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Evaluation of the Microbial, Proximate, and Sensory Properties of Biscuits Fortified with *Pleurotus ostreatus* at Various Inclusion Levels

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Abstract:- To satisfy consumer desires for more health advantages, food products must be fortified. The sensory, proximate, and microbiological characteristics of biscuits enriched with Pleutotus ostreatus at different inclusion levels were assessed. The manufacturing of wheat flour, baking of mushroom powder biscuits (MPB), microbiological examination of MPB, proximate analysis, and sensory evaluation of MPB were all done using standard procedures. The fortified mushroom biscuit had varying mushroom compositions, ranging from 50-100% (sample A-F). The results showed that the composition with 90% mushrooms had the highest bacteria count (62 CFU/ml) while 50% had 1 CFU/ml. Various microorganisms were tentatively identified from MPB, including Staphylococcus warnei, Bacillus niacin, Kluyuera georgiana, B. circulans, B. licheniforms, B. megaterium, B. barbaricue, B. cereus, B. simplex, Aspergillus flavus, A. niger, and P. ostreatus. According to the study, the nutritious value of wheat cookies supplemented with mushrooms rose as mushroom powder concentration rose. The value of the carbohydrates was the highest at 73%, while the value of the ash content was the lowest at 2%. Sample D, which contained 50% each of flour and mushrooms, had the highest crude fibre level. Higher mushroom powder concentrations reduced the fortified biscuit's overall acceptability (OA). Sample E (10% mushroom) had the highest OA, scoring 9.30 0.21 for both texture and flavour. Based on the findings, it is recommended that the commercial production of biscuits be carried out at a 50% level of mushroom inclusion for the best nutritional, essential mineral and sensory efficacy.

Keywords:- *Mushroom, Biscuit, Proximate, Sensory, Nutritional, Mineral.*

I. INTRODUCTION

The most popular sort of snack food among bakery goods, biscuits are created from simple, affordable, and easily accessible raw materials. Because of their flavour and lengthy shelf life due to their low water activity, they are frequently consumed (Caleja et al., 2017). They are among the most well-known bakery goods in Nigeria (Bello et al., 2017). They are healthy snacks produced from dough that is not particularly appetising but is transformed into a pleasant treat by baking it (Kure *et al*., 1998). The main components of biscuit dough are water, sugar, oil, and soft wheat flour. To create a dough with a strong gluten network, they are combined with a few other ingredients (including baking powder, skim milk, emulsifier, and sodium metabisulphite) (Blanco et al., 2016). The need to fortify food products like biscuits has increased due to the increased global demand for more functional agricultural and innovative food products, especially as biscuits are one of the most popular snack meals (Liyanage and Hettiarachchi, 2011).

In order to meet consumer requests for additional advantages, food fortification plays a significant role in boosting the amount of functional components that promote health in bakery products (Wieca et al., 2017; Adeboboye et al., 2020). When plant-based, macrofungal, and other protein supplies are used with wheat flour, the protein concentration of baked foods is greatly boosted (Ugwuona and Obeta, 2016). Some macrofungi, such as mushrooms, include high-quality digestible protein (10–40%), carbohydrates (3-21%), and dietary fibre (3–35%), depending on the species (Mallavadhani *et al*., 2006). Because they are rich in proteins, vitamins, minerals, chitin, and vital amino acids and contain few calories and fat, mushrooms are consumed as a source of nutrition around the world. In addition to protein and dietary fibre, mushrooms also contain vitamins, minerals like potassium, phosphorus, and iron, as well as carbs and carbohydrates (Valverde et al., 2015). Additionally, mushrooms contain vitamins including vitamin C and the B-vitamin family (thiamin, riboflavin, biotin, niacin, pyridoxine, and panthotenic acid), as well as vital amino acids like lysine (Adedayo, 2011; Okafor *et al*., 2012).

Pleurotus ostreatus is a tasty mushroom with a low fat and carbohydrate content, a variety of proteins, and minerals (Ca, P, Fe, Mg), making it a superior nutritional meal (Silva et al., 2002). After *Agaricus bisporus*. *P. ostreatus* is the second most popular edible mushroom for cultivation and has a substantial economic impact (Sanches, 2010). It has been demonstrated that some grown mushrooms produce less and grow more slowly than this species. *P. ostreatus* has witnessed a large increase in international cultivation in recent decades because of its notable tolerance of a range of agro-climatic conditions. (Sanches, 2010; Kholoud *et al*., 2014). *Pleurotus ostreatus* has a content of nutrients that is

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readily digestible proteins, mineral salts, vitamins, and compounds with potent pharmacological activities, e.g., lovastatin and pleuran, *P. ostreatus* is an important mushroom species of dietary and medical significance (Anandhi *et al*., 2013; Muszyńska *et al*., 2014; Caz *et al*., 2015).

Biscuits are often produced using wheat flour and fat, which might be unhealthy if consumed frequently, especially in excess, according to Caleja et al. (2017). A class of microbial agents used to fortify food and food products is represented by mushrooms. They have thus been employed as good fortificants because they have been shown to have high levels of protein, dietary fibres, vitamins, minerals (such as potassium, phosphorus, and iron), carbs, chitin, and vital amino acids, as well as low levels of fat and calories (Valverde *et al.*, 2015). The purpose of the study is to isolate and identify microorganism from biscuit fortified with mushroom *(P. ostreatus)*, fortify biscuit with mushroom *(P. ostreatus)* at various levels of inclusion, and assess the proximate and sensory composition of the fortified biscuit. Therefore, the purpose of this study is to assess the microbiological, proximate, and sensory qualities of biscuits enhanced with mushroom *(P. ostreatus)* at different levels of inclusion.

II. METHODS

A. Cultivation of Mushroom

➢ *Tissue Culturing*

PDA was produced in accordance with the manufacturer's instructions. A 500 ml conical flask was filled with precisely 5g of potato dextrose agar, 100ml of water, and the conical was gently shaken to help the agar dissolve. A combination of sterile cotton wool and aluminium foil were used to cap the conical flask. The agar was sterilized at 121°C for 60 minutes and allowed to cool to 45°C pour plating. Pour plating was done aseptically into Petri dishes. The agar was allowed to completely fill the bottom of the Petri dish, immediately covered and kept aside. After solidification, tissue of each species of *Pleurotus* was aseptically cultured into the different Petri dishes. Briefly, the basidiocarp of the fruiting body of a *Pleurotus* species was sanitized using absolute ethanol, longitudinally cut into two halves and tissue bits from collar regions was picked using forceps and transferred to pre sterilized Potato Dextrose Agar (PDA). The Petri plates were kept in an incubator for a week at a temperature of 25°C plus 2°C. To create pure cultures, mycelium from developing edges was carefully transferred to PDA slants and cultured for two to three weeks (Hsu *et al*., 2018).

➢ *Preparation of Grain Spawn*

The millet grains for spawning were thoroughly washed and soaked for 24hours in water, and then sieved. After this, the grains were filled halfway (to create air space for welling and shaking) into heat resistant. The autoclave was used to sterilise the bottles for 15 minutes at 121°C. After chilling, the grains in the bottles were inoculated with 6 cm mycelial discs of the desired Pleutorus species and cultured for roughly 2 weeks at 27 °C. The bottles were shook three days apart during the incubation period to prevent tissue clumping and to promote even and quick colonisation of the grains by the mycelium (Adeokun *et al*., 2012).

B. Preparation of Mushroom Powder

The procedure outlined by Okeke *et al.* (2003) was used in the laboratory to create fresh *P. ostreatus* mushrooms from fresh mushrooms. They were collected, cleaned, and then sliced into around 3 mm-thick slices that were subsequently dried for eight hours at 60°C. The dried mushroom sample was separately processed in an electric grinder and sieved through an 80-mesh screen to produce fine powders. The resulting powder was refrigerated, hygienically wrapped, and stored in an airtight container for later use.

