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Evaluation of phytochemical and antioxidant properties of *Pleurotus pulmonarias* flour supplemented cookies

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Abstract

Mushrooms are valuable food plants sources which possess different kinds of bioactive components suitable as health beneficial compounds for the management of diseases. Limited knowledge and ignorance about its benefits to man has led to low uses of *Pleurotus pulmonarius* in Nigeria. Flours were developed from *Pleurotus pulmonarius* aseptically and use to compound composite flour with wheat flour for production of cookies.

Phytochemicals and antioxidant properties of the cookies produced from *Pleurotus pulmonarius* supplemented flours were investigated using standard analytical methods.

In this study, composite flour was obtained from blends of wheat and *Pleurotus pulmonarius* at ratio 100:0, 90:10, 85:15, 80:20 and 75:25 (w/w) to produce cookies. The Diphenyl picryl hydroxyl (DPPH) radical scavenging, Iron chelating assay, total phenolics content, and Ferric reducing antioxidant power (FRAP) assay of the wheat-mushroom cookies were determined using water extract.

Phytochemical results of the cultivated mushroom cookies, shows varying quantities of alkaloids (50-90%), saponins (11.05-12.09 µg/mL), terpenoids (8.03-12.05µg/mL), tannins (0.01-0.03 µg/mL), and steroids (0.39-1.72 µg/mL). The total flavonoid, FRAP, ABTS scavenging activity, Vitamin C, DPPH and iron (Fe^{2+}) chelating ability of the cookies produced increased significantly ($p < 0.05$) with increasing levels of mushroom flour. The phytochemical and antioxidant activities of the wheat-mushroom flour blended cookies showed several antioxidant properties which makes it a prospective functional food.

This study revealed that bioactive improved cookies produced as a food-based approach are responsible for inhibiting the oxidative damage caused by free radicals on health in developing countries.

Keywords: Phytochemical; Mushroom; FRAP; DPPH; Antioxidant; Supplemented

1. Introduction

Cultivated *Pleurotus pulmonarius* are prominent edible forest food products that are relatively simple, low-cost, and highly profitable (Gupta *et al*., 2019, Raman *et al*., 2021). *Pleurotus pulmonarius* is an excellent food supplement with

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phytochemicals that have as an anti-inflammation, anti-carcinogenic, hypocholesterolemic, hypoglycaemic, and immunostimulant effects (Oloruntola and Omotosho, 2019; Goswami *et al*., 2020). *Pleurotus pulmonarius* has been recognized as a therapeutic valuable mushroom with an important source of food and medicine to man. It can be cultivated on large and small scales or collected in the wild (Bamigboye *et al*., 2021).

Edible mushrooms have been widely utilized as human food for centuries and are appreciated for their texture, flavour, as well as dietary supplements, medicinal and tonic attributes (Oghenemaro *et al*., 2020, Manzi *et al*., 2001). Many bioactive compounds have been identified in mushroom extracts and are available for use Mushrooms are rich in dietary sources with important bioactive compounds and micronutrients that are known to decrease in humans as they age (Beelman *et al*., 2019). Mushrooms are important plant-based diet with high nutritional (protein and fibre) and medicinal values (Akinwande *et al*., 2020).

Cookies are leavened chemically baked products containing a high quantity of fat and sugar (Sannake *et al*., 2021). They are one of the largest consumed categories of snacks among baked foods all over the world (Asefa *et al*., 2017). Consumption of snack like biscuits, cookies, wafers and shortbread as foods is very common, especially among children, in Nigeria. The rigging cost of wheat flour has led to high price of production and cooking in Nigeria. This concern has attracted attention of food scientist on the possibility of augmenting the cooking composition with other materials. This development opens opportunity for incorporation *P. pulmonarius* in the cookie's formulations.

Conversion of this wheat-mushroom flour into natural products like snacks can serves as food and medicine. In this study, the phytochemical and antioxidant activities of the cookies produced from *Pleurotus pulmonarius* and wheat flour were determined using an established method. The aim of this study is to evaluate phytochemical and antioxidant properties of cookies produced from *Pleurotus pulmonarius* and mushroom flour.

2. Materials and methods

2.1. Materials

Wheat flour was purchased from a super market shop in Ogbomoso, Nigeria and freshly harvested *Pleurotus pulmonarius* mushroom fruiting bodies were obtained from a Mushroom Farm at Ilupeju-Offatedo, Osogbo, Osun State, Nigeria. The *Pleurotus pulmonarius* was characterized and registered with the National Center for Biotechnology Information - GenBank Database under the accession number MK751847.

