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Nutritional and Techno-Functional Properties of Flours Processed from *Dioscorea bulbifera* Fermented with Edible *Calocybe indica* and *Pleurotus ostreatus*

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Abstract

A medicinal and nutritional value product that is underutilised is *Dioscorea bulbifera* (air yam). Its usage as a potential and functional foods have been compromised due to traditional belief and preparation challenges. This study aimed to enhance the nutritional, functional, and antioxidant properties of *D. bulbifera* by fermenting it with edible mushrooms, *Calocybe indica* and *Pleurotus ostreatus.* The treatment of *D. bulbifera* bulbils were segregated into four; A control thus unfermented (UDB), submerged fermented (FDB), fermented with *C. indica* (DBC), and fermented with *P. ostreatus* (DBP). The phytochemical composition, nutritional composition, protein digestibility, amino acid profiles, starch digestibility, antioxidant activity, colour indices, and functional properties of the samples were evaluated using standard analytical methods. The nutritional and functional qualities of *D. bulbifera* flours were improved significantly through fermentation. The amino acid phenylalanine and Leucine increased by 15.1 % and 32.5–32.8 % in DBC and DBP respectively as compared to UDB. In addition, the slowly digestible starch (SDS) content rose by 8.3–11.49 % in DBC and DBP as shown in UDB. The functional properties were also elevated in the treatment as compared to the control, with water absorption capacity and water solubility index increased by 131.3 % and 76.1 % in DBP as compared to UDB. However, there was decrease in the Bulk density of the fermented samples implying the flour has low texture. The study suggests that fermented *D. bulbifera* flours could be used to manage chronic conditions such as diabetes and oxidative stress because of enhanced antioxidant activity observed in the treatment ($p < 0.05$). Fermenting *D. bulbifera* flour with edible mushrooms have shown an improvement in nutritional and functional properties of the *D. bulbifera* flour and could be use as functional food. The study also sought to address nutritional and health challenges in relation to chronic conditions such as diabetes and oxidative stress.

Keywords: *Dioscorea bulbifera*, Fermentation, *Calocybe indica*, *Pleurotus ostreatus*, Nutritional properties, Functional properties

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1. Introduction

Nutrients in food are important for maintaining health, supporting growth, and preventing diseases [1]. Fermented foods, edible mushrooms, and herbal products are gaining attention for their health benefits including managing conditions such as osteoporosis, metabolic syndrome, heart diseases, obesity, and diabetes [2]. Many people globally suffer from malnutrition which leads to serious deficiencies in nutrient and energy in areas where there is scarcity in food [3]. According to [4], the swift increase in population across globe has worsened food insecurity, prompting interest in underutilized crops such as air yam (*Dioscorea bulbifera*). Food security and health of individuals could improve through the use of underutilized *D. bulbifera* alongside edible

mushrooms such as *Calocybe indica* and *Pleurotus ostreatus*. *D. bulbifera* contains compounds with medicinal benefits for ulcers, diabetes, and dysentery [5]. Mushrooms on the other hand have health-promoting properties and can manage chronic diseases like diabetes without side effects [6].

Fermentation, a common food processing method boosts the nutritional and medicinal qualities of these ingredients. It increases nutrient absorption, removes harmful compounds, and extends shelf life [7]. Many people across the globe avoid *D. bulbifera* because of the misconception associated with its edibility and challenges in the preparation process [8], despite its numerous benefits. In Asia and Africa to be specific, the major problem remains malnutrition and food insecurity. According to [9], rapid growth in population in Asia and Africa, have widen gap between food supply and demand. In order to resolve these issues, different ways of producing food are needed, this includes creating functional foods that could reduce malnutrition and manage chronic conditions such as obesity and diabetes. This study aims to create a functional food by fermenting *Dioscorea bulbifera* with *Calocybe indica* and *Pleurotus ostreatus* through solid-state fermentation. The study contributes to global goals like SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), and SDG 12 (Responsible Consumption and Production). It promotes sustainable food production, better nutrition, and solutions to chronic diseases.