➢ *Production of Wheat Flour and Mushroom Powder Biscuit*

Mushroom Powder (MP) was substituted for varied percentages of the wheat flour in biscuits, including 10%, 20%, 30%, 40%, and 50%. The market-purchased 0% MP biscuit was utilised as the control according to the procedure of (Bello *et al*., 2017),

➢ *Baking Process*

The blended formulations were applied to the baking process using Chauhan *et al.* (2012)'s methodology with a few minor modifications. In the beginning, a Kenwood mixer (model HM 430) was used to whip the oil and aspartame till frothy. Skimmed milk and egg white were added while mixing for around 40 minutes. The right amount of flour, baking powder, salt, nutmeg, and vanilla flavour were gradually added to the batter. Then, after completely combining with water, consistent dough was made. The acquired dough was kneaded on a smooth, clean surface for about 5 minutes, thinly flattened with a rolling pin to a uniform thickness of 5 mm on a wooden board, and then cut out into the appropriate shapes and sizes. The dough pieces were cut out, placed on a prepared baking sheet, and baked for 15 minutes at 160°C. The biscuits were maintained at 4°C until needed for sensory evaluation and other studies after being properly cooled and packaged in airtight polythene. As a control, samples of biscuits made using white wheat flour were used.

C. Microbiological Analysis

➢ *Culture Media and Reagents*

The media used for the isolation and enumeration of the microorganisms associated with the fortified bread are nutrient agar (NA), which is used for the isolation and counting of the total viable mesophilic bacterial count, and potato dextrose agar (PDA), which is used for the isolation of fungi. According to the manufacturer's instructions, each medium was sterilised and prepared. The method was used to manufacture the reagents for the chemical analysis and biochemical characterization tests in accordance with the specifications of each analysis Isong *et al.* (2013).

➢ *Microbiological Examination and Determination of Total Viable Count*

Following baking, the biscuits were stored at 21°C and 50% relative humidity for five days while the physical changes were noted and the microbiological count was repeated. This procedure was based on the methodology of Isong *et al* (2013). 1g of each sample was taken aseptically and blended for 2 minutes in 9ml of sterile, distilled water. Serial dilutions (1ml of each dilution) were used to plate fungi and bacteria on potato dextrose agar and plate count agar, respectively, in sterile petri dishes. Bacteria were incubated for 24 hours at 37 oC and fungi for 8 hours at 20 oC. Using a colony counter (Gallenkamp), visible colonies were counted and represented as log Cfu/g of the biscuit sample.

➢ *Identification of Bacteria*

On fresh nutrient agar, representative bacterial colonies were chosen and sub-cultured until pure cultures were obtained. For further research, the colonies were afterwards kept on nutrient agar slants at a temperature of 5 oC in the refrigerator. Biochemical tests, morphological traits, and colony characteristics were used to identify the bacteria. According to Oyetayo and Oyedeji (2018), morphological characteristics were observed for each bacterial colony after 24 hours of growth under the microscope while biochemical characterizations were carried out. The appearance of the colony of each isolate on the agar media was studied, and the characteristics observed include: shape, elevation, edge, optical characteristics, consistency colony surface, and pigmentation.

➢ *Identification of Fungi*

On Potato Dextrose Agar (PDA), the microbial colonies were subcultured. The isolates were recognised based on their microscopic and morphological characteristics. A clean glass slide was coated with two drops of cotton-blue-in-lactophenol, and a little piece of mycelium free of medium was extracted and applied to the stain using a sterile inoculating needle. A clean cover slip was carefully applied over the mycelium after it had been delicately removed with a needle. This was fixed on the microscope, and a 100x oil immersion objective lens was used to study it.

D. Proximate Analysis

Routine analysis of food and food product is termed the proximate. The carbohydrate determination is given differences in which the values of crude protein, moisture, fat, ash and crude fibre is subtracted from 100. The analyses were carried out on the fortified and unfortified biscuit.

➢ *Moisture Content Determination*

The Petri dishes were cleaned, properly labelled, dried in the oven, and weighed (W1). To avoid absorbing moisture from the air, two gram of each sample were weighed onto the corresponding plates (W2), spread out uniformly, and then immediately put into desiccators. The dishes containing the samples were placed in an oven set to 105 oC for three hours of drying. They were then reweighed after cooling for 30 minutes in the desiccators. This procedure was carried out repeatedly until the weight (W3) became constant. Next, the percentage moisture content was determined (AOAC, 2012).

$$\% \text{ Moisture content} = \frac{\text{Loss in weight during drying}}{\text{Weight of sample taken}} \times 100$$

$$= \frac{W_1 - W_3}{W_2 - W_1} \times 100$$

➢ *Total Ash Determination*

Pristine, dried, crucibles were weighed (W1). The clean, dried, pre-weighed crucibles were filled with about 1g of each sample, and they were then reweighed (W2). The crucibles were then heated in the muffle furnace (Gallenkamp) for three hours at 550 degrees Celsius. Heating was continued until a light grey or white ash was formed. The crucibles were taken out of the furnace, brought to room temperature in desiccators, and weighed (W3). After reaching a steady weight, cooling and weighing were both continued (AOAC, 2012). The ash content was calculated with the formula below;

$$\% \text{ Ash} = \frac{W_1 - W_3}{W_2 - W_1} \times 100$$

➢ *Crude Fat Determination*

1g of each sample was weighed into a filter paper, which was then weighed (W1) and weighed (W2) after being neatly wrapped in thread. Petroleum ether (b.pt 40-60oC) was poured into a round bottom flask until it reached the third-quarter mark. Reflux condenser was fixed to the Soxhlet extractor, and the heat source was changed such that the solvent slowly boils. Petroleum ether (40–60% boiling range) was used for the 6-hour extraction process under reflux after the filter paper containing the sample was placed inside the soxhlet device. After the extraction was complete, the filter paper and its contents were dried for an hour at 100°C in an oven, then chilled in a desiccator and weighed again (W3) (AOAC, 2012).

$$\text{Crude Fat \% (W/w)} = \frac{\text{Loss in weight by sample (extracted fat)}}{\text{Weight of sample}} \times 100$$

$$= \frac{W_1 - W_3}{W_2 - W_1} \times 100$$

➢ *Crude Protein Determination*

About 1g of sample was weighed into 50 ml micro Kjehldal digestion flask and 15 ml of concentrated H_2SO_4 was added into the flask and a tablet of selenium catalyst was also added. In the block digester of a fume cupboard, the mixture was heated to 105 oC until a clear solution was produced. To ensure that the digest was clear, the flask was periodically spun. After allowing the digest to cool, the solution was diluted to 50 ml with distilled water, and 5 ml of this was added to the distillation apparatus. A 100 ml conical flask (the receiver flask) was filled with 5 ml of 2% boric acid before 5 drops of mixed indicator (0.016 g methyl red + 0.083 g bromocresol green in 100 ml alcohol) were added. The reaction vessel's digest was added 1.5 ml of 4%

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NaOH through a funnel to make sure it was alkaline and create a murky solution. All of the exits were closed to prevent suckback as steam from the steam generator was delivered into the reaction vessel. With the delivery tube below the acid level in the receiver flask, the distillation was conducted into the acid solution. The receiver flask's pink solution changed to blue during distillation, indicating the presence of ammonia. Up until 50 cc of distillate were collected into a receiving flask, the distillation process was continued. The distillate was then titrated to a pink end point against 0.1M HCl. The total nitrogen content was calculated as:

$$\% \text{ of Nitrogen} = \frac{\text{Titre value} \times 0.1\text{M HCl} \times 0.014 \times 100 \times 50/5}{\text{Original weight of sample}}$$

$$\% \text{ Crude protein} = \% \text{ Nitrogen} \times 6.25 \text{ (protein conversion factor)}$$

