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ACCEPTABILITY AND ANTIOXIDANT PROPERTIES OF CONVENIENCE FOOD PRODUCTS FORTIFIED WITH MUSHROOMS

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ABSTRACT

This study sought to address the challenge of increasing availability of mushrooms to consumers in Nigeria by formulating them into convenience food products. Fresh oyster mushroom (*Pleurotus ostreatus*) and powder prepared from the dried form were used to develop, mushroom biscuits and mushroom instant soup premix respectively. The soup premix was thickened using "achi" powder. Biscuits were prepared according to the buttermilk biscuits formula. Physical properties of the food products, their acceptability, antioxidant properties and their shelf lives were evaluated. Mushroom biscuits were high in moisture (28 to 47 %), in proportion to quantity of mushroom used while moisture levels in the soup premix averaged 17%. Sinkability of the mushroom soup indicated its ease of reconstitution and a good mouth feel. Sensory analyses showed that both food products were acceptable to a wide range of Nigerians. Antioxidant capacities found in both food products are proportionately comparable to capacities found in studies of *Pleurotus ostreatus* mushrooms. The mushroom biscuits stored 3 – 4 days while the soup premix stored for up to 35 days. These food products, which can be served at home or through quick service restaurants, will increase availability and hence consumption of mushrooms among Nigerians with associated health benefits

Key words: Mushrooms, convenience foods, Acceptability to consumers, antioxidant properties

INTRODUCTION

Mushrooms are macrofungi that contain a suite of under-consumed nutrients; not found across plant and animal-based foods. They are also sustainable foods and therefore provide a unique health benefiting food source (Feeney *et al*., 2014). Mushrooms are important sources of antioxidants in foods (Dai et al. (2015; Beelman *et al*. (2019; Zhang *et al*. (2020). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many

non communicable diseases (NCDs) such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing.

Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Halliwell and Gutteridge, 2003). Oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical compounds such as α-tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione, usually protect most organisms against free radical damage (Mwangi et al., 2022). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant-containing foods may be used to help the human body to reduce oxidative damage (Halliwell and Gutteridge, 2003; Mwangi et al., 2022)

Because of health benefits derived from eating mushrooms, the global mushroom market has a compound annual growth rate (CAGR) of 6.41% and is projected to reach 20.84 MT by 2026 (Global Market Analysis, Insights and Forecast, 2019-2026). In Nigeria, from a study done in Southeast (SE) Nigeria, there appears to be widespread awareness about the use of mushrooms as food (Ogbo *et al*., 2022a). In another study, Ogbo *et al*. (2022b), also found that half of population of SE Nigeria have knowledge that mushroom consumption could ameliorate NCDs (Ogbo *et al*., 2022b). However, consumption of mushrooms, remain at a very low level in Nigeria. Adedokun & Okomadu (2017) in a survey found that only 12.2% of Nigerians claimed to consume mushrooms all year-round while 57.8% consumed mushroom only once in a while. Ogbo *et al* (2022a) found in a survey conducted in Southeast Nigeria, that the major reason respondents did not eat mushrooms more frequently was because of its unavailability.

A few farms located mostly in the South west of Nigeria are making efforts to cultivate mushrooms. However, it does appear that they are being limited by several factors. Ayafunke (2019*)* has estimated that 72.7% of mushroom farmers consider poor sales as the major constraint they faced. Poor sales are of course exacerbated by the perishability and difficult-to-store or distribute nature of mushrooms after harvest. The challenge of increasing availability of mushrooms to consumers in Nigeria may be addressed by processing, not only to extend shelf life, to ensure profits to farmers but also to formulate novel products of added value, which will have more convenience than the traditional methods of offering mushrooms for consumption as well as deliver health benefits to consumers who eat them over a reasonable period of time. Clinical trial data obtained by Dai *et al*. (2015), showed that consuming 5 -10 g of *Lentinula edodes* mushrooms daily over a period of four weeks resulted in improved immunity. Also, Beelman *et al*. (2019), has recommended that consumption of about 100 g of fresh button mushrooms or around 25 g of fresh specialty mushrooms such as shiitake, oyster, or maitake mushrooms, per day, will deliver 3 mg/d of L- ergothioneine. The aim of this project is therefore, to develop novel healthy convenience food products using mushrooms, which will extend shelf life after harvest, increase its availability to consumers as well as ensure that Nigerians obtain health benefits associated with eating mushrooms. It is expected that these improvements will drive

the mass consumption and consequently, cultivation of mushrooms in Nigeria.

