotus ostreatus.*

Table 1. Mineral composition of *Pleurotus ostreatus* and *Calocybe indica* (mg/g)

Parameter	Sample A	Sample B
Sodium	102.97±0.05^{a}	96.02±0.01^{a}
Potassium	78.23±0.06^{b}	80.20±0.02^{ab}
Calcium	32.00±0.03^{c}	40.00±0.03^{b}
Magnesium	78.00±0.01^{b}	99.60±0.02^{a}
Iron	2.21±0.03^{d}	2.32±0.03^{c}
Manganese	0.20±0.04^{e}	0.22±0.01^{d}
Copper	0.20±0.02^{e}	0.26±0.01^{d}
Zinc	0.20±0.02^{e}	0.22±0.02^{d}

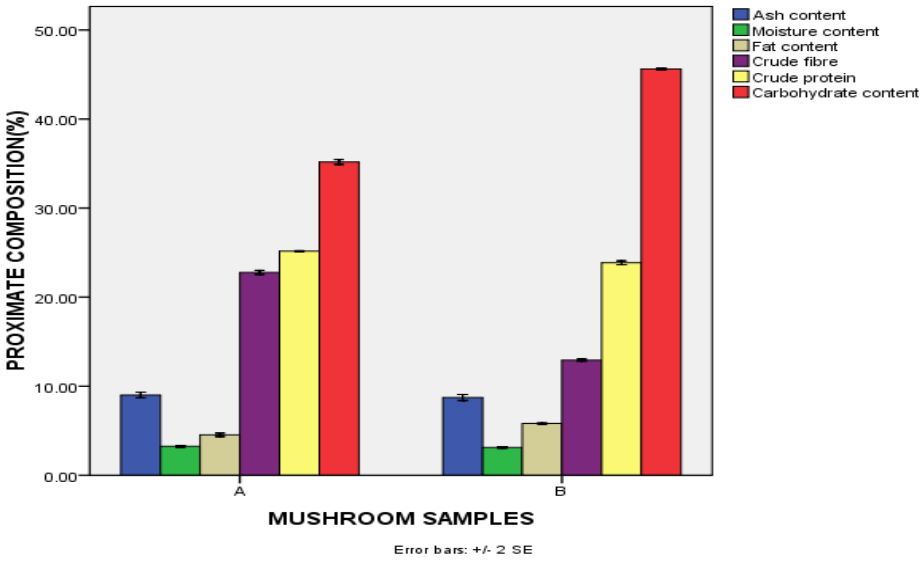

Fig. 1. Proximate composition of *Pleurotus ostreatus and Calocybe indica*
Key: A= Pleurotus ostreatus, B= Calycybe indica

The proximate composition of bread fortified with *Pleutotus ostreatus* and *Calocybe indica* on the day of production, is presented in Tables 2 and 3. A general decrease in the carbohydrate content was observed as the concentration of the mushroom was increased. Sample F (containing 20% *Pleutotus ostreatus*) and sample J (containing 20% *Calocybe indica*) having the lowest of 29.23% and 31.23% respectively. The protein content increased with increase in the mushroom supplementation, bread with 20% supplementation of *Pleurotus ostreatus* and 20% *Calocybe indica* both contains the highest protein contents of 28.7% and 29.8% respectively. Similar result was observed after storage for five days (Tables 4 and 5).