2.2. Sample Preparation Method

The freshly harvested mushrooms were washed thoroughly under running tap water to remove adhering soil particles and then drained in a sieve. Then, the mushrooms were sorted and sliced manually into 3±1 mm thickness using a stainless-steel knife (Tolera and Abera, 2017). The sample was dried at 40 ^{o}C for 24 h using a heat drying oven, (Model DHG-9053A). The dried mushroom was milled into flour using an electric blender (Universal heavy duty, 1100watts, INDIA-421302) and sieved (150 µm), then packed in airtight opaque zip-lock low-density polyethylene bags, labelled and kept inside a plastic container. The container was stored inside a freezer at -4 ^{o}C

2.3. Mushroom cookies formulation

The composite flour samples were produced from wheat and mushroom flours at different ratios of 90:10, 85:15, 80:20 and 75:25, respectively to form a blend (Table 1) (Biao *et al*., 2020). Cookies were prepared using the method described by Kolawole *et al*. (2020) with slight modifications. Other ingredients added were egg albumin, milk, salt, baking powder, cocoa butter and sugar. The dough was prepared in the laboratory using a manual mixing method. The egg albumin, cocoa butter and sugar was creamed together in a bowl using a stirrer until a soft and spongy texture was formed. The formulated blend of refined wheat flour and mushroom flour was added alongside, milk, salt, and baking powder and mixed gently with the creamed mass of shortening and sugar powder mixture (Kolawole *et al*., 2020). The dough was prepared by adding the required amount of water to the mixture. The dough was rolled out and sheeted by a rolling pin and cut using a rectangular cutter. The formed batter was baked in an oven at 160 °C for 15-20 minutes, cooled and stored in air-tight pouches for further analyses. The Flow chart for the wheat-mushroom cookies is shown in Figure 1.

Table 1 Formulation of Composite Flour Blends

Sample code	Wheat flour (%)	*Pleurotus plumonarius* (%)
WFC	100	
WMC10	90	10
WMC15	85	15
WMC20	80	20
WMC25	75	25