➢ *Crude Fibre Determination*

A 50ml conical flask that was clean, dry, and well labelled was filled with one gram of the sample and weighed (W1). The sample in the conical flask received 200 millilitres of 1.25% H2SO4 and cooked for 30 minutes. The solutions were thoroughly washed with hot distilled water after filtering (to remove fat and sugar). With a spatula, the residue was scraped back into the conical flask. Each sample then received 200 ml of a 1.25% NaOH solution, which was brought to boil for 30 minutes. The thoroughly cleaned residue from each sample was scraped into a crucible, dried in an oven at 105°C, cooled in a desiccator, and weighed (W2). The boiled sample was then filtered through muslin cloth, rinsed twice with industrial methylated spirit and once more with 10% HCl. The sample was subsequently heated to 500°C for three hours in the muffled furnace (Gallenkamp). The samples were taken out of the desiccators, chilled, and weighed once more (W3). (AOAC, 2012)

$$\% \text{ Crude fibre} = \frac{W_1 - W_3}{W_1} \times 100$$

➢ *Carbohydrate Content Determination*

The Nitrogen-free Extractive (N.F.E.), also known as soluble carbohydrate, is produced using difference rather than being determined directly.

100 - (% Ash + % Crude Protein + % Crude Fat + % Crude Fibre + % Moisture) = % Carbohydrate

E. Determination of Mineral Elements

Wet aching was used to determine the mineral content (potassium, sodium, calcium, magnesium, zinc, iron, and copper) of each sample, and the mineral content was then measured using a spectroscope. One gramme of each sample, in triplicate, was heated to 450 degrees Celsius in a muffle furnace for 5 to 6 hours. The samples were removed from the ash and silica dishes and put into the desiccators to cool before being dissolved in 1ml of 0.5% HNO3. A little amount of distilled water was combined with number 43 Whatman filter paper before being filtered into a clean, compact plastic bottle. Atomic adsorption spectrophotometer (Buck 201, VGP) was used in determining the mineral content (AOAC, 2012). The mineral content was calculated using the formula below:

$$\text{Mineral (mg/100g)} = \frac{R \times V \times D}{W_t}$$

Where R = Solution concentration,

V = Volume of sample digested,

D = Dilution factor,

W_t = Weight of sample

F. Sensory Evaluation of Biscuit

The biscuit products were subjected to organoleptic analysis using the method described by Oyetayo and Oyedeji, (2018). A total of 10 untrained panelists consisting 5 males and 5 females, within the age range of 19 – 29 were drawn from students of Federal University of Technology, Akure who familiar with biscuit, participated in the evaluation.

The samples were divided into equal portions and coded before being presented to the panellists at room temperature (28±2 °C) under lit fluorescent fixtures. The panellist consumed the biscuit while rating each sample on a 9-point Hedonic scale (Larmond, 1977), with 1 denoting a strong dislike and 9 denoting a strong liking. The qualities that are assessed include acceptability overall, colour, texture, taste, and odour.

G. Statistical Analysis

All experiments were carried out in triplicate. One-way analysis of variance (ANOVA) was used to analyse the data, and Duncan's New Multiple Range test (SPSS 16.0 version) was used to compare the means. At a p-value of 0.05, differences were deemed significant.

III. RESULTS

A. Microbiological Examination and Determination of Total Viable Count

The result of the microbiological examination of total viable bacteria count of mushroom biscuit composition is shown in Figure 1. The composition with 90% mushroom had the highest bacteria count followed by the composition with 60% mushroom (60/40). The least viable count was recorded in the 50/50 and 100% composition respectively.

B. Biochemical Identification of Bacteria

The result for the biochemical characteristics of bacterial isolates is shown in Table 1. The organisms tentatively identified after biochemical test includes; *Staphylococcus warnei, Bacillus niacin, Kluyuera georgiana, Bacillus circulans, Bacillus licheniformis, Bacillus megaterium, Bacillus barbaricue, Bacillus cereus,* and *Bacillus simplex*. The most prominent of the organisms isolated was *Bacillus* sp.

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C. Macroscopic Identification of Fungi

Table 2 shows the macroscopic identification of fungi and the fungal genera isolated were identified as *Aspergillus flavus*, *A. niger*, and *Pleurotus ostreatus.*

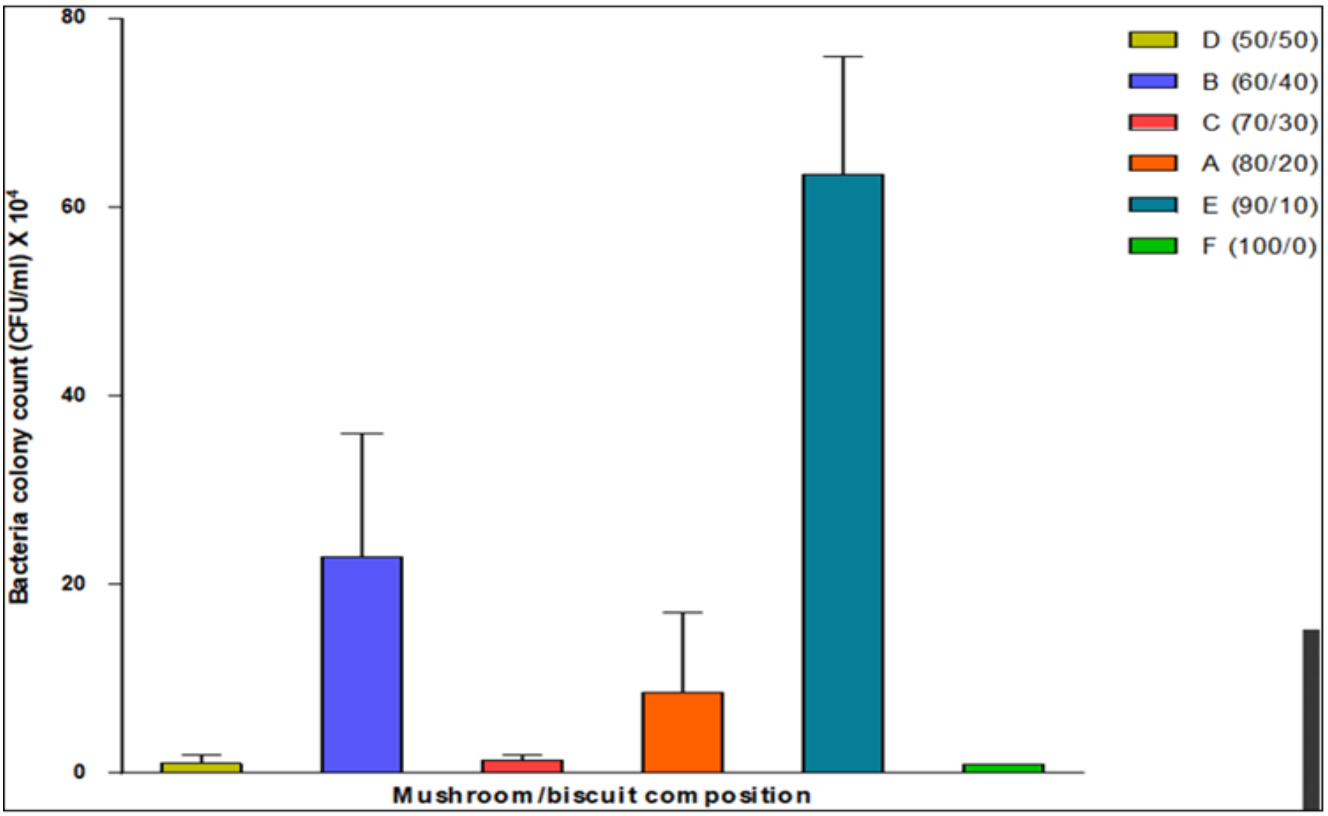

Fig 1 Microbiological Examination of Total Viable Bacteria Count of Various Mushroom Biscuit Compositions