MATERIALS AND METHODS

Raw Materials

Fresh oyster mushrooms (*Pleurotus ostreatus*) was purchased from local producers in Awka, while the dried oyster was purchased from LTV Farms, Lagos. Other ingredients including, sugar, butter, "achi"thickener, consisted of locally available brands and were also purchased from Eke Awka market. "Achi" is a local soup thickener prepared by grinding dried and roasted seeds of *Brachystegia eurycoma* into powder (Nwosu, 2012).

Food Products

Two food products, mushroom biscuits and mushroom instant soup premix, which may be served at home or through quick service restaurants, to suit increasing urbanized and rapid lifestyles of Nigerians, were selected for this project.

Formulation of Mushroom instant soup premix

The instant soup premix was prepared according to the method of Srivastava *et al*. (2019) with adaptation of ingredients to locally available materials. The dried mushrooms were ground into powder in a locally made grinding mill (LOTTOJ-PENTA, Osogbo, Osun state, Nigeria) followed by sieving through 0.5mm sieve to convert into a fine powder.

Five formulations of mushroom instant soup premix containing mushroom powder, 10 g; Seasoning (commercially available cubes containing monosodium glutamate and other ingredients, used to prepare soups), 1 g and varying proportions of "achi" powder thickener were prepared by dry mixing of all ingredients (Table 1). Mushroom soup can be prepared from this premix by heating it with 200 – 300 ml of water (according to taste), at low flame for 2 minutes with stirring.

Table 1: Formulations of Mushroom Instant Soup Premix

Formulation	Mushroom Powder (g)	Achi (g)	Seasoning (g)
S5	10	2.5	1
S4	10	2	1
S3	10	1.5	1
S2	10	1	1
S1	10	0.5	1

Formulation of Mushroom Biscuits

Mushroom biscuits were prepared according to the Easy Buttermilk Biscuits formula (Anonymous, 2023a). Buttermilk and Edible mushrooms have been reported to have comparable compositions (Anonymous, 2023b). The Easy Buttermilk Biscuits formula is comprised as follows: flour 250 g; baking powder, 29 g; baking soda 1.5 g; sugar 8 g; salt, 0.5 g; butter, 85 g, and buttermilk, 182 g.

Washed, fruit bodies of fresh oyster mushrooms were separated from the bunch and ground into slurry using a laboratory mill. This was used to substitute buttermilk in the biscuit formula at varying percentages as described in Table 2. Biscuits were baked according to instructions of owners of formula.

Table 2: Formulations of Mushroom Biscuits

Formulation	Mushroom Slurry (g)	Flour (g)	% Substitution of Flour
B1	0	400	0 %
B2	70	362	15%
B3	90	342	20%
B4	110	322	25%
B5	130	302	30%
B6	150	282	35%
B7	182	250	40%

Physical Properties of the Food Products

Moisture of the mushroom instant soup premix and mushroom biscuits were determined by AOAC (2000). Sinkability of the mushroom instant soup premix was done according to National Dairy Research Institute as reported by Yaakub *et al*. (2019), using 100 mg sample. Sinkability is usually expressed as milligrams of powder that sink per minute per square centimeter of surface area.

Acceptability of Food Products

The mushroom instant soup premix was prepared for eating as described in methodology for its formulation. Acceptability of the prepared soup and the mushroom biscuits were then evaluated by assessing their different sensory attributes using an untrained panel of 40 members drawn randomly from staff and students of the Faculty of Biosciences, Nnamdi Azikiwe University Awka. Sensory attributes like colour & appearance, body or chewiness, mouth feel, aroma, taste and overall acceptability of coded samples were assessed using a nine point hedonic scale. Hedonic scale was in the following sequence: like extremely – 9, like very much - 8, like moderately - 7, like slightly - 6, neither like nor dislike - 5, dislike slightly 4, dislike moderately - 3, dislike very much - 2, dislike extremely – 1. Data was analyzed using the one way ANOVA and the Tukey HSD Test (Watts *et al*., 1989).

Antioxidant Properties of Food Products

Extraction of Antioxidants

Food products were further dried in the oven at 50ºC overnight. Ten grammes of dried samples were ground into a fine powder in a mill, then, mixed with 100 ml methyl alcohol at room temperature at 150 rpm for 24 h. The residue was re-extracted under the same conditions until the solution was colorless. The extract was filtered over Whatman filter paper and methyl alcohol removed by evaporation at room temperature overnight (Sifat *et al*., 2020).

Extraction yield was obtained by weighing extract of each food sample. The dry extract was then collected in plastic bottles and stored at 4ºC during analyses. Methanolic extracts of food product samples used in analyses were prepared by dissolving extract in 10 ml methanol such that 1 ml of extract was equivalent to 1 g of food product sample.

