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Research Article
Sanitary and Nutritional Quality Assessment of Edible Mushrooms from Benin

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Abstract

Background and Objective: Edible mushrooms represent an invaluable food resource consumed worldwide, including in Africa and Benin. However, their sanitary quality and nutritional value remain poorly documented in Beninese ecosystems. This study aimed to assess the sanitary, nutritional and physicochemical quality of three widely consumed edible mushroom species: *Lactifluus gymnocarpoides*, *Lentinus squarrosulus* and *Volvariella volvacea*. **Materials and Methods:** Standard physicochemical, nutritional and microbiological analyses were conducted on three edible mushroom species. Microbial tests screened for hygiene indicators and pathogens, while metal presence was analyzed using HACH 12 and complexometric methods. Aflatoxins were quantified by ELISA and nutrients (proteins, lipids, sugars, ash) were measured using Kjeldahl, Soxhlet, colorimetry and AACC 08-0117 standards. Data were statistically analyzed using ANOVA in SAS v9.2. **Results:** Chemical contamination levels were generally low, with Lentinus squarrosulus showing the lowest aflatoxin B1 and cadmium concentrations. Lactifluus gymnocarpoides met microbiological safety standards, while all species were nutritionally rich. Volvariella volvacea exhibited the highest protein and iron contents among the three-mushroom species. **Conclusion:** These findings highlight the potential of these mushrooms to improve food and nutrition security in Benin. Their valorization through appropriate conservation and processing strategies offers a sustainable pathway to enhance food availability and quality.

Key words: Edible mushrooms, sanitary quality, nutritional value, Benin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The rapid development of low- and middle-income countries is accompanied by a growing tendency to adopt the dietary habits of industrialized nations. These habits, characterized by high consumption of added sugars, salt and carbonated beverages, are considered nutritionally imbalanced and contribute to the rising prevalence of chronic diseases. Simultaneously, traditional African food systems, based on the use of local food resources, are declining. These resources, often neglected or underutilized, hold substantial potential for diversifying and enhancing dietary intake.

Local food resources include plants (edible wild plants, seeds, tubers, herbs and spices), animal products and organisms such as edible mushrooms, which are notable for their rich nutritional composition due to the diversity of their natural constituents[1]. Among these overlooked resources, edible mushrooms exhibit remarkable ecological diversity and offer health benefits that remain largely underexplored, including antitumor, immunostimulatory, anti-inflammatory, antidiabetic properties and promotion of gastrointestinal health[2]. Despite their importance, only about 14% of the global fungal diversity has been described, with the total number of species estimated to 5.1 million[3].

Mushrooms, immobile organisms lacking leaves, roots, stems and chlorophyll, belong to a distinct biological kingdom: Fungi. They are categorized into three main ecological groups: (i) Symbiotic fungi, which form mutualistic relationships with other organisms, such as lichens (fungus-algae associations), mycorrhizae (associations with plants) and termitophiles (associations with termites); (ii) Saprotrophic fungi, which grow by decomposing organic matter and (iii) Parasitic fungi, which feed on living hosts, including plants, animals, or other fungi[4].

Due to their nutritional profile and health-promoting properties, edible mushrooms represent a valuable global food resource, particularly in Africa. Their sustainable exploitation and large-scale valorization could improve food security on the continent and mitigate malnutrition-related risks[1].

In Benin, fungal diversity is particularly rich, with an estimated 18,000 species, including 3,600 higher fungi[3]. Among them, *Lactifluus gymnocarpoides* is an ectomycorrhizal symbiotic species associated with trees of the *Caesalpiniaceae* and *Phyllanthaceae* families in the Sudanian-Guinean region of northern Benin. *Lentinus squarrosulus* is a ubiquitous saprotrophic species, whereas *Volvariella volvacea* is a saprotrophic species primarily found in palm oil-producing regions in southern Benin.