2. Materials and Methods

2.1. Collection of Samples

The Spawns of *Pleurotus ostreatus* and *Calocybe indica* were obtained from the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos, Nigeria. These spawns were grown on potato dextrose agar (PDA) medium and kept in a dark chamber at 25 ± 2°C for seven days. Bulbils of *Dioscorea bulbifera* (Air Yam) were purchased from Akpan Market in Gboko, Benue State, Nigeria.

2.2. Sample Preparation

The bulbils were washed and divided into four groups, unfermented Sample (UDB), Submerged Fermented Sample (FDB), Solid-State Fermentation with *C. indica* (DBC), and Solid-State Fermentation with *P. ostreatus* (DBP). The UDB sample was prepared by peeling the bulbils, sun-dried for seven days and ground into powder. A 250 g of peeled bulbils fermented in a medium for 3 days, sun-dried, and ground into powder to formulate FDB. A 250 g of peeled bulbils fermented with *C. indica* mycelium grown in sterilised potato dextrose broth, cooled, and left in a dark place for seven days before drying and grinding into powdered form to formulate DBC. The DBP process was similar to DBC but used *P. ostreatus* mycelium instead. Each sample well labelled accordingly.

2.3. Protein Digestibility Studies

The method by [10] was adopted for the protein digestibility was test. 250 mg of each sample was mixed with 15 mL of 0.1 M hydrochloric acid containing 1.5 mg pepsin. The mixture was incubated for two hours at 37°C in a shaking water bath. After incubation, the solution was neutralised with 0.5 M sodium hydroxide until the pH was 7.0. The suspension was then treated with 4 mg pancreatin dissolved in 7.5 mL of 0.2 M sodium phosphate buffer (pH 8.0) containing sodium azide. It was incubated for two hours and filtered. The filtrate was stored in a freezer overnight for protein precipitation. The precipitate was dried and analysed for protein content using the Kjeldahl method [11]. Protein digestibility-corrected amino acid scores (PDCAAS) were calculated as described by [11]. Amino acid profiles were determined using AOAC method 999.13 with a Sequential Multi-Sample Amino Acid Analyser (TSM).

2.4. Determination of Total Starch Content

The method by [12] was used to determine starch content. Residue from the protein digestibility test was washed with 80% ethanol, dried, and treated with 5 mL of water and 6.5 mL of 52% perchloric acid. The extract was diluted to 100 mL, and 0.2 mL of the supernatant was taken. The supernatant was adjusted to 1 mL with water, and 4 mL of anthrone reagent was added. The mixture was heated in a boiling water bath for eight minutes, cooled, and its colour intensity measured at 630 nm. Starch content was calculated by multiplying the glucose content by 0.9.

2.5. Resistant Starch (RS) Analysis

The resistant starch was determined using the method by [12]. A 100 mg sample was treated with 20 mg pepsin to remove proteins. Afterward, pancreatic amylase in a Tris-maleate buffer was used for hydrolysis at 37°C for 16 hours. The residue was treated with amyloglucosidase from *Aspergillus niger* at 60°C for 45 minutes. Glucose content was measured using a glucose oxidase-peroxidase kit, and RS was calculated as glucose content (mg) multiplied by 0.9.

2.6. Rapid Digestible Starch (RDS)

The determination of RDS was done by weighing 5 g of the sample, treated with alpha-amylase and amyl glucosidase enzymes for 30 minutes. Enzyme activity was stopped by heating the mixture. Glucose content was measured using a glucose assay kit, and the RDS percentage was determined based on glucose content and the sample weight, as described by [13].

2.7. Slowly Digestible Starch (SDS)

The SDS was determined by using alpha-amylase to digest 5 g of the sample. The enzymatic activity is inhibited by heating, and the SDS content was measured using spectrophotometry. Therefore, the percentage of the SDS was determined following the method by [13].

2.8. Phytochemical Analysis

2.8.1. Total Phenols

The Folin-Ciocalteu procedure described by [14] was used to determine the total phenolic content. An amount of 125 µl of sample extract was mixed with distilled water and Folin-Ciocalteu's reagent. Sodium carbonate solution (7 %) was added after Six (6) minutes of mixing. This was allowed to stand for 90 minutes. A SpectrumLab70 spectrophotometer was used to take the absorbance at a wavelength of 760 nm. The results were reported as gallic acid equivalents (GAE).