Table 2. Proximate composition of bread fortified with *Pleutotus ostreatus* on the day of production

S/N	Protein	Carbohydrate	Crude fibre	Ash	Moisture content	Fat
A	9.1^{a}	52.7^{e}	1.2^{a}	1.8^{a}	31.1^{c}	6.1^{a}
B	17.3^{b}	38.4^{d}	2.2^{b}	2.4^{a}	32.9^{d}	6.5^{a}
C	18.5^{c}	37.4^{cd}	1.9^{b}	3.8^{b}	29.6^{b}	8.6^{b}
D	19.6^{c}	36.9^{c}	2.3^{b}	3.9^{b}	29.0^{bc}	8.1^{b}
E	22.3^{d}	33.5^{b}	3.1^{c}	4.2^{b}	28.1^{a}	8.5^{b}
F	25.6^{e}	27.9^{a}	4.2^{d}	4.5^{b}	28.1^{a}	8.9^{b}

Key: A=Positive control, B= Negative control, C=5% Pleurotus ostraetus, D=10% Pleurotus ostraetus, E= 15% Pleurotus ostraetus, F= 20% Pleurotus ostraetus

Table 3. Proximate composition of bread fortified with *Calocybe indica* on the day of production

S/N	Protein	Carbohydrate	Crude fibre	Ash	Moisture content	Fat
A	9.1^{a}	52.7^{e}	1.2^{a}	1.8^{a}	31.1^{e}	6.1^{a}
B	17.3^{b}	38.4^{d}	2.2^{a}	2.4^{b}	32.9^{d}	6.5^{a}
G	22.7^{c}	35.2^{c}	3.4^{ab}	3.6^{b}	28.0^{b}	6.7^{a}
H	24.2^{d}	32.7^{b}	3.9^{b}	4.3^{d}	27.3^{b}	6.7^{a}
I	25.6^{e}	31.1^{a}	4.5^{bc}	4.9^{e}	27.0^{b}	6.8^{a}
J	27.0^{f}	30.8^{a}	5.3^{c}	5.8^{f}	24.0^{a}	6.9^{a}

Key: A=Positive control, B= Negative control, G=5% Calocybe indica, H=10% Calocybe indica, I= 15% Calocybe indica, J= 20% Calocybe indica

Table 4. Proximate composition of bread fortified with *Pleutotus ostreatus* after storage

S/N	Protein	Carbohydrate	Crude fibre	Ash	Moisture content	Fat
1	9.1^{a}	52.4^{e}	0.5^{a}	1.7^{a}	31.8^{b}	4.5^{a}
2	17.0^{b}	38.7^{d}	2.1^{bc}	2.5^{a}	33.0^{c}	6.4^{b}
3	18.5^{c}	37.1^{c}	1.8^{b}	3.8^{b}	29.5^{a}	8.5^{c}
4	19.4^{c}	37.4^{c}	2.9^{cd}	3.9^{b}	28.7^{a}	8.1^{c}
5	22.1^{d}	33.2^{b}	3.2^{d}	4.3^{b}	28.4^{a}	8.5^{c}
6	25.0^{e}	27.8^{a}	4.3^{d}	4.4^{b}	29.0^{g}	8.9^{c}

Key: A=Positive control, B= negative control, C=5% Pleurotus ostraetus, D=10% Pleurotus ostraetus, E= 15% Pleurotus ostraetus, F= 20% Pleurotus ostraetus

Table 5. Proximate composition of bread fortified with *Calocybe indica* after storage

S/N	Protein	Carbohydrate	Crude fibre	Ash	Moisture content	Fat
1	9.1^{a}	52.4^{e}	0.5^{a}	1.7^{a}	31.8^{d}	4.5^{a}
2	17.0^{b}	38.7^{d}	2.1^{b}	2.5^{a}	33.0^{e}	6.4^{a}
3	22.6^{c}	34.0^{c}	3.3^{bc}	3.6^{b}	29.7^{c}	6.8^{a}
4	24.7^{d}	32.3^{b}	3.9^{c}	4.5^{c}	27.7^{b}	4.8^{a}
5	25.7^{d}	30.3^{a}	4.2^{cd}	4.9^{cd}	25.1^{a}	6.7^{a}
6	27.0^{e}	29.9^{a}	5.3^{d}	5.7^{d}	25.0^{a}	6.9^{a}

Key: A=Positive control, B= negative control, G=5% Calocybe indica, H=10% Calocybe indica, I= 15% Calocybe indica, J= 20% Calocybe indica