Despite this diversity, data on the sanitary quality and nutritional potential of these edible mushrooms remain scarce. This study aims to evaluate the sanitary quality and nutritional value of three edible mushroom species consumed in Benin. This approach will enhance understanding of their role in local diets. Sanitary assessment will include the detection of chemical and microbiological contaminants posing potential health risks. Physicochemical and nutritional characterization will determine moisture, fiber, protein, lipid, carbohydrate, sugar, ash, vitamin B2, phenolic compounds and mineral content.

Such a study provides valuable insights for the valorization of these local mushrooms, whether for direct consumption or incorporation into processed food products, thereby contributing to improved food security in Benin.

MATERIALS AND METHODS

Study area and sampling design: Samples were collected from three regions in Benin representing sites of mushroom fruiting and drying: Gbessakpérou in the North, Bantè in the Central Region and Covè in the South. Dried edible mushroom samples were collected randomly, with three samples per targeted species. *Lactifluus gymnocarpoides* samples were obtained from Gbessakpérou, Kalalé (Borgou Department, Northern Benin), from three different female vendors. *Lentinus squarrosulus* samples were collected from three separate vendors in Gbessakpérou (North), Bantè (Collines Department, Central Benin) and the "Ahito" market in Covè (Zou Department, South).

Volvariella volvacea samples were collected from three different female vendors at the "Ahito" market in Covè (South). Vendors sourced their mushrooms from multiple suppliers, including cultivators and wild collectors. In total, twenty-seven dried mushroom samples were collected. All samples were visually free from mold and contamination by other species. Samples were transported to the laboratory in sterile packaging and stored at 4°C until analysis. Physicochemical, nutritional and microbiological analyses were performed in triplicate for each sample. The various samples of *Lactifluus gymnocarpoides*, *Lentinus squarrosulus* and *Volvariella volvacea* were collected from November 5 to 30, 2024.

Sanitary quality assessment: Sanitary quality was evaluated through both toxicological (toxic metals: Arsenic, lead, cadmium, aluminum, mycotoxins: total aflatoxins and aflatoxin B1) and microbiological analyses (pathogens and indicator organisms: *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, yeasts and molds, mesophilic aerobic bacteria and enterobacteria).

Microbiological analyses: Microbiological analyses targeted both pathogenic microorganisms and hygiene indicator organisms in food products (*Salmonella* spp., *E. coli*, *Staphylococcus* spp., yeasts and molds, mesophilic aerobic bacteria, enterobacteria)[5]. Appropriate culture media were prepared according to the manufacturer's instructions.

A stock dilution was prepared by homogenizing 10 g of each sample in 90 mL of tryptone salt broth using a stomacher bag, followed by further decimal dilutions. Inoculations were performed following established standards: Total mesophilic aerobic bacteria were enumerated on plate count agar (PCA) after incubation at 37°C for 72±2 hrs[6], *Staphylococcus aureus* counts were performed on baird-parker agar (BPA) supplemented with egg yolk and potassium tellurite, incubated at 37°C for 24-48±2 hrs[7], Escherichia coli detection used Tryptone Bile X-Glucuronide (TBX) agar poured into Petri dishes, incubated at 44°C for 24±2 hrs[8], *Salmonella* spp., were tested according to ISO-6579-1[9]. In addition, yeasts and molds were enumerated on Sabouraud agar supplemented with chloramphenicol, incubated at 25°C for 4 days[10] while Enterobacteria were enumerated on Eosin Methylene Blue (EMB) agar after incubation at 30°C for 24±2 hrs[11]. Microbial counts were conducted in accordance with the corresponding ISO and national standards.

Toxicological analyses (mycotoxins and metals): Metal concentrations were determined on mineralized samples using methods developed by HACH[12] and complexometric approaches. Arsenic was quantified by the HACH 8013 method using silica diethyldithiocarbamate. Cadmium was measured via the HACH 8017 method employing DithiVer Metals, a stable dithizone powder. Aluminum was determined using the HACH 8326 method with Eriochrome cyanine R as the reagent. Lead was analyzed by a complexometric method using 0.0200 mol/L ethylenediaminetetraacetic acid (EDTA).