2.8.2. Flavonoids

The flavonoid content was determined by measuring 0.25 g of extract and dissolved in 1 ml of distilled water. A 5% $NaNO_2$ solution, 0.150 ml of fresh aluminium chloride ($AlCl_3$), and 1 M NaOH were added to the solution. The

solution was allowed to stand for 5 minutes and a SpectrumLab70 spectrophotometer was used to take the absorbance at 510 nm. The result was expressed as quercetin equivalents (QE) following [14].

2.9. Antioxidant Assays

2.9.1. DPPH Scavenging Assay

The DPPH assay evaluated the antioxidant activity of the extracts, based on methods by [15] and [16]. A 0.3 mM DPPH solution (1 ml) was mixed with 2.5 ml of extract at concentrations of 50, 100, 150, 200, and 250 µg/ml, as well as a standard ascorbic acid. The solution was incubated in dark at room temperature for 30 minutes. Absorbance was read at 517 nm, and percentage inhibition was calculated as:
DPPH radical scavenging activity (%) = [(Abs control – Abs sample)]/ (Abs control) ×100

Where Abs control is the absorbance of DPPH radicals + methanol; Abs sample is the absorbance of DPPH radical + sample or standard.

2.9.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measured the reducing power of the extracts using method described by [17] with modifications. A 0.5 ml sample or ascorbic acid (50, 100, 150, 200, 250 µg/ml) was added to the FRAP reagent. This reagent contained 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ, and 20.0 mM $FeCl_3 \cdot 6H_2O$. The mixture was incubated at 50°C for 20 minutes, and absorbance recorded at 700 nm. Higher absorbance showed greater reducing power.

2.10. Color Analysis

The color of the samples was determined using ColorTec-PCM (model SN 3000421, USA) instrument. The device was calibrated using a white paper (L* = 93.24, a* = 0.96, b* = 2.75). A 3 g sample was placed on paper, and the sensor recorded the L*, a*, and b* values. Chroma (C) and hue angle (h) were calculated using [18].

2.11. Functional Properties

- ***Water Absorption Capacity***

One (1) gram of sample was mixed with 10 ml distilled water in a centrifuge tube. After standing for 1 hour, it was centrifuged at 3500 rpm for 30 minutes. The unabsorbed water was drained, and absorbed water weight was calculated according to the method of [19]

$$\text{WAC} = \frac{w2-w1}{w3} \times \frac{100}{1}$$

Where:
W1= weight of sample
Weight of empty tube + sample used
W3=weight of empty tube + sample + water absorbed

- ***Water Solubility Index***

Five grams of sample was mixed with 25 ml distilled water. The mixture was incubated at 25°C for 60 minutes, then filtered. The insoluble residue was dried at 105°C. The water solubility index (WSI) was calculated as:

$$WSI = \frac{\text{weight of soluble solid}}{\text{weight of flour sample}} \text{ X } 100$$

Where:
Weight of soluble solids= initial weight of flour sample- the weight of insoluble residue after drying according to the method of [19].

- ***Swelling Capacity***

The swelling capacity of the sample was determined by the method described by [19]. A 100ml graduated cylinder was filled with each sample to 10ml mark, it was topped up with distilled water to make a volume of 50ml. The graduated cylinder tightly was covered and inverted to aid mixing the sample and the distilled water. The suspension was inverted again after 2 min and left to stand for 8 min. The volume occupied by the sample was taken after the 8th minute.

- ***Bulk Density***

Bulk density was determined according to the method described by [20]. Ten grams (10g) of the sample was weighed into a 25ml 25-graduated measuring cylinder. The sample was gently tapped continuously on a laboratory table to eliminate spaces b/w flour particles until a constant volume was obtained. The experiment was done in triplicate and mean was taken. Bulk density was calculated as:

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

2.12. Statistical Analysis

Data were shown as mean ± standard deviation (SD). Analysis was done with IBM SPSS Statistics v23. Differences in means were assessed using one-way ANOVA and Duncan's multiple range test. Significance was set at $p<0.05$.