The mineral composition of bread fortified with *Pleurotus ostreatus* and *Calocybe indica* on the day of production is shown in Tables 6 and 7. The sodium content of the bread was observed to increase with increase in the concentration of the mushroom powder added, with sample F and sample J having the highest of 60.80 mg/g and 72.60 mg/g respectively this was equally the case with the potassium calcium and magnesium content, giving sample J (20% *Calocybe indica*) the highest values of 89.20 mg/g, 32.00 mg/g and 42.04 mg/g respectively. There was a negligible increase in the iron and manganese content of the bread samples. However, zinc content in the bread was observed to decrease as the concentration of mushroom supplement was increased. Similar result was obtained after five days of storage (Tables 8 and 9).

4. DISCUSSION

The proximate composition of the mushrooms (Fig. 1) shows *Calocybe indica* has relatively higher carbohydrate content than Pleurotus *ostreatus*. *Pleurotus ostreatus* however contains a higher percentage of protein (28%), while *Calocybe indica* had a protein level of 24%. This suggest that *Pleurotus ostreatus* as a better option for the protein enhancement of bread. This was however lower than 36% protein content of *Pleurotus ostreatus* reported by [7]. *Pleurotus ostreatus* had significantly higher fiber content of 23% than *Calocybe indica* which had 14% fiber. These results were similar to those reported by [15] and [16].

Table 6. Mineral composition of bread fortified with *Pleurotus ostreatus* on the day of production (mm/g)

Parameter	A	B	C	D	E	F
Sodium	33.50 ± 0.08^{a}	34.49 ± 0.02^{a}	48.00 ± 0.08^{b}	50.11 ± 0.05^{c}	51.30 ± 0.01^{d}	60.80 ± 0.03^{e}
Potassium	66.50 ± 0.04^{bc}	58.40 ± 0.02^{a}	61.56 ± 0.01^{ab}	68.40 ± 0.09^{bc}	70.10 ± 0.01^{c}	70.30 ± 0.09^{c}
Calcium	12.00 ± 0.05^{b}	10.00 ± 0.06^{a}	16.00 ± 0.09^{c}	18.07 ± 0.04^{d}	20.00 ± 0.02^{e}	24.05 ± 0.06^{f}
Magnesium	16.80 ± 0.09^{a}	26.80 ± 0.01^{b}	28.80 ± 0.01^{c}	36.04 ± 0.04^{d}	36.04 ± 0.04^{d}	38.88 ± 0.07^{e}
Iron	2.46 ± 0.02^{bc}	1.84 ± 0.09^{ab}	2.00 ± 0.02^{ab}	2.08 ± 0.03^{ab}	1.34 ± 0.05^{a}	3.04 ± 0.03^{c}
Manganese	0.48 ± 0.08^{a}	0.23 ± 0.05^{a}	0.43 ± 0.04^{a}	0.58 ± 0.03^{a}	0.42 ± 0.07^{a}	0.35 ± 0.03^{a}
Copper	0.12 ± 0.01^{a}	0.18 ± 0.03^{a}	0.19 ± 0.04^{a}	0.22 ± 0.01^{a}	0.17 ± 0.01^{a}	0.19 ± 0.01^{a}
Zinc	0.95 ± 0.03^{a}	0.86 ± 0.06^{a}	0.93 ± 0.04^{a}	1.04 ± 0.01^{a}	0.74 ± 0.01^{a}	0.74 ± 0.01^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: A=Positive control, B= negative control, C=5% Pleurotus ostraetus, D=10% Pleurotus ostraetus, E= 15% Pleurotus ostraetus, F= 20% Pleurotus ostraetus

Table 7. Mineral composition of bread fortified with *Calocybe indica* on the day of production (mm/g)