Table 1 Biochemical Identification of Bacteria

Gram reaction	Catalase	Motility	Gas Production	Nitrate	Citrate	H_2S	Glucose	Lactose	Sucrose	Arabinose	Mannitol	Raffinose	Fructose	Galactose	Mannose	Maltose	Oxidase	Indole	MR	V.P	Starch hydrolysiss	Probable organisms
+cocci	+	+	-	+	+		-	+	+	+	-	-	-	-	-	-		-	+	-	-	*Staphylococcus warnei*
+rod	+	-	-		+	-	-	+	+	-	-	+	+	-	+	-		-	+	-	-	*Bacillusniacini*
-rod	+	+	-	+	+	-	+	+	+	-	+	+	+	+	-	+		+	+	-	-	*Kluyuera georgiana*
+rod	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+		-	-	-	+	*Bacillus circulans*
+rod	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	_	+	+	+	*Bacillus licheniforms*
+rod	+	-	-		+	-	+	+	+	+	+	+		+	+	+		-	+	-	+	*Bacillus megaterium*
+rod in chains	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Bacillus barbaricue*
+rod	+	+	-	+	+	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	+	*Bacillus cereus*
+rod in chains	+	+	-	-	-	-	+	-	+	+	-	-	-	-	+	-		-	-	-	-	*Bacillus simplex*
+rod	+	+	-	+	+	-	+	+	+	-	-	-	+	+	+	-		-	-	-	+	*Bacillus cereus*

Keys: + = Positive; - = Negative

Table 2 Macroscopic and Microscopic Features of Fungal Isolates

S/N	Macroscopic Description	Microscopic characteristics	Fungi
1	Black and powdery colonies	Conidiophores terminates in vesicles, smooth walled colorless with brownish shade	*Aspergillus niger*
2	Yellowish green colonies	Conidiophores are coarsely roughened, uncolored with vesicles, spherical and metulae covering nearly the entire vesicles	*A. flavus*
3	Thick, Smooth, Cream, broad fan shaped	In clusters and interwoven with whitish gills running down and a nearly absent stem	*Pleurotus ostreatus*

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D. Proximate Analysis of Mushroom

The result for the proximate composition for the total mushroom below in Figure 2 showed that carbohydrate is the highest composition at 73%, while ash content was the lowest at about 2%. Fibre content also contributes significantly to the mass of the mushroom at 17%, while moisture and nitrogen were measured at 10% and 8% respectively.

➢ *Moisture Content Determination*

The result of the determination of the moisture content of the different mushroom biscuit sample formulations in Figure 3 showed that sample C (70% mushroom) had the highest moisture content, while sample A (80% mushroom) had the significantly lowest even when compared with the control sample F (100% mushroom). Meanwhile Sample B (60% mushroom) and D (50% mushroom) had close range of moisture content.

➢ *Total Ash Determination*

The result of the determination of the ash content of the different mushroom biscuit sample formulations showed that the addition of mushroom to the biscuit composition generally increased the ash content of the resultant biscuit with sample D (50%) mushroom having the highest ash content, while control group i.e., sample F (100% mushroom) had the least observed ash content (Figure 4).

➢ *Crude Fat Determination*

Figure 5 presents the result of the determination of crude fat content for different mushroom biscuit sample formulations. It showed that there was only little difference between the fat contained within all the various mushroom biscuit formulation. However, sample D (80%) and B (60%) mushroom showed the same and the lowest content of fat when compared with the control group (0%) mushroom.

➢ *Crude Protein Determination*

The result of the determination of the protein content of the different formulations of biscuits fortified with mushroom as presented in Figure 6 showed that sample B (60% mushroom composition) had the highest protein content, which was followed by sample C (70%), while the least was observed in sample E (90% mushroom composition). Generally, the result showed that the higher the mushroom content, then the lower the protein content estimated in the mushroom biscuit.

➢ *Crude Fiber Determination*

The result of the determination of the fiber content of the different formulations of biscuits fortified with mushroom when compared with the control group which had (0%) mushroom composition showed that the addition of mushroom to the biscuit greatly increased its overall fibre content. However, the highest crude fibre content was observed in sample D which had equal mushroom and flower content at 50% each (Figure 7).

➢ *Carbohydrate Content Determination*

The result of the determination of the carbohydrate content of the different formulations of biscuits fortified with mushroom showed that carbohydrate content of the mushroom decreased with increased addition of mushroom to its formulation with the slight exception of sample D (80% mushroom composition), while the carbohydrate content level of other percentages in A, B, C, and E were observed to be extremely low (Figure 8).