3. Results and discussion

3.1. Impact of Fermentation on the Essential Amino Acids in Dioscorea bulbifera *with* C. indica *and* P. ostreatus

The essential amino acids in unfermented and fermented *Dioscorea bulbifera* are shown in Table 1, with fermentation significantly enhancing the nutritional profile. Fermentation with *C. indica* (DBC) and *P. ostreatus* (DBP) increased leucine levels to 73.00% and 74.00%, respectively, compared to 55.67% in unfermented samples (UDB), representing over 32% improvement. Phenylalanine rose by 15.1%, while valine increased by 25.1%, demonstrating the ability of fermentation to enhance amino acid content. There was significant difference ($p < 0.05$) between the control (UDB) and the treatments (DBC and DBP). Studies by [21] and [22] observed that leucine and valine are essential for blood sugar regulation and muscle growth. Lysine levels were slightly lower in fermented samples, suggesting minor degradation, possibly due to microbial metabolism, but remained nutritionally adequate. Similar report by [23], who saw that fermentation, could alter amino acid profiles. The improved leucine and valine levels in DBP highlight its potential for supporting muscle repair and protein synthesis, making fermented yam a valuable functional food for protein-deficient populations. Fermentation with edible fungi effectively enhances the essential amino acid composition of *D. bulbifera*, supporting its use in dietary supplementation.

3.2. Protein Digestibility of Fermented Dioscorea bulbifera

The protein digestibility of unfermented and fermented *Dioscorea bulbifera* combined with *C. indica* and

P. ostreatus are shown in Table 2. Fermented samples, DBC (0.88 ± 0.10) and DBP (0.87 ± 0.00), exhibited significantly higher protein digestibility compared to FDB (0.83 ± 0.12) and UDB (0.81 ± 0.07). These results reflect 8.6% and 7.4% improvements in digestibility for DBC and DBP, respectively. Among the samples, DBC achieved the highest protein digestibility-corrected score (40.93 ± 0.21), a 12% increase over UDB (36.54 ± 0.00), with DBP showing a comparable score of 40.57 ± 0.00. This improvement aligns with studies reporting that fermentation enhances protein quality by breaking down complex proteins into simpler peptides and amino acids, thereby improving bioavailability. Solid-state fermentation (DBC, DBP) proved more effective than submerged fermentation (FDB), likely due to the specific enzymatic activity of the mushrooms used. Similar studies on legume and cereal fermentation, where enzymatic hydrolysis during solid-state processes improves protein digestibility and nutritional value [24]. The higher mean observed in DBC and DBP suggest their potential as nutrient-rich options to address dietary protein deficiencies, particularly in malnourished populations.

3.3. Phytochemical Analysis of Unfermented and Fermented Dioscorea bulbifera

The quantitative phytochemical analysis of unfermented and fermented *Dioscorea bulbifera* with *Calocybe indica* (DBC) and *Pleurotus ostreatus* (DBP) revealed a substantial increase in bioactive compounds after fermentation, as shown in Table 3. The total phenol content in DBC (53.99 ± 0.02 mg/g) was significantly higher than that of unfermented *D. bulbifera* (UDB, 34.52 ± 0.02 mg/g), representing a 56.4% increase. Similarly, DBP also showed elevated phenol levels (51.99 ± 0.02 mg/g). Flavonoid content was highest in DBC (15.71 ± 0.01 mg/g), exceeding UDB (13.68 ± 0.01 mg/g) by 14.8%. These increases could be because of the enzymatic activities of the mushrooms during fermentation, which release bound phytochemicals, as supported by studies showing enhanced phenolic and flavonoid levels in fermented foods. Higher levels of these compounds correlate with increased antioxidant potential, suggesting that fermented samples, especially DBC, may be more effective in mitigating oxidative stress [25]. This study revealed that fermented products could serve as functional food and may have potential to managing oxidative stress-related health issues.