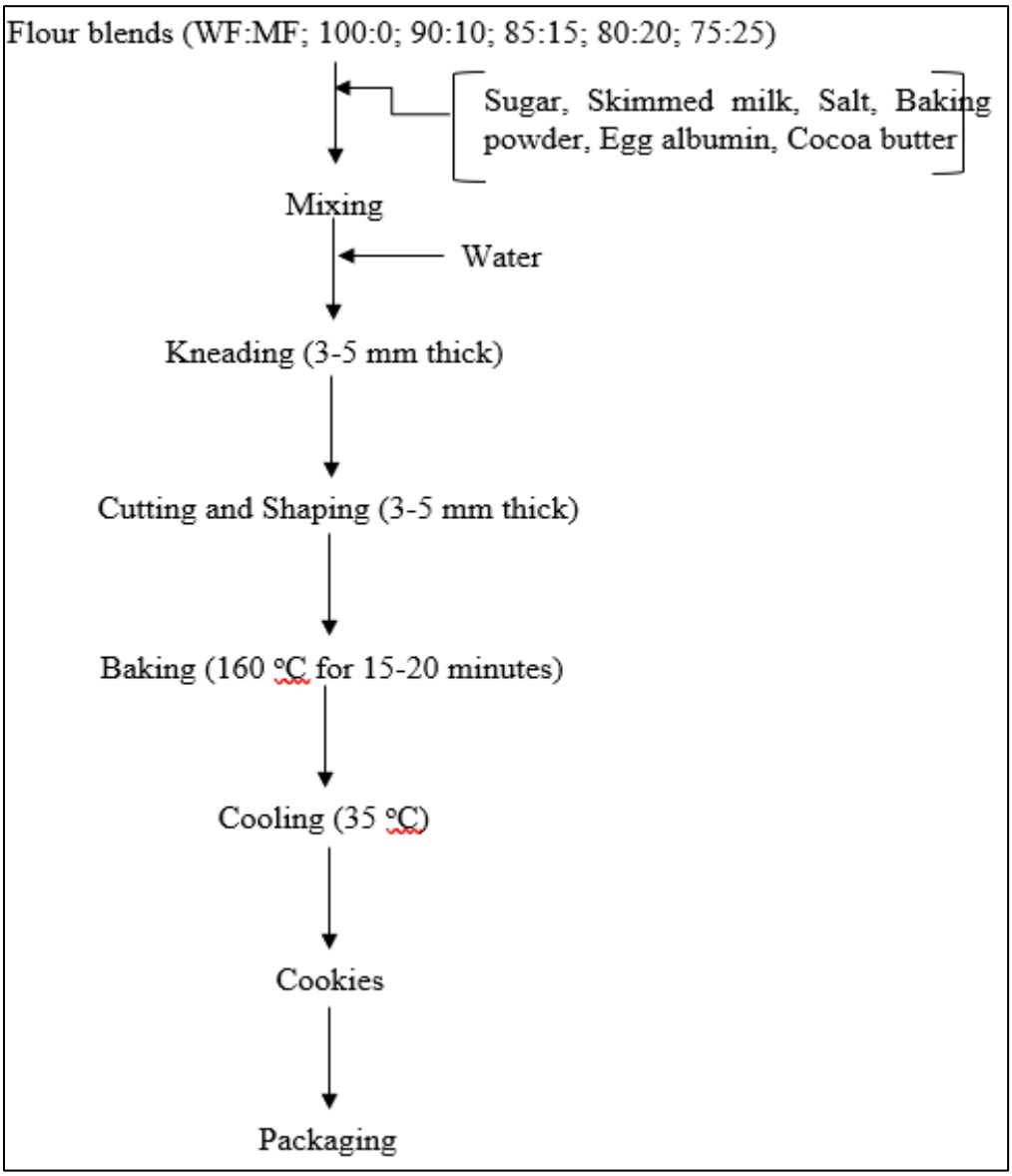

Figure 1 Flow chart for preparation of cookies

2.4. Determination of Phytochemical Components of Wheat-Mushroom Cookies

The wheat mushroom cookies were screened for phytochemical constituents such as alkaloids, flavonoids, tannins, steroids, saponins, and terpenoids. The quantitative analysis of phytochemical constituents was carried out using standard procedures (Ogidi *et al*., 2019).

2.4.1. Alkaloids

Forty (40) mL of 10% acetic acid in ethanol was added to 1 g of the wheat-mushroom cookies sample. It was covered and allowed to stand for 4 h. Then the filtrate was concentrated in a water bath to get $^1/_4$th of its original volume. Concentrated ammonium hydroxide was added dropwise to the sample until the precipitation was completed. The solution was allowed to settle for few minutes and the precipitate was collected and washed with dilute ammonium hydroxide and then it was filtered to obtain the residue which was dried and weighed (Ogidi *et al*., 2019).

2.5. Flavonoids

The total flavonoid content of the wheat-mushroom cookies sample was determined by following the Aluminium chloride method. The samples were mixed with $NaNO_2$ solution, distilled H_2O, and $AlCl_3$ solution after 6 mins, NaOH solution and distilled H_2O were added to the mixture to achieve the final volume. The mixture was vortexed extensively and stood for another 15 minutes. The optical density of the mixture was recorded using a spectrophotometer at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoids. The total flavonoids were calculated using the standard curve and expressed as rutin equivalent in mg/g of the sample Ogidi *et al*. (2019) as shown in equation 1.

$$Total\ Flavonoid = \frac{A_S \times St_c}{A_{st} \times Sa_c} \quad (1)$$

Where A_s = Absorbance of Sample; St_c = Standard Concentration; A_{st} = Absorbance of Standard and Sa_c = Sample Concentration

2.5.1. Tannins

Total tannins were determined by a slightly modified Folin and Ciocalteu method. Wheat-mushroom cookies of 0.5 g were added to 3.8 mL of distilled water, 35% sodium carbonate solution, and 0.25 mL of Folin Phenol reagent. The absorbance was measured at 725 nm. Tannic acid dilutions from 0 to 0.5 mg/mL were used as standard solutions. The result of tannins was expressed in terms of tannic acid in mg/mL of the sample (Ogidi *et al*., 2019).

2.5.2. Total terpenoid

The terpenoid content of the wheat-mushroom cookies samples was determined using Ogidi *et al*. (2019) method. The wheat-mushroom cookies sample (1 g) was macerated with 50 mL of ethanol and filtered. The filtrate 2.5 mL of 5 % aqueous phosphoric acid solution and concentrated H_2SO_4 was added and mixed, then allowed and left to stand for 30 minutes, 12.5 mL of ethanol solvent was used to make up and absorbance was taken at 700 nm on the spectrophotometer.

2.5.3. Saponins

Two (2) mL of 80 % methanol, 72 % sulphuric acid solution, and vanillin in ethanol solvents were dissolved with the pulverized wheat-mushroom cookies and mixed thoroughly and heated in a water bath at 60 °C for 10 minutes, the absorbance of 554 nm was measured against reagent blank. Diosgenin was used as standard material and assay compared with Diosgenin equivalents.

2.5.4. Steroids

The steroid content of the wheat-mushroom cookies sample was determined using the method described by Trease and Evans (2009). A portion of 2 mL was taken from a solution of 2.5 g of wheat-mushroom cookies prepared in 50 mL of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 mL of 0.1M NaOH (pH 9) and later mixed with 2 mL of chloroform and 3 mL of ice-cold acetic anhydride followed by the cautious addition of two drops of concentrated H_2SO_4. The absorbance of both sample and blank was measured using a spectrophotometer at 420 nm.