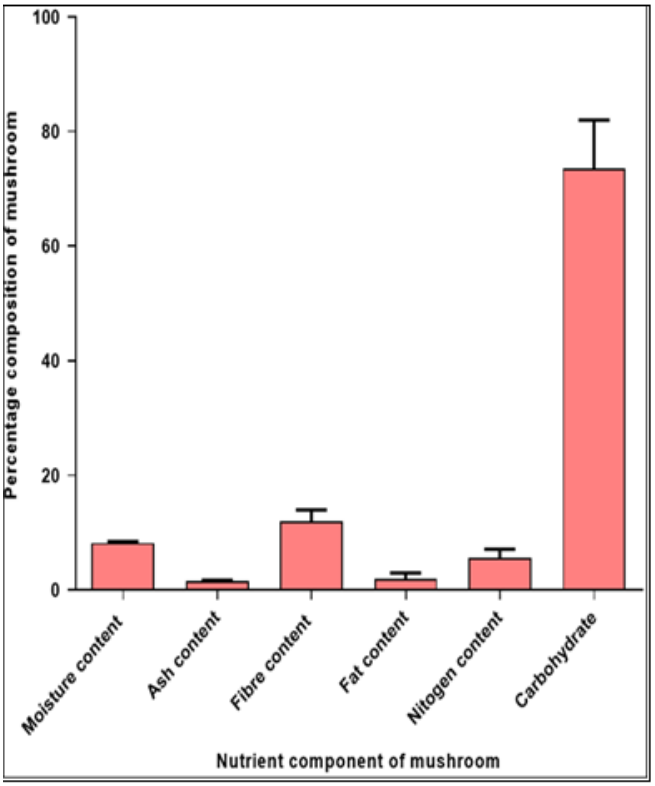

Fig 2 Percentage Proximate Composition of Mushroom *Pleurotus oestratus*

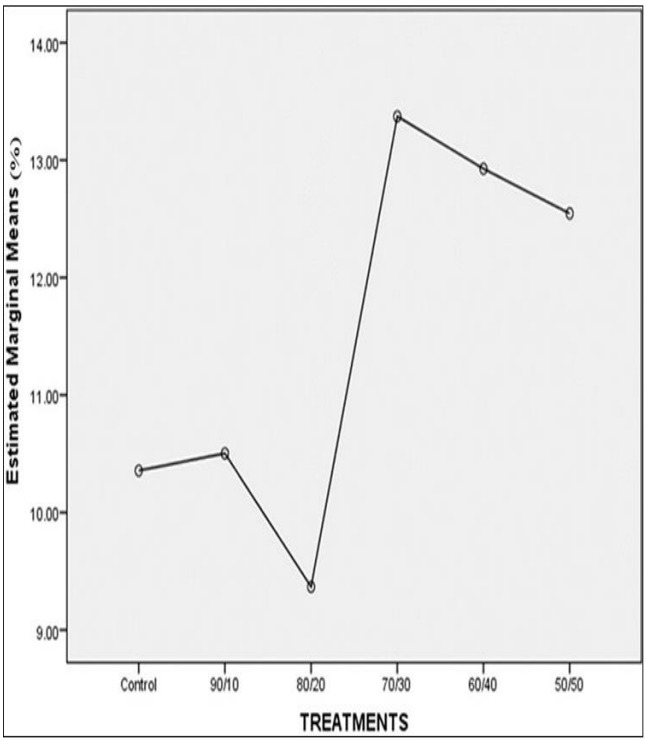

Fig 3 Percentage Content of Various Mushroom Biscuit Formulations

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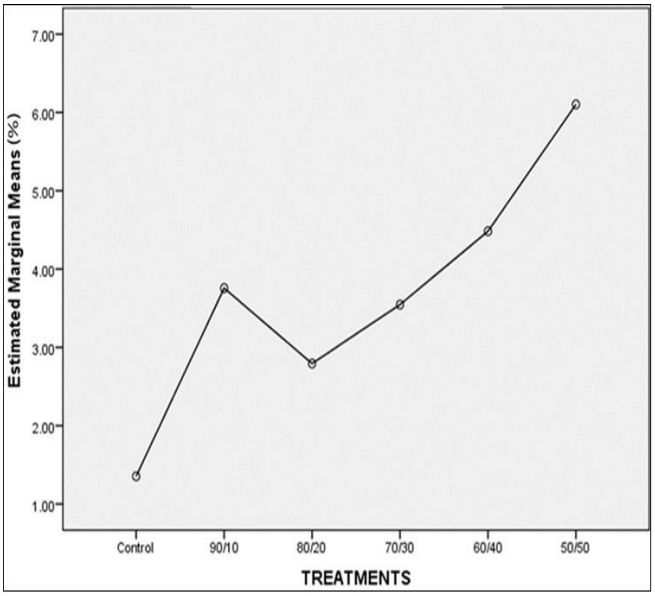

Fig 4 Percentage Ash Content of Various Mushroom Biscuit Formulations

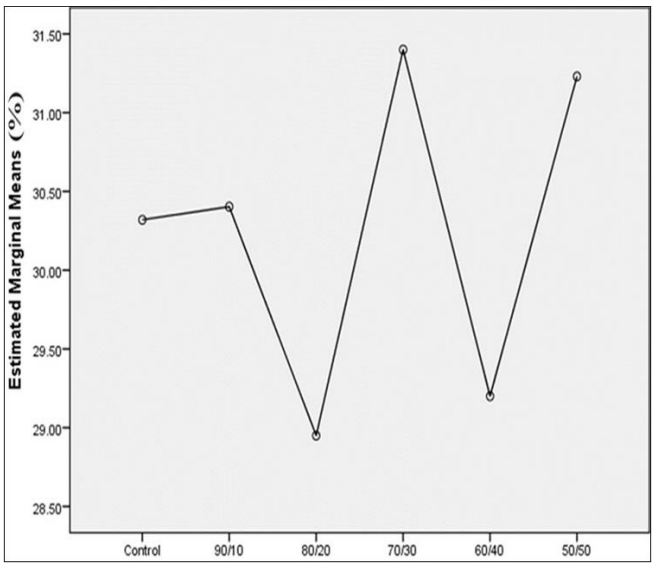

Fig 5 Percentage Fat Content of Various Mushroom Biscuit Formulations

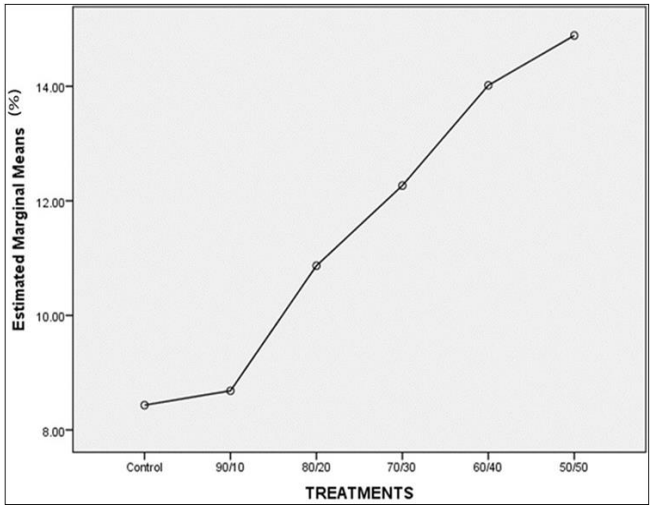

Fig 6 Percentage Protein Content of Various Mushroom Biscuit Formulations

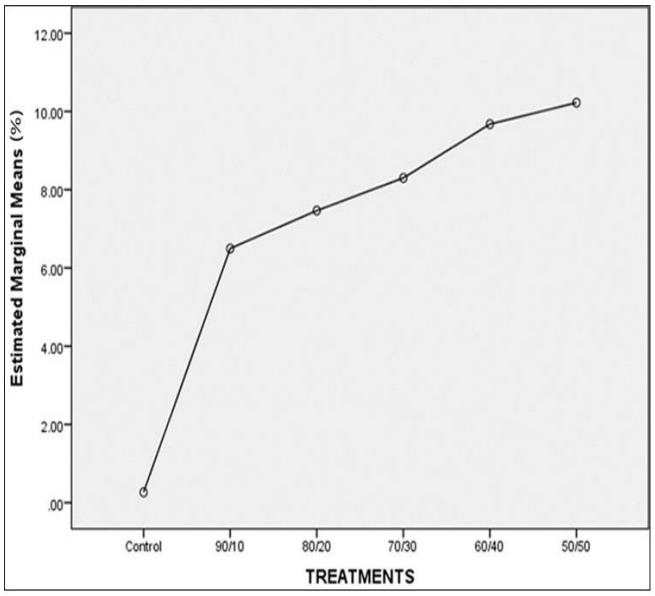

Fig 7 Percentage Crude Fiber Content of Various Mushroom Biscuit Formulations

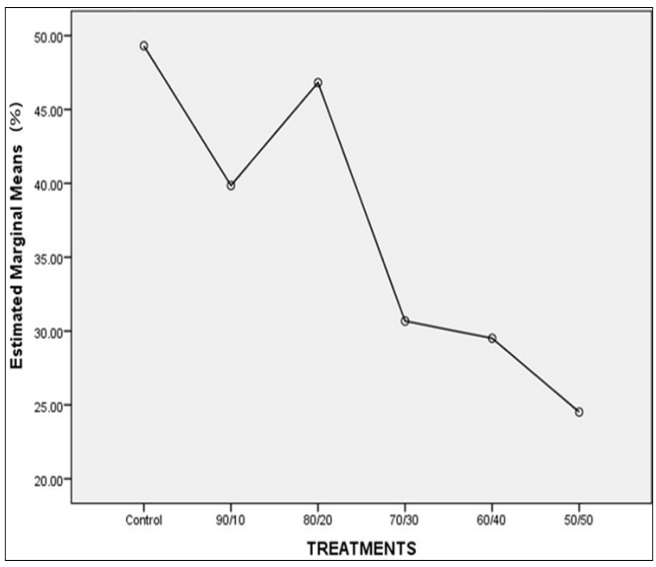

Fig 8 Percentage Carbohydrate Content of Various Mushroom Biscuit Formulations

➢ *Determination of Mineral Elements*

Figure 9 present the result of the determination of the mineral composition of the different formulations of biscuits fortified with mushroom, it showed that the most abundant mineral in the biscuit are Na, K and Ca while Fe and Pb were low significantly. Potassium (K) was the overall highest mineral content in the fortified biscuits, with sodium (Na) observed to also have the second overall highest for mineral nutrient content and generally, lead (Pb) was only present in trace amount. 50% mushroom biscuit formulation was generally observed to contain the highest potassium and sodium content at 19.5±0.5 and 10.15±0.15 ppm respectively. The calcium content of fortified mushroom biscuit showed that no significant increase when compared with the control (0% mushroom).