3.4. Starch Digestibility of Unfermented and Fermented Dioscorea bulbifera

The starch digestibility of unfermented and fermented *Dioscorea bulbifera* with *C. indica* and *P. ostreatus* showed a significant decrease in total starch content. It dropped from 60.54 ± 0.18 g/100 g in UDB to 57.52 ± 0.12 g/100 g in DBC and 57.65 ± 0.13 g/100 g in DBP, marking a 4.9% reduction. This study shows that fermentation can reduce the total starch content of tubers. The Slowly Digestible Starch (SDS) increased notably in both DBC (52.65 ± 0.21%) and DBP (54.16 ± 0.21%) compared to UDB (48.58 ± 0.20%), indicating an enhancement of 8.37% and 11.49%, respectively. This increase in SDS suggests that fermentation with *C. indica* and *P. ostreatus* makes the starch more resistant to rapid digestion. On the other hand, the percentage of Rapidly Digestible Starch (RDS) was lower in DBC (39.53 ± 0.21%) and DBP (38.30 ± 0.24%) than in UDB (46.16 ± 0.18%). This is shown in Table 4 below. The reduction in RDS highlights the potential of fermented *Dioscorea bulbifera* for better glycemic control, as lower RDS is associated with slower glucose release. These findings suggest that fermentation can modify the starch structure, leading to a product with a lower glycemic index. This is consistent with other studies that have shown fermented foods often have slower digestibility [26, 27]. which may benefit individuals needing to control blood sugar levels. Therefore, fermented Dioscorea bulbifera products are suitable for low-glycemic diets, which could help in managing conditions like diabetes.

3.5. Antioxidant Composition of Dioscorea bulbifera

The antioxidant composition of *Dioscorea bulbifera*, both unfermented and fermented with *C. indica* and *P. ostreatus*, was measured in terms of DPPH and FRAP activities (Table 5). The DPPH activity in the unfermented Dioscorea bulbifera (UDB) and fermented Dioscorea bulbifera (FDB) remained the same (82.54 mg AA/100 g), indicating no significant effect from fermentation on hydrogen-donating capacity. In contrast, the DPPH activity was slightly lower in both DBP (76.50 mg AA/100 g) and DBC (74.87 mg AA/100 g), which suggests fermentation with P. ostreatus and C. indica reduced this antioxidant activity. However, when examining the FRAP activity, DBP showed a notable increase (58.42 mg TE/100 g) compared to UDB (56.21 mg TE/100 g), marking a 3.9% improvement in reducing power. This indicates that fermentation, especially with *P. ostreatus*, enhances the reducing power of the product. Fermented products, particularly DBP, showed balanced antioxidant properties, making them potentially useful for managing oxidative stress-related diseases [30]. While UDB and FDB displayed higher DPPH values, DBP's superior FRAP value suggests it may have stronger antioxidant activity, possibly due to an increase in phenolic content. These findings align with previous studies, showing that fermentation can alter antioxidant mechanisms, improving some properties like reducing power, while slightly decreasing others like hydrogen-donating capacity [28, 29]. This highlights the potential of fermented *Dioscorea bulbifera* products in preventing chronic diseases through their natural antioxidant properties.

3.6. Color Index for Unfermented and Fermented Dioscorea bulbifera

The colour index results for unfermented and fermented *Dioscorea bulbifera* with *C. indica* and *P. ostreatus* are shown in Table 6. The fermented samples, including DBP, had a higher L* value (73.82 ± 0.11) compared to unfermented DB (71.65 ± 0.26), meaning they were brighter and more visually appealing. DBC exhibited the highest hue angle (81.98 ± 0.03), indicating a more attractive colour tone. The fermented samples also had higher Chroma values, with DBC (19.33 ± 0.03) and DBP (19.07 ± 0.06) outperforming unfermented DB (18.56 ± 0.05). This suggests that fermentation increases the saturation and intensity of the colour, making the product more appealing to consumers [30]. These results are consistent with other studies, which show that fermentation can improve the colour of food products by modifying pigments. Enhanced colour properties of fermented *Dioscorea bulbifera* make it suitable for use in food products where visual appeal is important.