Determination of total antioxidant potential

The phosphomolybdate assay system was used to determine the total antioxidant activity of the methanol extract of the food products (Sifat *et al*., 2020). To the phosphomolybdate reagent solution; sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM); 100 µl of each sample was added and incubated at 95°C in a water bath for 90 min. After cooling to room temperature; absorbance was recorded at 765 nm against

reagent blank. The total antioxidant capacity was determined by reference to an ascorbic acid standard curve plotted from varying dilutions of this substance reacted similarly with phosphomolybdate reagent under same conditions. Results expressed in mg of ascorbic acid equivalent (AAE) per 100 g of food product.

Determination of hydroxyl radical (*OH) scavenging ability

Hydroxyl radical (#OH) scavenging ability of the of the food products were determined according to the procedure outlined by Elmastas *et al.* (2007). Briefly, 1 mL of FeS04 solution (1.5 mM) was added to 1 mL extract/standard (ascorbic acid) solution of each concentration (0.5-2.5 mg/mL). The reaction mixture was then added to a solution containing 0.7 mL of H_20_2 (6 mM) and 0.3 mL of sodium salicylate, followed by incubation for 1 h at 37 °C. Absorbance of the hydroxylated salicylate complex was measured at 562 nm, and the percentage of scavenging activity was calculated using the following equation:

% radical scavenging capacity =

$$[1- (A1- A2)/A0] \times 100\%$$

Where A0, A1, and A2 are the absorbance of the control (without extract), presence of extract, and without sodium salicylate, respectively.

Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of extracts of foods was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) following the method of Elmastas *et al.* (2007). In brief, 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts in ethanol at different concentration (5, 10, 15, 20, 25, 30 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm. using spectrophotometer (UV-VIS Spectronic). Ascorbic acid was used as reference compound. DPPH scavenging effect (%) or Percent inhibition was calculated from the equation;

$$A0 - A\ 1 / A0 \times 100.$$

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample. The IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve.

Shelf life Studies

The products were packaged in polythene bags and stored at ambient conditions (25 – 30^{o} C and 60 – 85 % RH). The mushroom biscuits were drawn daily, while the mushroom instant soup premix was examined at two-weekly intervals over a period of 72 weeks. Both food products were evaluated for various sensory characteristics i.e. colour & appearance, aroma, taste and overall acceptability using 9 point hedonic scale by a panel of 10 selected from the Biotechnology Research Center, Nnamdi Azikiwe University, Awka until they became unacceptable for human consumption (Kumari *et al*., 2021).

Statistical analyses

All experiments were done in triplicate. Statistical tools of ANOVA and Regression analyses were applied as appropriate to particular data to validate them.

3. RESULTS AND DISCUSSION

Formulation of Food Products

Two food products, mushroom biscuits and mushroom instant soup premix were formulated to achieve the twin objectives of making mushrooms available as well as provide health benefits to Nigerians. These foods may be served at home or through quick service restaurants, thus are convenient to suit increasing urbanized and

rapid lifestyles of Nigerians. Food products were also formulated to contain sufficient quantities of mushrooms per serving to ensure that a consumer derives health benefits by eating them for a reasonable period of time. Clinical trial data obtained by Dai *et al*. (2015), showed that consuming 5 -10 g of *Lentinula edodes* mushrooms daily over a period of four weeks resulted in improved immunity. Also, Beelman *et al*. (2019), has recommended that consumption of about 100 g of fresh button mushrooms or around 25 g of fresh specialty mushrooms such as shiitake, oyster, or maitake mushrooms, per day, will deliver 3 mg/d of L- ergothioneine.

There are some reports about the formulation of instant soup premix using mushrooms (Hafeel *et al.,* 2013; Srivastava *et al*., 2019). These ready to drink dehydrated soup mixtures consist of different ingredients, mostly corn starch, which serves as thickener, spices, salt, flavours and flavour enhancers (Hafeel *et al.,* 2013). As far as we know, this is the first report where "achi' has been used to thicken mushroom soup. "Achi"is also rich in protein and fibre content and would also contribute to the nutrition of the product (Nwosu, 2012).

Several authors including Desayi et al. (2012) and Bello et al. (2017) have reported the utilization of mushrooms for the production of biscuits with enhanced nutritional characteristics. However, all these biscuits have been produced using mushroom flour. In this study, we have used fresh mushrooms, which will eliminate the processing of fresh mushrooms into flour. This process usually involves several steps that are not only time consuming, but also expensive.