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➢ *Sensory Evaluation of Biscuit*

The result of the determination of the sensory evaluation of the various formulations of biscuits fortified with mushroom in Table 3 showed that sample E (10% mushroom) had the best overall acceptability at 9.50±0.17, with texture and taste both at 9.30±0.21 which was only a little lower than the values for standard biscuits (control) on a general measurement scale of 1-10. Sample A containing 50% mushroom had the poorest acceptance at 5.40±0.85. However, sample D (20% mushroom) had the best aroma production valued at 9.10±0.28.

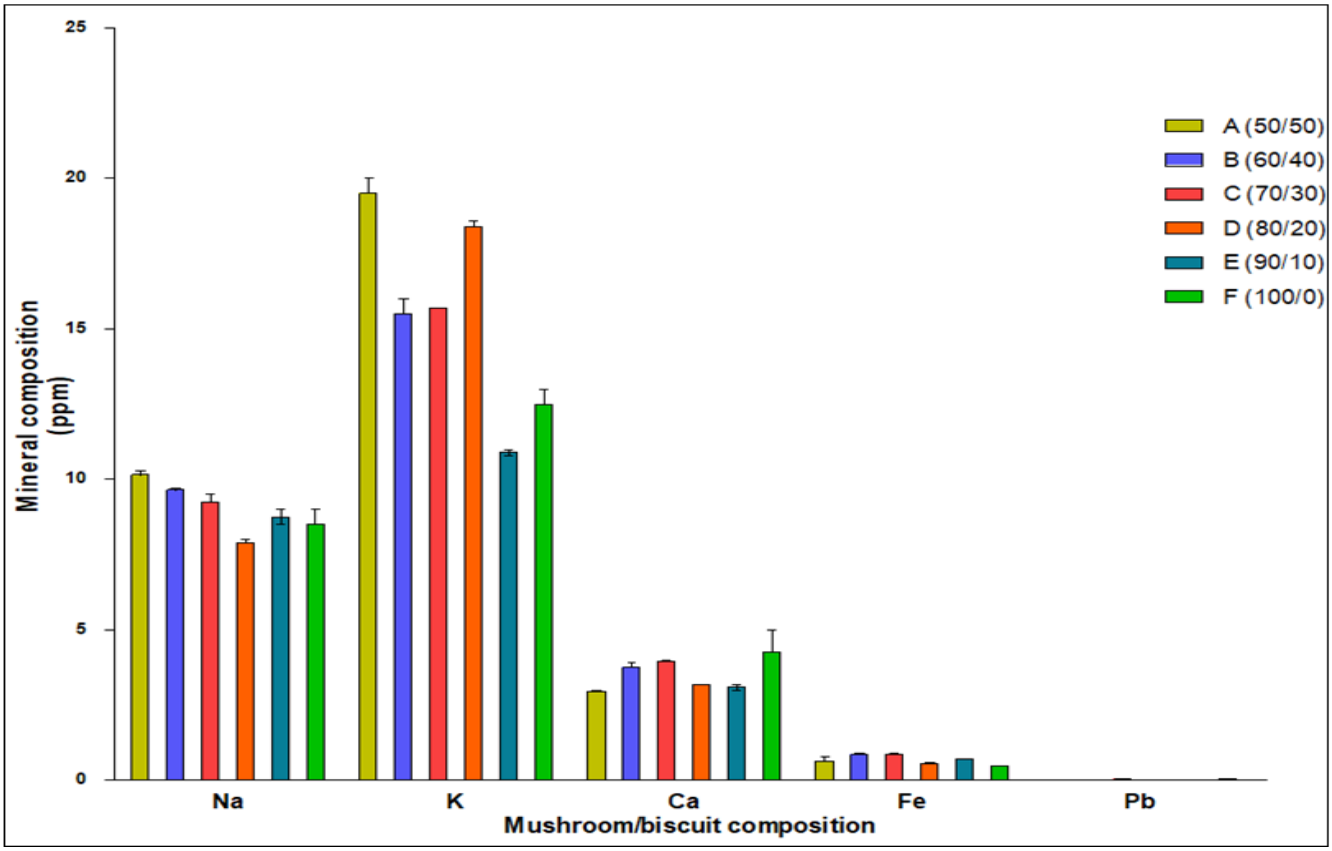

Fig 9 Mineral Composition of Various Mushroom Biscuit Formulations in Ppm

Table 3 Sensory Evaluation of Mushroom Biscuit

	Quality evaluated				
Samples	**Aroma**	**Colour**	**Texture**	**Taste**	**Overall acceptability**
A	8.10±0.43	7.40±0.45	7.10±0.59	5.50±0.40	5.40±0.85
B	8.30±0.30	7.80±0.29	7.90±0.57	6.30±0.52	5.90±0.86
C	7.90±0.91	8.90±0.28	8.40±0.22	6.90±0.87	7.40±0.92
D	8.50±0.31	9.10±0.28	9.40±0.22	7.80±0.39	8.80±0.20
E	8.80±0.33	8.40±0.31	9.30±0.21	9.30±0.21	9.40±0.16
F	8.00±0.42	9.20±0.29	9.60±0.16	9.50±0.22	9.50±0.17

Key: Mushroom compositions, A; (50/50), B; (60/40), C; (70/30), D; (80/20), E; (90/10) and F; Control (100/0)

IV. DISCUSSION

Baked products such as biscuits is among the highly consumed products acceptable by all (Wan Rosli *et al*., 2012) due to the fact that they are popular, convenient, inexpensive and its long shelf-life, however the primary ingredient (wheat) used in its production is deficient in protein and some other essential nutrients. A study by Nieburg (2012) have shown that replacing the wheat flour with mushrooms at different levels in formulations could have a substantial improvement in the protein content and other essential nutrients such as the vitamins, minerals and dietary fiber without affecting its physical and sensory properties.

From this study, 4 bacteria genera (*Bacillus* spp, *Pseudomonas* spp, *Staphylococcus* spp and Kluyvera) and fungi (*Aspergillus flavus* and *Aspergillus niger*) were isolated from the cultivated mushroom (*Pleurotus ostreatus*), however the dominant bacteria is the *bacillus* spp. This observation is similar to Mariusz and Stanislaw (2019) who also recorded a high number of Bacillus spp. This could be attributed to fact that Bacillus and Pseudomonas are growth promoting bacteria due to their competitive and antagonistic activity against several

pathogens (Company *et al*., 2010). Some of the isolates could also be associated with the substrate on which the mushroom was cultivated as it is related to the report of Obire and Amadi (2013) who (similarly isolated Aspergillus and Mucor spp. from fermented sawdust) isolated from the substrate in which the mushroom was grown.

The proximate composition of the *Pleurotus ostreatus* shows that it has percentage protein and crude fiber of 21% and 10% respectively. Singh and Thakur (2016) also reported similar work, having a 13% protein content, however Oyetayo and Ariyo (2013) and Bello *et al*. (2017) reported a higher percentage of protein (20- 24%) and crude fiber (17-30%) respectively in *P. ostreatus* cultivated on different wood substrates. The variation in nutrient from different study could be due to the nutrient composition of the substrates on which the mushrooms was cultivated. The protein, crude fiber and ash content from the proximate analysis of the biscuit from this study is similar to the low values of (10, 2 and 1% respectively) reported by Adegbanke *et al*. (2020) in wheat biscuit.