Table 1: Digestibility-corrected Amino acid score for essential amino acids of unfermented and fermented *Dioscorea bulbifera* with C.indica and P.ostreatus as percentage

Treatment	Essential Amino acid						
	Leucine	**Lysine**	**Isoleucine**	**Phenylalanine**	**Valine**	**Methionine**	**Threonine**
UDB	55.67±0.89^{b}	72.33±1.45^{a}	47.67±1.20^{b}	59.67±0.89^{ab}	61.00±1.00^{b}	53.67±1.77^{a}	70.00±0.00^{a}
FDB	53.33±0.89^{b}	72.33±1.45^{a}	40.67±0.33^{c}	57.33±1.77^{a}	54.00±2.08^{c}	52.33±0.67^{a}	61.00±1.00^{c}
DBC	73.00±1.73^{a}	62.33±1.45^{b}	50.00±0.58^{a}	62.67±0.67^{b}	72.00±1.53^{a}	53.00±0.58^{a}	63.00±0.58^{c}
DBP	74.00±2.65^{a}	65.67±0.68^{b}	51.33±0.89^{a}	68.67±0.67^{a}	76.33±0.33^{a}	55.33±0.89^{a}	66.67±0.89^{b}

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus*

Table 2: Protein Digestibility of Unfermented and Fermented *Dioscorea bulbifera*

Treatment	Digestibility	Digestibility- Corrected Protein
UDB	0.81±0.07^{b}	36.54±0.00^{d}
FDB	0.83±0.12^{b}	36.99±0.00^{c}
DBC	0.88±0.10^{a}	40.93±0.21^{b}
DBP	0.87±0.00^{a}	40.57±0.00^{a}

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus*

Table 3: Phytochemical composition of unfermented and fermented *Dioscorea bulbifera*

Treatment	Total Phenols	Total Flavonoids
UDB	34.52±0.02^{d}	13.68±0.01^{c}
FDB	36.22±0.02^{c}	12.87±0.01^{d}
DBC	53.99±0.02^{a}	15.71±0.01^{a}
DBP	51.99±0.02^{b}	14.55±0.01^{b}

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus*

Table 4: Starch Digestibility of Unfermented and Fermented *Dioscorea bulbifera*

Treatment	Total starch(g/100g)	Resistant Starch (%)	Rapidly Digestible Starch (%)	Slowly Digestible Starch (%)
UDB	60.54±0.18^{a}	5.26±0.96^{a}	46.16±0.18^{a}	48.58±0.20^{b}
FDB	59.31±0.57^{b}	6.45±0.01^{b}	43.65±0.70^{a}	49.90±0.07^{b}
DBC	57.52±0.12^{c}	7.82±0.48^{b}	39.53±0.21^{b}	52.65±0.21^{a}
DBP	57.65±0.13^{c}	7.54±0.23^{b}	38.30±0.24^{b}	54.16±0.21^{a}

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus.*

Table 5: Antioxidant Composition of Unfermented and Fermented *Dioscorea bulbifera*

Treatment	**DPPH** (mg/AA/100g)	**FRAP**(mg/TE/100g)
UDB	82.54±0.00	56.21±0.00
FDB	82.54±0.00	54.76±0.00
DBC	74.87±0.00	56.48±0.00
DBP	76.50±0.00	58.42±0.00

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus.*

Table 6: Color Index for Unfermented and Fermented *Dioscorea bulbifera*

Treatment	L	a*	b*	Chroma (C)	Hue angle (h)
UDB	71.65±0.26^{c}	3.62±0.15^{c}	18.21±0.08^{d}	18.56±0.05^{d}	78.66±0.21^{d}
FDB	70.45±0.15^{d}	4.36±0.03^{a}	18.77±0.06^{c}	19.65±0.03^{a}	81.45±0.00^{b}
DBC	76.68±0.15^{a}	3.88±0.05^{b}	19.20±0.03^{a}	19.33±0.03^{b}	81.98±0.03^{a}
DBP	73.82±0.11^{b}	3.08±0.03^{d}	18.83±0.02^{c}	19.07±0.06^{c}	80.68±0.18^{c}

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus*.