Physical Characteristics of the Food Products

Physical characteristics of the products studied are given in Table 3. The mushroom biscuits, which contained more ingredients showed higher moisture levels than the soup premix. Furthermore, an increase in the content of mushroom slurry resulted in increased moisture of biscuits. The functional properties of foods and flours, including their water binding capacity are influenced by their components, especially the carbohydrates, proteins, fats and oils, moisture, fibre, ash, and other ingredients or food additives added to the food (flour), as well as the structures of these components (Awuchi *et al*., 2019).

Sinkability defines how well a food powder will dissolve and form a stable suspension. Results obtained for the mushroom soup indicates that it will reconstitute well to give a soup with a good mouth feel.

Table 3: Physical Characteristics of Food Products

Food Product	% composition, Mushroom Powder	Moisture (%)	Sinkability mean (mg/cm^2 of surface area		
Mushroom Biscuit	0	28.5 ± 0.9			
	15	34.8± 1.0			
	20	37.1± 0.7			
	25	39.4 ± 1.3	-		
	30	46.8± 1.2			
	35	47.1± 0.9			
	40	47.7± 1.1			
			0 min.	2 min.	4 min.
Mushroom instant soup premix	100	17.4 ± 0.7	4.73±.01	5.62±.03	13.70±.02

Acceptability of Food Products

Results in Table 4 indicate that the colour and apprearance of the biscuits to which no mushrooms were added (0% substitution) was more acceptable to the panelists than those containing mushrooms. This would probably be because they are more accustomed to this formulation and this biscuit was better leavened than those containing mushrooms (Plate 1). However, their scores for other characteristics, aroma, taste and chewiness were higher for biscuits, which contained mushrooms. Edible mushrooms are one of the major sources of umami flavor, which imparts a pleasant savory taste to foods. Today, umami flavor is recognized as the 5th basic taste and one of the most effective flavor enhancers used in different food products (Khan *et al*., 2020). Overall, the biscuits were acceptable to the panelists, with biscuits substituted at 25% showing the highest rate of acceptability. It would seem that higher concentration of mushrooms resulted in saltiness, which is a characteristic also associated with unami taste.

Sensory attributes of the instant soup as shown in Table 5 also indicated a good overall acceptability of this product. Unami taste as discussed earlier would also have contributed to the good taste and flavor of the product. Comparison of the different formulations made by the panelists served to determine the effect of the thickener on particularly the mouth feel of soup. "Achi" thickener added at the rate of 2 g/10 g of mushroom powder gave the best results and also sored highest in overall acceptability. The prepared soup of this formulation is shown in Plate 2.

Table 4: Sensory attributes of Mushroom Biscuits

Characteristics	Mean panelists Scores For different Mushroom Biscuit formulations						
% mushroom Substitution of flour in formulation	0	15	20	25	30	35	40
Colour & Apprearance	7 ± 1.0	5 ± 0.5	5 ± 0.4	5 ± 1.4	5 ± 0.6	4 ± 0.7	3 ± 0.8
Aroma	6 ± 1.6	6 ± 1.0	7 ± 0.8	7 ± 1.0	7 ± 0.8	7 ± 0.5	5 ± 1.4
Taste	5 ± 2.0	5 ± 2.4	6 ± 1.0	7 ± 0.8	7 ± 1.0	6 ± 0.8	5 ± 1.3
Chewiness	5 ± 1.0	6 ± 0.9	6 ± 2.0	7 ± 0.7	6 ± 1.5	5 ± 1.1	5 ± 2.0
Overall Acceptability	5 ± 0.9	5 ± 0.7	6 ± 0.7	7 ± 1.3	4 ± 1.0	4 ± 0.4	4 ± 1.3

Plate 1: Colour and Appearance of Mushroom Biscuit formulations according to % mushroom slurry content

Table 5: Sensory attributes of Mushroom Soup

Characteristics	Mean Panelists Scores for different Mushroom soup formulations				
Quantity(g) "achi"/10 g mushroom powder	0.5	1	1.5	2	2.5
Colour & Apprearance	5 ± 0.4	4 ± 0.4	4 ± 0.8	5 ± 0.6	5 ± 0.7
Aroma	6 ± 1.0	6 ± 0.8	7 ± 1.4	7 ± 0.5	7 ± 0.9
Taste	6 ± 1.1	6 ± 1.4	6 ± 0.7	6 ± 0.7	6 ± 0.9
Mouth feel	4 ± 0.4	4 ± 0.7	5 ± 0.7	7 ± 0.7	5 ± 1.0
Overall Acceptability	5 ± 0.6	5 ± 1.0	5 ± 1.0	8 ± 1.1	6 ± 0.8

Antioxidant Properties of Food Products

The yield of extracts from 10 g of each of the food products and their total antioxidants in mg of ascorbic acid equivalent (AAE) per 100 g is given in Table 6. Generally, as the content of mushrooms increased in the products, both the extract and the total antioxidant capacity of the products also increased. Other antioxidant activities including hydroxyl radical (*OH) scavenging ability and the DPPH radical scavenging ability determined for the formulated food products showed similar trends (Table 7). The antioxidant capacities found in these products are proportionately comparable to capacities found by Sifat et al., 2020 in *Pleurotus ostreatus* mushrooms in Bangladesh. They are also comparable to activities obtained by Elmastas et al. (2007), who studied wild mushrooms in Turkey.