2.6. Determination of Antioxidant Activities of Wheat-Mushroom Cookies

The Diphenylpicryl hydrazyl (DPPH) radical scavenging, Iron chelating assay, total phenolics content, and Ferric reducing antioxidant power (FRAP) assay of wheat-mushroom cookies were determined using standard methods. An aqueous solution was prepared by weighing 2 g of each sample into a reagent bottle and mixed with 50 mL of distilled

water. It was vigorously shaken for 45 hr using an orbital shaker and then filtered with Whatman filter paper. The aqueous extract was used for the antioxidant analysis.

2.7. Diphenyl picryl hydroxyl (DPPH) radical scavenging activity

A quantitative DPPH antioxidant assay was performed on the aqueous extract of the wheat-mushroom cookies at 0.5 mg/mL in triplicates to ascertain their degrees of activity following the standard and modified method by Okonkwo *et al*. (2019). Six different concentrations were obtained by two-fold serial dilution, and the stock solution 1 mg/mL prepared for the wheat-mushroom cookies were used for the quantitative DPPH antioxidant assay to determine their respective inhibition concentration capable of scavenging 50% of the free radicals produced from DPPH solution (IC_{50}).

Two (2) mL of each of the test concentrations in a test tube wrapped with an aluminium foil to prevent exposure to light, 2 mL of 0.02% DPPH solution in methanol was separately added and the mixtures were shaken and incubated in the dark for 30 minutes. The absorbance of the reaction between DPPH solution and the wheat-mushroom cookies were measured at 516 nm against a blank (distilled water) using a spectrophotometer. All determinations were carried out in triplicate and he radical scavenging activity was calculated using the formula below in equation 2.

$$Inhibition\ of\ DPPH\ radical\ (\%) = \frac{[A_0 - A_1]}{A_0} \times 100\% \qquad (2)$$

Where: A_0= Absorbance of reference (control solution containing all reagents except the test extract) and

A_1= Absorbance of the sample. The IC50 value is the inhibitory concentration of aqueous wheat-mushroom cookies scavenged 50% DPPH radicals and it was obtained by extrapolation from the regression curve.

2.7.1. Iron chelating assay

The aqueous wheat-mushroom cookies ability to chelate the ferrous ions (Fe^{2+}) in ferrous chloride was estimated by a standard method by Okonkwo *et al*. (2019). Two (2) mL of 200 µM ferrous chloride were added separately to 2 mL of varying concentrations of the aqueous solution (20, 40, 60, and 80 µl). Reaction occurred after adding 0.25% phenanthroline. The mixture was shaken strongly and left to stand at room temperature for 10 minutes before measuring the absorbance at 510 nm. The percentage inhibition of Iron chelating potential was calculated using the formula in equation 3.

$$Iron\ chelating\ potential(\%) = \frac{[A_0 - A_1]}{A_0} \times 100 \qquad (3)$$

Were

A_0= Absorbance of reference (control solution containing all reagents except the test extract); and A_1= Absorbance of the sample. The IC50 value is the inhibition concentration of the extract that resulted in 50% iron chelating action and it was obtained by extrapolation from the regression curve.

2.7.2. Total phenolic content

The concentration of phenolic compounds in the aqueous extract of the wheat-mushroom cookies was determined using gallic acid as standard by the method of Mukhia *et al*. (2014) with slight modifications using Folin–Ciocalteau's reagent. The extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 mL) were mixed with 1.0 mL of tenfold diluted and 0.8 mL of 7.5% sodium carbonate solution. After standing for 30 minutes at room temperature, the absorbance was measured at 765 nm. The estimation of phenolic compounds in the fractions was carried out in triplicates and the results were averaged in mg/g as shown in equation 4.

$$Total\ Phenol = \frac{A_S \times St_c}{A_{st} \times Sa_c} \qquad (4)$$

Where As = Absorbance of Sample; Stc = Standard Concentration; Ast = Absorbance of Standard and Sac = Sample Concentration

2.7.3. Ferric reducing antioxidant power (FRAP) assay

The Ferric reducing activity of the aqueous extract of the wheat-mushroom cookies was determined according to the method of Katalinic *et al*. (2004). About 1.5 mL of FRAP reagent containing TPTZ (10 mM in 40 mM HCl), $FeCl_3$ (20 mM), and acetate buffer pH 3.6 (300 mM) in a ratio of 10:1:1 (v/v/v) was pre-incubated at 37 ^oC for 8 minutes and then mixed with 150 µl of deionized water and 50 µl of the test sample. The mixture was left to stand at 37 ^oC for 8 minutes and the absorbance was measured at 593 nm using a spectrophotometer (JENWAY 6405 Model, UK, and England). A calibration curve was obtained from various concentrations of $FeSO_4.7H_2O$ (0.1 – 1 mM). The FRAP value was expressed as µM of Fe^{2+} equivalents per gram of extract and calculated using the formula in equation 5.