Parameter	A	B	G	H	I	J
Sodium	33.50±0.08^{a}	33.49±0.02^{a}	57.00±0.08^{b}	67.50±0.01^{c}	66.50±0.01^{c}	72.60±0.01^{d}
Potassium	66.50±0.04^{b}	58.40±0.02^{a}	70.5±0.03^{c}	72.50±0.01^{d}	85.3±0.01^{c}	89.20±0.09^{f}
Calcium	12.00±0.05^{b}	10.00±0.06^{a}	14.00±0.06^{c}	19.20±0.07^{d}	20.02±0.02^{d}	32.00±0.06^{e}
Magnesium	16.80±0.09^{a}	26.80±0.01^{b}	32.40±0.01^{c}	31.20±0.04^{d}	38.41±0.07^{e}	42.04±0.03^{f}
Iron	2.46±0.02^{a}	1.84±0.09^{a}	1.96±0.02^{a}	2.12±0.00^{a}	2.06±0.05^{a}	2.46±0.03^{a}
Manganese	0.48±0.08^{a}	0.23±0.05^{a}	0.41±0.02^{a}	0.51±0.01^{a}	0.39±0.01^{a}	0.64±0.05^{a}
Copper	0.12±0.01^{a}	0.18±0.03^{a}	0.35±0.09^{a}	0.68±0.21^{b}	0.35±0.12^{a}	0.21±0.03^{a}
Zinc	0.95±0.03^{a}	0.86±0.06^{a}	0.80±0.01^{a}	0.70±0.03^{a}	0.68±0.04^{a}	0.88±0.07^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: A=Positive control, B= negative control, G=5% Calocybe indica, H=10% Calocybe indica, I= 15% Calocybe indica, J= 20% Calocybe indica

Table 8. Mineral composition of bread fortified with *Pleurotus ostreatus* after storage (mm/g)

Parameter	A	B	C	D	E	F
Sodium	33.45±0.03^{a}	33.40±0.02^{b}	48.00±0.03^{c}	52.11±0.04^{d}	50.30±0.03^{e}	60.30±0.07^{f}
Potassium	66.40±0.08^{b}	56.45±0.01^{a}	66.45±0.04^{b}	68.41±0.08^{c}	68.09±0.03^{c}	70.08±0.01^{d}
Calcium	11.82±0.01^{b}	9.08±0.05^{a}	15.31±0.05^{c}	20.00±0.07^{d}	24.05±0.04^{f}	13.45±0.02^{c}
Magnesium	16.53±0.01^{a}	26.80±0.04^{b}	28.50±0.01^{b}	36.00±0.03^{d}	31.45±0.04^{c}	38.55±0.02^{e}
Iron	2.39±0.01^{a}	1.37±0.09^{a}	1.81±0.03^{a}	2.00±0.03^{a}	1.30±0.04^{a}	2.78±0.02^{a}
Manganese	0.44±0.01^{a}	0.20±0.01^{a}	0.40±0.08^{a}	0.48±0.01^{a}	0.78±0.05^{a}	0.50±0.02^{a}
Copper	0.08±0.01^{a}	0.12±0.02^{a}	0.18±0.01^{a}	0.22±0.04^{b}	0.60±0.04^{b}	0.26±0.04^{b}
Zinc	1.65±0.04^{b}	0.75±0.04^{a}	0.75±0.03^{a}	1.07±0.04^{a}	0.65±0.07^{a}	0.90±0.04^{ab}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: A=Positive control, B= negative control, C=5% Pleurotus ostraetus, D=10% Pleurotus ostraetus, E= 15% Pleurotus ostraetus, F= 20% Pleurotus ostraetus

Table 9. Mineral composition of bread fortified with *Calocybe indica* after storage (mm/g)