The proximate composition of biscuit fortified with *Pleurotus ostreatus* showed increase in moisture content, total ash, crude protein and a significant increase in the crude fiber, no significant increase was seen in the fat content compare with the control; however, a decrease was observed in the percentage carbohydrate. Bello *et al*., (2017) and Prodhan *et al*., (2015) also recorded a similar trend of proximate composition of Pleurotus *sajur-caju* fortified biscuit.

The protein content at different percentage of mushroom flour inclusion ranges from 9% to 15% and a control of 8.4%. There was a significant increase in all the samples compared with the control. The increase in the protein content could be attributed to the high protein content of the mushroom added and this reveals that the fortified biscuit would be a better-quality compare with the unfortified one (Bello *et al*., 2017). Also, in agreement is Ayo *et al*. (2014) who enriched a malted soy biscuit, however Mepba and Iboh (2007) reported a decrease in protein content in wheat plantain biscuit. The significant increase in the crude fiber compares with the control ranges between 6.5% to 10% which could be attributed to the mushroom used, been a rich source of fiber which eventually increases the fiber content of the biscuit with increase in the level of addition. Bello *et al*. (2017) reported that a high intake of dietary fiber helps prevent constipation, reduce cholesterol level in the blood, slow digestion and sudden release of energy thus making blood level stable. The ash content of a food material could be used as an index of mineral constituents of the food; therefore the increase in the ash content at all level of addition indicates the enriched biscuit to be a good source of mineral.

The carbohydrate content was found to decrease with increase in the mushroom flour, however there was no significant decrease in the (80/20) concentration compare with the control. A similar trend was presented by Ng *et al*. (2017) and Nordiana *et al*. (2019) who fortified biscuit with mushroom flour, however Aishah (2013) documented that the carbohydrate content increases with increase in mushroom flour, though this may be due to the different formulations of the cakes. The moisture content of biscuit and mushroom pre-determined before mixing was 10.4%, the mixture after production has increased moisture content. Aishah (2013) and Nordiana *et al*. (2019) has documented similar concurrent increased moisture content, this could be that the mushroom flour possess high water content and water holding capacity, also the sugar, starch and dietary fibre in the formulation could have also contributed as they contain high amount of water (Mohammed *et al*., 2010) In contrast to this report is Ng *et al*. (2017) who recorded a lower moisture as compared to the control , this may be as a result of the very high temperature used in baking thereby reducing the water content.

The most abundant mineral in the biscuit are Na, K and Ca while Fe and Pb were present in trace amount. Adegbanke *et al*. (2020) also reported Na, K, Ca, P and Zn in significant amount and Cu, Pb, Fe and Mn were reported to be found in trace amount in wheat biscuit. The mineral composition of the fortified biscuit shows significant increase in Na and K, a slight increase was also observed in Fe content while a general decrease was observed in the Ca content and Pb remain insignificant. The mineral composition obtained from this study shows that the fortified biscuit is a good source of mineral. The most abundant mineral is K followed by Na, this is in line with the report that the most abundant mineral in biscuit is K and Na and both are required to maintain osmotic balance in the body fluid, pH of the body, to regulate muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (Arinathan *et al*., 2003). The fortified biscuit is also a good source of Fe, which is a major component of hemoglobin that carries oxygen to all part of the body and it also plays a critical role in overall cell function (Bello *et al*., 2017). The decrease in the Ca content when compared with the control, indicates that the fortified biscuit is not a good source of Ca and Ca is involved in the overall body formation and functions. The results of Bello *et al*. (2017) appears similar with the result of this study's mineral content, however it recorded a steady decrease in Na and then a slight increase in the 30% addition of the mushroom flour.

The result of the sensory evaluation of the fortified biscuit after production indicates that the biscuit fortified with 10% and 20% mushroom flour does not show any significant difference from the control, while there is a significant difference between the control and the biscuits fortified with 30%, 40% and 50% mushroom flour and the overall acceptability of the fortified biscuit decreases with increase in the mushroom flour. This could be as a result of the obvious and unpalatable taste from adding larger portion of the mushroom flour, this aligned with the study of Prabhasankar *et al*. (2009), Aishah (2013) and Bello *et al*. (2017) who reported that the panelist preferred lower percentages of oyster mushroom flour, and sensory scores were reduced significantly when more mushroom powder is added as compared to the control.

V. CONCLUSION

The study suggests that the nutritional value of wheat biscuits enriched with mushroom *(P. ostreatus)* improved with the addition of more mushroom powder. However, with high addition levels of the mushroom powder, the general acceptance of the enriched biscuit declines. For the optimum nutritional, essential mineral, and sensory qualities, it is advised that commercial biscuit manufacture be done at a 50% level of mushroom inclusion. The production of biscuit using other types of flour in synergy with mushroom (*P. ostreatus*) could be studied to determine its nutritional value and sensory acceptability warrants industrial application. Malnourished persons, communities and societies can be provided these fortified biscuits to alleviate consumption of foods with low levels of essential nutrients because of its high nutritional composition.

➢ *Authors' Contribution*

Author Victor O. Oyetayo supervised the study and provided administrative support. Author Rosemary A. Ajayi performed the literature search, laboratory work, data analysis and wrote the first draft of the manuscript. All authors read and approved the final draft.

REFERENCES

[1]. A.O.A.C. (2012). Official methods of analysis. 19th edition. Washington DC by Association of Official Analytical Chemist: 35-60.

[2]. Adeboboye, C. F., Oladejo, B. O., and Adebolu, T. T. (2022). The effect of *Lactobacillus fermentum* from indigenous dairy product on inflammatory-associated anemia and leucocyte homeostasis in carrageenan-induced inflammation in Wistar rats. *Bacterial Empire*, 5(1): e327. https://doi.org/10.36547/be.327

[3]. Adeboboye, C. F., Oladejo, B. O., and Adebolu, T. T. (2020). Immunomodulation: a broad perspective for patients' survival of COVID-19 infection. European Journal of Biological Research, 10(3), 217–224. https://doi.org/10.5281/zenodo.3956771

[4]. Adedayo, M.R. (2011). Proximate analysis of four edible mushrooms. *Journal of Applied Science and Environment Management*. 5(1): 9-11.

[5]. Adegbanke, O.R., Osundahunsi, O.F., and Enujiugha, V.N (2020). Chemical and Mineral Composition of Biscuit Produced from wheat and Bambara groundnut flour. Acta Scientific Nutritional Health 10(4) 03-09.

[6]. Aishah, M. S. 2013. Nutritional composition, antioxidant properties and sensory acceptability of carbohydrate-based products formulated with *Pleurotus sajor-caju.* Malaysia: Universiti Sains Malaysia, MSc thesis.

[7]. Anandhi, R., Annadurai,T., Anitha, T.S., Muralidharan, A.R., Najmunnisha, K., Nachiappan, V., Thomas, P.A. and Geraldine, P. (2013). Anti-hypercholesterolemic and antioxidative effects of an extract of the oyster mush-room, *Pleurotus ostreatus*, and its major constituent, chrysin, in Triton WR-1339-induced hyper-cholesterolemic rats. *Journal of Physiology Biochemistry*, **69**:313–323.

[8]. Arinathan V, Mohan VR, De Britto AJ (2003). Chemical composition of certain tribal pulses In South India. International Journal of Food Sciences and Nutrition. 54(3):209–17.