$$FRAP\ value = \frac{A_S \times St_c}{A_{st} \times Sa_c} \quad (5)$$

Where: As = Absorbance of Sample; Stc = Standard Concentration; Ast = Absorbance of Standard, and Sac = Sample Concentration

2.8. Statistical analysis

Data obtained from this study were subjected to the Statistical Package for Social Sciences (SPSS, IBM SPSS Statistical version 21) using on-way analysis of variance (ANOVA). The mean was separated using the Least Significant Difference (LSD) test of all determined parameters. Multiple comparisons, mean separation of all test parameters, and follow-up tests were carried out using Duncan Multiple Range Test. All statistical test was carried out at a 95% confidence level.

3. Results and Discussion

3.1. Phytochemical components of wheat-*P. pulmonarius* cookies

The phytochemical quantitative analysis of the wheat-*P. pulmonarius* cookies (Table 2) revealed the presence and the number of alkaloids, saponins, terpenoids, tannins, and steroids.

Alkaloid content of cookies produced from wheat- *Pleurotus plumonarius* flour ranged from 50 to 90%. The cookies with (0, 10, 15, 20, 25%) mushroom inclusion had value ranged from 50 to 90%. Sample WMC10 had the highest value (90%) while wheat flour cookies had the lowest value (50%). The alkaloids decreased with an increase in the *Pleurotus plumonarius* flour may be due to the effect of processing into snacks. The alkaloids are the class of nitrogenous compounds produced by numerous plants as secondary metabolites for defense, herbivory, and to protect from pathogenic organisms and harmful insects (Shantabi *et al*., 2014). According to Chaves-Valadao *et al*. (2015), the pharmaceutical properties of mushrooms may be attributed to the presence of alkaloids which have been reported to be active against malaria, arrhythmia, hypertension, cardiovascular disorders, cancer and immunodeficiency virus in man. Also, alkaloids are known to have the ability to interchange with DNA and terminate the division of cells (Alafid *et al*., 2019).

The saponin content of the wheat mushroom cookies samples ranged between 11.05 and12.09 µg/mL. The 100% wheat sample (WFC) had the highest value (12.09 µg/mL), while sample WMC25 had the lowest value (11.05 µg/mL). The saponin content decreases with an increase in the *Pleurotus plumonarius* mushroom flour maybe due to the reaction that occur in the composite flour.

Table 2 Phytochemical contents of wheat-*P. pulmonarius* cookies

Samples	Alkaloid (%)	Saponin (µg/mL)	Terpenoid (µg/mL)	Tannin (µg/mL)	Steroid (µg/mL)
WFC	50.00±0.01^e	12.09±0.21^a	12.05±0.41^a	0.01±0.03^c	1.72±0.58^a
WMC10	90.00±0.00^a	11.97±0.39^a	11.06±0.56^b	0.02±0.03^b	0.89±0.05^b
WMC15	80.00±0.00^b	11.56±0.98^{ab}	9.65±0.72^c	0.02±0.06^b	0.76±0.10^c
WMC20	76.00±0.01^c	11.20±1.39^b	9.36±0.64^d	0.03±0.02^a	0.54±0.13^d
WMC25	70.00±0.00^d	11.05±1.38^b	8.03±0.68^e	0.03±0.01^a	0.39±0.08^e

Values are of replicates ± standard deviation. The values within the columns with different superscripts are significantly ($P<0.05$) different. Wheat Flour Cookies (WFC) = 100%, Wheat Mushroom Cookies (WMC) WMC10 = 10% MF and 90% WF; WMC15= 15% MF and 85% WF; WMC20= 20% MF and 80% WF; WMC25=25% MF and 75% WF.

The terpenoid contents of the various cookie samples ranged from 8.03 to 12.05 µg/mL. The 100% wheat sample (WFC) had the highest value (12.05 µg/mL), while WMC25 had the lowest value (8.03 µg/mL). The terpenoid content decreases with an increase in the *Pleurotus plumonarius* flour in the cookies, this may occur due to the biochemical reactions and processing technique. There was a significant difference among the samples. The range of values obtained as the tannin contents of various formulated cookie samples was between 0.01 and 0.03 µg/mL. The 100% wheat sample (WFC) had the lowest value (0.01 µg/mL), while sample WMC25 (25% mushroom inclusion) and sample WMC20 (20% mushroom inclusion) had the highest value (0.03 µg/mL). The tannin content increased with an increase in the *Pleurotus plumonarius* mushroom flour. Tannin is a bioactive substance that hasten the healing of wounds and treatment of diseases (Ogidi *et al*., 2021). The steroid contents of the various cookie samples ranged from 0.54 to 1.72 µg/mL. The steroid content decreased with an increase in the *Pleurotus plumonarius* flour. The presence of steroids is in agreement with the reported characterization of ergosterol derivatives from Oyster mushroom species (Afieroho and Ugoeze, 2014). According to Ajaiyeoba *et al.* (2006), the antimalarial activity of the plant might be induced by the presence of bioactive compounds (phytochemicals) like alkaloids.