From the revelations of Halliwell and Gutteridge (2003), Mwangi *et al*. (2022) and the clinical trial data of Dai *et al*. (2015) and Zhang *et al*. (2020), it is expected that if Nigerians consume mushrooms more regularly, they will obtain the benefits of reducing oxidative damage to their bodies, particularly when the natural mechanism of antioxidant protection becomes unbalanced by factors such as ageing. This will in the long run reduce the incidence of NCDs and accelerated ageing.

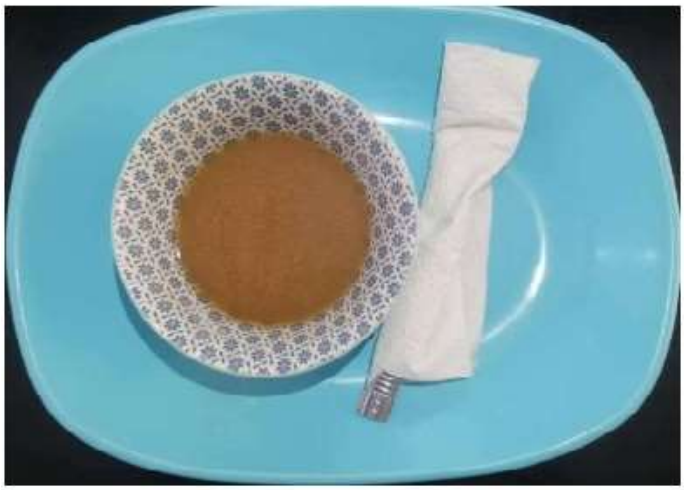

Plate 1: Colour and Apprearance of Mushroom Soup (2 g "achi" thickener)

Table 6: Extraction Yield (%) in ethanol solvent and Total Antioxidants from formulations of Food Products

Food Product	% composition Mushroom Powder	Extraction Yield (%)	Total Antioxidants (mg) of ascorbic acid equivalent (AAE) per 100 g
Mushroom Biscuit	0	8.2 ± 0.2	14 ± 1.1
	15	9.5 ± 0.1	40 ± 3.1
	20	11.7 ± 0.7	52 ± 2.4
	25	11.8 ± 0.3	73 ± 1.7
	30	11.4 ± 1.2	85 ± 1.2
	35	13.1 ± 0.3	91 ± 1.9
	40	14.5 ± 0.1	96 ± 1.2
Mushroom instant soup premix	100	34.2 ± 0.7	172 ± 2.2

Table 7: Other Antioxidant Activities of Preferred Food Product Formulations (IC_{50} values, mg/ml)

Food Product	% composition Mushroom Powder	Hydroxyl radical (*OH) scavenging ability	DPPH radical scavenging ability
Mushroom Biscuit	20	0.24 ± 0.03	0.1 ± 0.01
	25	0.31 ± 0.06	0.13 ± 0.03
	30	0.38 ± 0.01	0.15 ± 0.01
Mushroom instant soup premix	100	1.11 ± 0.04	0.48 ± 0.02

Shelf life Studies

The mushroom biscuits could be stored successfully at ambient temperature for between three and four days, for 0% mushroom substitution and biscuits fortified with mushrooms respectively. This is not surprising considering the high nutrient and moisture content of these food products. However, Calcium propionate and other chemical preservatives permitted in Nigeria for the preservation of bread and other baked goods may be applied to extend shelf life considerably.

The instant mushroom soup premix was stored successfully for up to 35 days. This product had less moisture and its packaging was more secure. It may be possible to dry the product further to extend shelf life even over a longer period of time.

CONCLUSION

It has been shown in this study that food products which are acceptable to many Nigerians can be developed using mushrooms. It has also been shown that these foods can carry the nutritional and functional components, including antioxidants obtained by eating mushrooms. The convenience of these foods and their enhanced shelf life will increase the ease of their distribution and hence increase availability of mushrooms and their health benefits to Nigerians.

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COMPETING INTERESTS
Authors have declared no competing interests.

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