[9]. Ayo, J.A., Ayo, V.A., Popoola, C., Omosebi, M., Joseph. L. (2014). Production and evaluation of malted soybean-acha composite flour bread and biscuit. African Journal of Food Sci. and Tech. 5(1):25-28

[10]. Bello, M. O. Oluwamukomi and Enujiugha, V. N. (2017). Nutrient Composition and Sensory Properties of Biscuit from Mushroom-Wheat Composite Flour. *Archives of Current Research International*. 9(3): 1-11.

[11]. Caleja, C. L., Barros, A. L., Antonio, *et al*. (2017). A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits". *Food Chemistry*, **216:** 342-346.

[12]. Caz, V., Gil-Ramírez, A., Largo, C., Tabernero, M., San-tamaría, M., Martín-Hernández, R., Marín, F.R., Reg-lero, G. and Soler-Rivas, C. (2015). Modulation of cholesterol related gene expression by dietary fiber fractions from edible mushrooms. *Journal on Agriculture. Food Chemistry,* **63**: 7371–7380.

[13]. Chauhan, G.S., Zillman, R. R. and Eskin, N. A. (2012). Dough mixing and bread making properties of quinoa-wheat flour blends. *International Journal of Food Science Technology*, **27**:701-705.

[14]. Compant, S., Clement, C. and Sessitsch, A. (2010). Plant growth promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanism involved and prospect for utilization. *Soil Biology and Biochemistry*, 42(5): 669-678.

[15]. Isong, N. B., Akpan, M. M., Udota, H. I. and Barber, L. (2013). Antibacterial and microbial assessment of breads in Abak Local Government area, Nigeria, West Africa. *Nigeria Journal of Microbiology*, **3**:155-159.

[16]. Kholoud, M., Alananbah, N., Bouquellah, Nadia, S.A. (2014). Cultivation of oyster mushroom *Pleurotus ostreatus* on date-palm leaves mixed with other agro-wastes in Saudi Arabia. *Saudi Journal of Biological Science*, 21(6): 616-625

[17]. Kong, W.S. (2004). Mushroom grower's handbook 1: Oyster mushroom cultivation; 1: 54-661.

[18]. Kure, O.A., Bahago, E.J. and Daniel, E.A. (1998). Studies on the proximate composition and effect of flour particle size on acceptability of biscuit produced from blends of soybeans and plantain flours. *Namida Tech. Scoped J*, **3**:17-21.

[19]. Liyanage, C. and Hettiarachchi, M. (2011). Food fortification. *Ceylon Medical Journal*, 56: 124-127.

[20]. Mallavadhani, U.V., Sudharkar, A.V., Satyanarayana, K.V., Mahapatra, A., Li, W. and Van Breemen, R.B. (2006). Chemical and analytical screening of some edible mushrooms. *Food Chemistry*, **95**:58-64.

[21]. Mepba, H.D., and. Iboh, D.E.B. (2007). Effects of processing treatments on the nutritional composition and consumer acceptance of some Nigeria edible leafy vegetables. *African Journal of Food Agriculture Nutrition and Development*. 7(1):35-41.

[22]. Muszyńska, B., Komendacki, P., Kała, K. and Opoka, W. (2014). L-Tryptophan and its derivatives in edible mushrooms species. *Medical International Rev*., **103:** 82–86.
[23]. Ng, S. H., Robert, S. D., Wan Ahmad, W. A. N. and Wan Ishak, W. R. (2017). Incorporation of dietary fibre-rich oyster mushroom (*Pleurotus sajor-caju*) powder improves postprandial glycaemic response by interfering with starch granule structure and starch digestibility of biscuit. Food Chemistry 227: 358–368.
[24]. Nieburg, O. (2012). Replacing wheat flour with lupin flour by up to 30% can increase protein and dietary fibre content without affecting taste, color, texture and flavor. Bakery and snacks.com Jan 2012.
[25]. Nordiana, A. B., Wan Rosli, W. I. and Wan Amir Nizam, W. A (2019). The effect of oyster mushroom (*Pleurotus sajor-caju*) flour incorporation on the physicochemical quality and sensorial acceptability of pasta. International Food Research Journal 26(4): 1249-1257
[26]. Obire, O. and Amadi, A.O. (2013). Cultivation of mushroom (*Pluerotus ostreatus*) and the microorganisms associated with the substrate used. e-Journal of science and technology (e-JST), 4(8), 50-59
[27]. Okafor, J.N.C., Okafor, G.I., Ozumba, A.U. and Elemo, G.N. (2012). Quality Characteristics of Bread made from Wheat and Nigerian Oyster Mushroom (*Pleurotus plumonarius*) Powder. *Pakistan Journal of Nutrition*. 11 (1): 5-10.
[28]. Okeke, J. N., Ozumba, A. U., Olatunji, O. O. and Odunfa, S. A. (2003). Effect of drying methods on some Commission, Kansas Association of Wheat qualities of Nigerian *Pleurotus pulmonarius* Growers, Manhattan, Kansas. Mushroom powder. *Nigeria Food Journal*, **21**:137-143.
[29]. Oladejo, Babayemi O. Adeboboye, Covenant F. Adiji, Precious I. and Adebolu, Tinuola T. (2023). Cytokine-mediated immunoregulatory activity of *Lactobacillus* species in a carrageenan-induced acute inflammatory model. *Journal of Biotechnology, Computational Biology and Bionanotechnology*. 104 (1): 53–63. https://doi.org/10.5114/bta.2023.125085
[30]. Cytokine-mediated immunoregulatory activity of Lactobacillus species in a carrageenan-induced acute inflammatory model BABAYEMI O. OLADEJO * , COVENANT F. ADEBOBOYE, PRECIOUS I. ADIJI, TINUOLA T. ADEBOLU.
[31]. Oyetayo, V.O and Ariyo, O.O. (2013). Micro and Macronutrient Properties of *Pleurotus ostreatus* (Jacq: Fries) Cultivated on Different Wood Substrates. *Jordan Journal of Biological Sciences*. 6(3)223 – 226
[32]. Oyetayo, V.O. and Oyedeji, R. R. (2018). Assessment of Microbial and Sensory Properties of Bread Fortified with Mushrooms (*Pleurotus ostreatus* and *Calocybe indica*). *Journal of Advances in Microbiology*, **2**: 1-7
[33]. Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R. and Miyashita, K. (2009). Edible Japanese seaweed, Wakame (*Undaria pinnatifida*) as an ingredient in pasta: chemical, functional and structural evaluation. Food Chemistry 115(2): 501–508.
[34]. Prodhan, U.K., Linkon, M.K., Al-Amin, M.F., Alam, M.J. (2015). Development and quality evaluation of mushroom (*pleurotus sajor-caju*) enriched biscuits. *Emirates Journal of Food and Agriculture*. 27(7): 542-547.
[35]. Sanches, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Applied Microbial Biotechnology*, **85:** 1321–13377.
[36]. Silva, S. O., Costa S. M. and Clemente, E. (2002). Chemical Composition of *Pleurotus pulmonarius* Substrates and Residue after Cultivation, *Brazilian Archives Biology Technology*, **45:** 531-535.
[37]. Singh, K. and Thakur, M. (2016). Formulation, organoleptic and nutritional evaluation of value-added baked product incorporating Oyster mushrooms (*Pleurotus ostearus*) powder. *International Journal of Food Science and Nutrition*. 1(6):16-20.
[38]. Swieca, M., Gawlik-Dziki, U., Dziki, D. and Baraniak, B. (2017). Wheat bread enriched with green coffee – In vitro bio-accessibility and bioavailability of phenolics and antioxidant activity. Food Chemistry, 221: 1451-1457.
[39]. Ugwuona, F.U. and Obeta, N.A. (2016). Quality characteristics of bread fortified with sesame seed. *Global Journal of Medical Research*, **1**:1-2.
[40]. Valverde, M. E., Hernández-Pérez, T. and Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, 14.