3.2. Antioxidant activities of wheat-*P. pulmonarius* cookies

The total phenol content obtained ranged from 2.61 mg·GAE/g to 5.77 mg·GAE/g (Table 3). Sample WMC25 had the highest value (5.77 mg·GAE/g) while the least value (2.61 mg·GAE/g) was obtained from WMC10. The total phenol content of the wheat-mushroom cookies increased with an increase in the mushroom content due to the high total phenol content of the *Pleurotus plumonarius* mushroom flour. Phenols are a major class of phytochemicals that are responsible for inhibiting the oxidative damage caused by free radicals generated inside the human body damage caused by free radicals generated inside the human body (Ihayere and Okhuoya, 2022). This may suggest that total phenol was the major antioxidant component in the *P. pulmonarius* and has contributed to their antioxidant activities (Azieana *et al*., 2017).

The range of values obtained as the Total flavonoid content (TFC) of the various samples was between 14.23 and 38.87 mg·QUE/g. Sample with100% wheat cookies sample (WFC) have the least value (14.23 mg·QUE/g) and 25% mushroom inclusion cookies having the highest (38.87 mg·QUE/g). All the samples were significantly different from one another and there was a significant increase in flavonoid contents with an increased level of *Pleurotus plumonarius* mushroom supplementation. This may be attributed to the high flavonoid content of the *Pleurotus plumonarius* mushroom flour since mushrooms contain flavoniods (Wang *et al*., 2022). Flavonoids are secondary metabolites produced by plants and have antioxidant and anti-inflammatory (Maleki *et al*., 2019).

The antioxidant properties as measured by FRAP inhibitory activities of the various cookie samples ranged between 0.39 and 0.56 mg AAE/g. The least antioxidant properties were obtained from (WFC) 100% wheat cookies (0.39 mg AAE/g) while the highest value (0.56 mgAAE/g) was (WMC25) cookies with 25% mushroom flour inclusion. It was observed that the FRAP of the cookies increased significantly with the enhancement in *Pleurotus plumonarius* mushroom flour supplementation level ($p<0.05$). Therefore, it was obvious that partially substituted mushroom flour greatly enhanced the FRAP. The higher FRAP activity of cookies might be due to the release of iron-chelated compounds. The vitamin C (ascorbic acid) of the various cookie samples as determined ranged between 2.31 and 3.47 mg AAE/100g. The result showed that the highest value 3.47 mg AAE/100g found in sample WMC25 while the lowest value (2.31 mg AAE/100g) was obtained from sample WFC. The vitamin C of the cookies increased with an increase in the mushroom content. There was a significant difference in the vitamin C content of the samples due to the effect of processing which can inhibit the activities in vitamin C through heat.

The antioxidant activities showing the inhibitory effect of the wheat-mushroom cookies on diphenylpicryl hydrazyl (DPPH) radical scavenging ability revealed that the trend in a concentration-dependent manner in WFC, WMC10, WMC15, WMC20 and WMC25 cookies samples has 60.87 mg/mL, 75.06 mg/mL, 86.95 mg/mL, 92.08 mg/mL and 97.39 mg/mL, respectively (Table 3). It was noted that the DPPH radical scavenging activity significantly increased with the increasing mushroom flour supplement levels ($P\leq 0.05$). The highest DPPH scavenging activity was observed in cookies with a 25% *Pleurotus plumonarius* mushroom flour addition (WMC25), while 100% wheat cookies samples had the lowest values. The result is similar to the report of Owheruo *et al.* (2023) that produce biscuit from wheat and Oyster mushroom flour. The DPPH method characterizes the antioxidant capacity of extracts against oxidation caused by free radicals (Kouassi *et al*., 2016). The DPPH method was used to evaluate the free-radical scavenging capacity of the mushrooms. It is described as a rapid, simple and convenient method for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors (Azieana *et al*., 2017).

Furthermore, the iron (Fe^{2+}) chelating ability in the WFC, WMC10, WMC15, WMC20 and WMC25 cookies samples has 48.93 mg/mL, 56.37mg/mL, 60.03 mg/mL, 65.98 mg/mL and 76.13 mg/mL, respectively (Table 3). The highest

chelating ability was found in cookies with a 25% *Pleurotus plumonarius* mushroom flour supplemented cookies (sample WMC25). The cookies produced from wheat-mushroom composite showed several antioxidant properties which makes it a prospective functional food. According to Olagunju *et al*. (2021), the potential of a food product to confer a health benefit is embedded in its ability to provide antioxidant properties. Ferrous ions are reported to be commonly found in food systems and the transition of metal ions promotes the formation of free radicals. However, the chelation of metal ions can reduce the production of reactive oxygen species by retarding metal-catalyzed oxidation. The antioxidant and phytochemical properties of the cookies suggest the ability of the wheat-mushroom cookies in the prevention and management of different diseases like malaria (Adetola *et al*., 2024), diabetics (Olagunju *et al*., 2021).