Table 7: Functional Properties of Unfermented and Fermented *Dioscorea bulbifera*

Treatment	Water absorption capacity (g/g)	Water solubility index (%)	Swelling capacity (%)	Bulk density
UDB	2.14±0.12[d]	33.69±0.01[d]	2.28±0.02[a]	0.63±0.02[a]
FDB	2.93±0.02[c]	42.42±0.21[c]	2.74±0.02[b]	0.54±0.02[b]
DBC	3.73±0.02[b]	53.75±0.23[b]	3.24±0.04[c]	0.34±0.00[c]
DBP	4.95±0.01[a]	59.35±0.23[a]	3.64±0.21[a]	0.32±0.01[c]

Values are means ± SE for samples. Values in the same column carrying the same superscript are not significantly different at ($p \leq 0.05$), UDB = Unfermented *Diocorea bulbifera*, FDB = Fermented *Dioscorea bulbifera*, DBC = *Dioscorea bulbifera* fermented with *C. indica* and DBP = *Dioscorea bulbifera* fermented with *P. ostreatus*.

Additionally, these improved colour traits suggest that fermented *Dioscorea bulbifera* could be a good choice for food formulations, offering both aesthetic and functional benefits, such as better mouthfeel and hydration.

3.7. Functional properties of unfermented and fermented Dioscorea bulbifera

The functional properties of unfermented and fermented Dioscorea bulbifera (DBP) with C. indica and P. ostreatus are shown in Table 7. DBP demonstrated the highest water absorption capacity (4.95 ± 0.01 g/g) and water solubility index (59.35 ± 0.23%) compared to unfermented Dioscorea bulbifera (UDB), which had 2.14 ± 0.12 g/g and 33.69 ± 0.01%, respectively. This improvement of 131.3% in water absorption and 76.1% in solubility aligns with findings from similar studies, where fermentation improved the hydration properties of starch-based materials (e.g., maize and cassava flour) due to enzymatic activity breaking down starches and enhancing water retention. The swelling capacity of DBP (3.64 ± 0.21%) was also higher, reflecting a 59.6% increase over UDB (2.28 ± 0.02%), similar to other fermented plant flours, which show improved swelling due to breakdown of complex carbohydrates during fermentation. Furthermore, DBP and DBC (0.32 ± 0.01 g/cm³ and 0.34 ± 0.00 g/cm³, respectively) had much lower bulk density than UDB (0.63 ± 0.02 g/cm³), indicating a lighter texture. This reduction in bulk density is consistent with research showing that fermentation leads to a more aerated product, contributing to a finer, lighter texture in food applications [31]. The increased water absorption and swelling capacity of fermented samples make them ideal for baked goods and thickened products, as these properties help with texture and structure [32]. Lower bulk density and higher swelling capacity in fermented samples further suggest that they may offer more desirable texture & improved palatability, similar to trends seen in other fermented plant-based flours [33].

4. Conclusions

This study explored the nutritional, antioxidant, and functional properties of flours made from *Dioscorea bulbifera* (air yam), which were fermented with edible mushrooms (*Calocybe indica* and *Pleurotus ostreatus*). The goal was to see how fermentation affects the nutritional value, amino acid profile, protein digestibility, starch properties, and antioxidant activity of the flour, and whether it could help fight malnutrition and chronic diseases. The results showed that fermentation improved the nutritional value of the flour. It increased the digestibility of amino acids and protein, especially in the samples fermented with *C. indica*, which had a 12% higher protein digestibility. Fermentation also boosted the levels of phenols and flavonoids, improving the flour's antioxidant properties. Additionally, fermentation modified the starch content, making it more suitable for managing diabetes due to a decrease in rapidly digestible starch and an increase in slowly digestible starch. The functional properties of the fermented flour also improved, making it more versatile for use in food products. This research highlights the potential of fermented *D. bulbifera* flour as a valuable food ingredient, especially for addressing malnutrition in developing countries.

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