Total aflatoxins and aflatoxin B1 were quantified using an Enzyme-Linked Immunosorbent Assay (ELISA). The procedure involved ethanol extraction, sample dilution, incubation at 40°C with ELISA strips and reading using the S-FLOW Reader and its associated S-FLOW SYMMETRIC software.

Nutritional quality: The physicochemical and nutritional composition of the mushroom samples was comprehensively characterized, including the determination of moisture, protein, lipids, total sugars, total carbohydrates, crude fiber, ash, minerals (K, Mg, Zn, Fe, Ca, Cu, Cr), vitamin B2 and phenolic compounds, following established standard methods[13].

Moisture and dry matter were assessed by weight loss after drying at 103±2°C until a constant mass was achieved[14]. Protein content was calculated from total nitrogen using the Kjeldahl method (6.25×N)[15], whereas lipids were extracted using the Soxhlet method with a hexane/alcohol solvent system[13]. Total sugars, expressed as glucose, were quantified colorimetrically by the phenol-sulfuric acid method[16], while total carbohydrates were estimated by difference, subtracting the sum of moisture, protein, crude fat, crude fiber and ash from 100%.

Energy density (kcal/100 g) was calculated using Atwater factors (4 kcal/g for protein, 4 kcal/g for carbohydrates and 9 kcal/g for lipids). Ash content was determined according to AACC 08-01[17], involving carbonization followed by incineration of 5 g samples in a muffle furnace at 550°C for 24 hrs.

Mineral analysis was performed using atomic absorption spectroscopy and complexometric techniques: Potassium, magnesium and zinc by atomic absorption spectroscopy (Varian SpectrAA-200, Varian Spectra v5.5), iron using the 1,10-phenanthroline spectrophotometric method, calcium by volumetric titration with EDTA and copper and chromium using HACH-developed methods (HACH 8026 for copper via bicinchoninate and HACH 8023 for chromium via 1,5-diphenylcarbazide).

Vitamin B2 content was determined based on absorbance measurements at 760 nm and 415 nm following an adapted AOAC[15] method and phenolic compounds were quantified using the Folin-Ciocalteu method[18-20].

Statistical analysis: All analytical results were coded, entered and processed using SPSS v20.0 for descriptive statistics, including means and percentages 19. Quantitative data were subjected to Analysis of Variance (ANOVA) using the PROC GLM procedure in SAS v9.2 20. Multiple mean comparisons were performed using the Student-Newman-Keuls test at the 5% significance level ($p<0.05$).

RESULTS AND DISCUSSION

Microbiological profile of edible wild mushrooms: Table 1 presents counts of mesophilic aerobic bacteria, enterobacteria, *Staphylococcus aureus*, yeasts and molds, *Salmonella* and *Escherichia coli* for the three edible mushroom species. The analysis indicates the absence of *Salmonella* spp. and *E. coli* in all three species. Among the species studied, *Volvariella volvacea* exhibited the highest microbial loads.

The results indicate that all three studied edible mushrooms were free of major pathogenic microorganisms such as *Salmonella* spp., consistent with findings by Hainghumbi *et al.*[23] on edible truffles, who reported the absence of *Salmonella* and low levels of hygiene indicator microorganisms such as total coliforms.

Regarding mesophilic aerobic flora, *Volvariella volvacea* exhibited the highest microbial load, significantly exceeding that of the other two species. Similar observations were reported by Hainghumbi *et al.*[23], who noted higher mesophilic aerobic counts in unwashed truffles compared to washed samples, highlighting the role of washing in reducing microbial contamination.

Volvariella volvacea was also significantly more contaminated with yeasts and molds than *Lactifluus gymnocarpoides* and *Lentinus squarrosulus*, aligning with the study by Öncül and Çiftçi[24], who detected yeast and mold contamination on fresh *Lactarius* spp. in Turkey. Comparable results were reported by Hainghumbi *et al.*[23] for truffles.