Table 3 Antioxidant activities of wheat-*P. pulmonarius* cookies

Sample	Total phenol (mg·GAE/g)	Total flavonoid (mg·QUE/g)	FRAP Inhibitory Activities (mg AAE/g)	Vitamin C (mg AAE/100g)	DPPH (mg/mL)	Fe^{2+} chelating activities (mg/mL)
WFC	3.63±0.11^{b}	14.23±0.17^{e}	0.39±0.02^{e}	2.31±0.15^{e}	60.87±0.01^{e}	48.93±0.03^{e}
WMC10	2.61±0.25^{e}	32.56±0.38^{d}	0.44±0.02^{d}	2.75±0.14^{d}	75.06±0.03^{d}	56.37±0.10^{d}
WMC15	3.57±0.16^{c}	35.06±0.38^{c}	0.49±0.03^{c}	2.99±0.17^{c}	86.95±0.02^{c}	60.03±0.01^{c}
WMC20	3.24±0.26^{d}	37.62±1.30^{b}	0.51±0.01^{b}	3.12±0.16^{b}	92.08±0.14^{b}	65.98±0.12^{b}
WMC25	5.77±0.64^{a}	38.87±0.25^{a}	0.56±0.01^{a}	3.47±0.14^{a}	97.39±0.04^{a}	76.13±0.02^{a}

Values are of replicates ± standard deviation. The values within the columns with different superscripts are significantly ($P<0.05$) different. Wheat Flour Cookies (WFC) = 100%, Wheat Mushroom Cookies (WMC) WMC10 = 10% MF and 90% WF; WMC15= 15% MF and 85% WF; WMC20= 20% MF and 80% WF; WMC25=25% MF and 75% WF.

3.3. Correlation analysis between phytochemicals and antioxidant activities of wheat-*P. pulmonarius* cookies

The relations between several phytochemicals and the antioxidant levels in the cookies made with *Pleurotus pulmonarius* flour were examined by using Pearson's correlation coefficients (r) as shown in Table 4. This study helps explain which bioactive substances give the strongest antioxidant effect and how they interact. A very high positive correlations were found between the antioxidant assays, FRAP, Vitamin C, DPPH radical scavenging activity and iron chelating ability in *Pleurotus pulmonarius* flour. That means the antioxidant content in the cookies is always affected by the mushroom flour. There is a very strong correlation between FRAP and Vitamin C, r = 0.995, between FRAP and DPPH, r = 0.985 and between FRAP and Iron Chelating, r = 0.98. A high significant relationship (r = 0.98) is found between Vit C and DPPH activity and between DPPH and Iron Chelating activity (r = 0.94).

This shows that the assays regularly reveal the antioxidant potential and actions observed in these samples. All antioxidant measures are strongly and positively correlated with Total Flavonoids (TF), with r values of 0.88 for FRAP, 0.91 for Vitamin C, 0.93 for DPPH and 0.83 for Iron Chelating. There are positive but moderate links between Total Phenolics and FRAP (0.645), Vit C (0.612), DPPH (0.508) and Iron Chelating (0.713). This finds that flavonoids in the samples are linked stronger to antioxidant activities than their class of total phenolics. Since flavonoids are categorized as phenolics with strong capacities to remove free radicals and bind to metals.

The flavonoid content and antioxidant capacity measured with different methods are highly associated with the tannin content (r = 0.91 – 0.94). Tannins, being polyphenols, are helpful antioxidants since they can capture free radicals and bind to metals, increasing their importance for antioxidant health. Alkaloids tend to have positive relationships with scores for Total Flavonoids (r = 0.73), FRAP (r = 0.34), Vitamin C (r = 0.41), DPPH (r = 0.44) and Iron Chelating (r = 0.26). That means alkaloids share some antioxidant properties with other compounds, but do not stand out as much as phenolics or flavonoids do.