Parameter	A	B	G	H	I	J
Sodium	32.45±0.03^{a}	33.40±0.02^{b}	56.55±0.03^{c}	65.41±0.04^{d}	65.92±0.03^{e}	72.40±0.01^{f}
Potassium	66.40±0.08^{b}	56.45±0.01^{a}	70.44±0.04^{c}	71.89±0.08^{d}	85.30±0.03^{e}	88.24±0.01^{f}
Calcium	11.82±0.01^{a}	9.08±0.05^{a}	13.49±0.05^{a}	18.20±0.07^{a}	19.99±0.04^{a}	18.71±0.02^{a}
Magnesium	16.53±0.01^{a}	26.80±0.04^{b}	32.05±0.07^{c}	31.20±0.06^{c}	38.40±0.04^{d}	41.57±0.02^{e}
Iron	2.39±0.01^{a}	1.37±0.09^{a}	1.90±0.03^{a}	2.05±0.05^{a}	2.00±0.09^{a}	2.40±0.02^{a}
Manganese	0.44±0.01^{a}	0.20±0.01^{a}	0.42±0.03^{a}	0.52±0.05^{a}	0.75±0.04^{a}	0.50±0.02^{a}
Copper	0.08±0.01^{a}	0.12±0.02^{a}	0.88±0.05^{a}	0.56±0.03^{a}	0.66±0.04^{a}	0.28±0.02^{a}
Zinc	1.65±0.04^{b}	0.75±0.04^{a}	0.80±0.03^{b}	0.82±0.04^{ab}	0.68±0.01^{ab}	0.75±0.06^{ab}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

Key: A=Positive control, B= negative control, G=5% Calocybe indica, H=10% Calocybe indica, I= 15% Calocybe indica, J= 20% Calocybe indica

The proximate composition of bread fortified with *Pleurotus ostreatus* and *Calocybe indica* after production (Tables 2 and 3) showed a general decrease in the carbohydrate content as the volume of mushroom was increased. Bread containing 20% of the mushrooms had the lowest carbohydrate content and the highest protein contents. This is in agreement with a previous report of [7], who recorded similar decrease in the carbohydrate content and attributed it to the decrease in the concentration of wheat replaced with the mushroom powder.

The resulting proximate composition of the bread samples after five days of storage (Tables 4 and 5) reviewed a relatively similar results to the proximate on the day of production. However, there was a slight increase in the protein content and a subsequent decrease in the carbohydrate content of the fortified bread were observed at day five of storage. This was equally observed by [15] who attributed this to the activity of the microorganism present in the bread during the period of storage.

The mineral composition of the fortified bread after production (Tables 6 and 7) shows an increase in the sodium content with increase in mushroom concentration, similar increase was observed in the potassium content. This can be attributed to the initial content of the mushrooms. Calcium and magnesium content of the bread was observed to increase as the mushroom content increased, these findings were supported by the report of [15], who reported similar increase in the sodium, potassium, calcium and magnesium content of bread fortified with *Pleurotus ostraetus*. However it can be deduced that fortification of bread with 20% mushroom powder was most effective in boosting the nutrient content of the bread. These minerals are beneficial to health and mushroom fortification could be useful in ameliorating the deficiency diseases of this nutrients, this has also been suggested by [7].

There was a similar trend in the mineral composition of the mushroom fortified breads on the day of production and also after storage (Tables 8 and 9), however, there is a slight decrease in some mineral contents. Bread sample containing 20% *Calocybe indica* decreased from 32.00 mg/g of calcium on the day of production to 18.24 mg/g after storage. This could be as a result of the utilization of some of these minerals by the microorganisms present in the bread.

5. CONCLUSION

This investigation shows that there was significant improvement in the bread protein content and nutritional quality on addition of mushroom powder supplementation. This was evident in the significant increase of over a 100% in the crude protein content of fortified bread sample. Also over 50% increase in the ash and crude fibre content was achieved. Mushroom could be used to improve the nutritional quality of bread which could help in reduction of protein-energy malnutrition prevalent in Nigeria and other developing Countries. Further studies on the amino acid in the fortified bread are the next research focus.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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