Table 4 Result of the Correlation analysis between phytochemicals and antioxidant activities of wheat-*P. pulmonarius* cookies

	TP	**TF**	**FRAP**	**VITC**	**DPPH**	**Iron Chelating**	**Alkaloid**	**Saponin**	**Terpenoid**	**Tannin**	**Steroid**
TP	1										
TF	0.250007	1									
FRAP	0.645117	0.884444	1								
VitC	0.612314	0.913496	0.995079	1							
DPPH	0.508278	0.935194	0.984918	0.982803	1						
Iron Chelating	0.71346	0.829184	0.979944	0.979814	0.940149	1					
Alkaloid	-0.35929	0.729922	0.337495	0.410923	0.443705	0.264203	1				
Saponin	-0.63166	-0.79895	-0.96608	-0.94243	-0.95403	-0.95035	-0.17572	1			
Terpenoid	-0.67831	-0.86121	-0.99789	-0.98864	-0.97738	-0.97487	-0.29761	0.964431	1		
Tannin	0.405006	0.909934	0.923695	0.934165	0.94198	0.92351	0.458072	-0.92325	-0.89833	1	
Steroid	-0.3665	-0.98937	-0.9363	-0.95865	-0.96726	-0.90002	-0.63328	0.867738	0.915681	-0.95158	1

TP- Total Phenolics; TF- Total Flavonoids; FRAP- Ferric Reducing Antioxidant Power; Vit C -Vitamin C content; DPPH-DPPH radical scavenging activity; Iron Chelating- Iron chelating activity.

Additionally, Saponins have reliable negative relationships with antioxidant assays such as FRAP (r = -0.966), Vitamin C (r = -0.94) and others. The strongest negative correlations with FRAP (r = -0.998), Vit C (r = -0.99), DPPH (r = -0.98) and Iron Chelating (r = -0.97) are shown by terpenoids. Steroids tend to have a negative relationship with antioxidant activities, FRAP (r = -0.94), Vitamin C (r = -0.96), DPPH (r = -0.96) and Iron Chelating (r = -0.90). Such significant adverse relationships suggest that these chemicals do not improve the cookies' ability to neutralize free radicals. Because of this, they might disturb antioxidant tests and their presence is related to a lack of antioxidant phytochemicals. Saponins and terpenoids have a strong positive correlation (r = 0.96) and tend to associate with steroids, too, but not in such a strong way. This shows that even if these are usually found together, each compound might behave differently as antioxidants.

A weak positive correlation (r = 0.25) between TF and TP in the phytochemical data which suggests reasons could include different ways of extracting compounds and the fact that some flavonoids may be different from others. There is a strong relationship between tannins and TF (r = 0.91) and tannins are also related to alkaloids (r = 0.46). The associations are negative between TF and saponins and between TP and terpenoids; the strength is -0.79 and -0.68, respectively. These findings point to possible mixed effects of different plant chemical substances in the composition of *Pleurotus pulmonarius*.

The main positive influence of flavonoids and tannins in the antioxidant effect of *Pleurotus pulmonarius* flour-enhanced cookies is clearly seen on the correlation matrix. Total phenolics play a useful role, but only to a minor extent. Saponins, terpenoids and steroids have negative effects on antioxidants which means that getting the right balance of phytochemicals is essential to boosting the functional benefits in bakery products.

The analysis of the correlation data found that Total Flavonoids and tannins demonstrate close and strong links to antioxidant activities, with FRAP (r = 0.88, r = 0.92), vitamin C (r = 0.91, r = 0.93), DPPH (r = 0.94, r = 0.94) and iron (r = 0.83, r = 0.92). An analysis showed that moderate positive correlations existed between Total Phenol and the antioxidant measures, indicating that total phenolics contribute to fighting oxidation. On the other hand, saponins, terpenoids and steroids were connected with below-zero correlations (-0.87 to -0.99) for antioxidant parameters, meaning they may have an inhibitory or weak effect on antioxidant signaling. Antioxidant activity was linked in a positive way to increasing alkaloid levels. It is clear from these results that flavonoids and tannins are the main contributors to the antioxidant power of *P. pulmonarius* flour in the cookies.

4. Conclusion

Phytochemical analysis of the cookies showed that mushroom is rich in different type of secondary metabolites which are essential for man's life. The study showed that the cookies produced from the edible mushroom (*Pleurotus pulmonarius*) can be used as more efficient and potent antimicrobial agents. The phytochemical and the antioxidant obtained in the cookies produced reveal the evidence of the possible therapeutic use of mushrooms in traditional medicine. The consumption of the mushroom (*Pleurotus pulmonarius*) cookies will aid good health and healthy living.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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Author Contributions

- *Rachel Oluwatoyin Adetola*: Writing-original draft; investigation; methodology; data curation, formal analysis; writing-review and editing.
- *Gladys Ayangbenjo Akanbi*: reviewing and editing of the manuscript
- *Bolanle A. Akinwande*: Conceptualization, project administration, supervision, validation, writing-review and editing, reading of final draft, reviewed manuscript.
- *Grace O. Babarinde*: Supervision, validation, writing-review and editing, reading of final draft, reviewed manuscript
- *Ganiyu Oboh*: Methodology, supervision, validation.

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