Analysis of *Staphylococcus aureus* revealed its presence in the mushroom samples, as similarly observed by Öncül and Çiftçi[24] and Gaglio *et al.*[25] also detected several microbial species in fresh wild edible mushrooms collected in Italy. The higher risk of contamination in wild mushrooms is attributed to their growth in open environments, where pathogens can be transmitted via animal feces, insects, or environmental exposure to ascocarps[24].

Importantly, *Salmonella* spp. and *Escherichia coli* were absent in all three species. ANOVA of microbial counts (mesophilic aerobic bacteria, enterobacteria, *Staphylococcus aureus*, yeasts, molds) revealed highly significant differences among species (Table 1). This variability may be influenced by the mushrooms' chemical composition (nutrient and bioactive compound content), surface properties, microstructure and moisture levels, which can promote microbial growth[26].

According to US Food and Drug Administration[27], staphylococcal enterotoxins are produced only above 10 CFU/g a threshold not reached in any of the species studied. Similarly, the risk of *Clostridium perfringens* multiplication, sporulation and enterotoxin production remain low below 10 CFU/g.

Comparing the results to microbiological criteria of the FCD[21] and those used by Ludewig *et al.*[22] (Table 1), as well as the analytical tolerance set at 3 m, *Lactifluus gymnocarpoides* exhibited microbial loads below critical thresholds and *S. aureus* concentrations fell between m and M (below 3 m), indicating acceptable hygiene, compliance with analytical tolerances and no presumed risk of enterotoxin production.

In contrast, *Volvariella volvacea* (with enterobacterial counts exceeding standard limits) and *Lentinus squarrosulus* (with elevated *S. aureus*) exceeded the M threshold (10^3 CFU/g) and the analytical tolerance (3 m = 3×10^2 CFU/g), rendering them unsatisfactory. Although concentrations remained well below the 10 CFU/g threshold for enterotoxin production according to Luxembourg standards, these findings suggest the need for improved hygiene during harvesting and careful thermal treatment to reduce microbial loads, as also recommended by Ludewig *et al.*[22].

These results further indicate that rural populations do not consistently follow good hygiene practices during mushroom processing. Uncontrolled environments are prone to contamination[28], emphasizing the potential for developing processing technologies aimed at reducing microbial loads.

Mycotoxin analysis: Results of mycotoxin screening are presented in Table 2. All mushroom samples tested positive for total aflatoxins and aflatoxin B1. Among the species, *Volvariella volvacea* exhibited the highest total aflatoxin concentration (9.07±0.04 µg/kg). Significant differences were observed in total aflatoxin levels among the three species.

Mycotoxin contamination: Analysis of the results revealed that *Volvariella volvacea* exhibited the highest total aflatoxin contamination. This observation is likely linked to the substrate in which this species grows; the sap from oil palms may favor mycotoxin production. Ajis *et al.*[26] and Magan and Aldred[30], reported that when water availability reaches a water activity (aw) of approximately 0.75-0.85, spoilage fungi proliferate. In addition, the slow rate of traditional drying further promotes mycotoxin development.

Similarly, Hainghumbi *et al.*[23] detected total aflatoxins in truffle samples, with the highest concentration in unwashed truffles (27.7 µg/kg) and the lowest in washed truffles (26.3 µg/kg). Compared to these results, the current study shows much lower contamination levels, all within the FAO and WHO[29] regulatory limits.

However, significant differences were observed for aflatoxin B1 contamination among the mushroom species. *Lactifluus gymnocarpoides* was the most contaminated, with a concentration of 4.49±0.04 µg/kg.

Heavy metals and aluminum: The concentrations of heavy metals and aluminum in the edible mushrooms are summarized in Table 3. Lead, cadmium, arsenic and aluminum were detected in all analyzed samples. Analysis of Variance (ANOVA) indicated significant differences in metal concentrations among the three mushroom species ($p<0.05$).

Table 1: Microbiological characteristics of the three studied edible wild mushrooms

Searched Parameters (CFU/g)	Mushrooms			F-value	Probability	Acceptability criteria
	Lactifluus gymnocarpoides	*Volvariella volvacea*	*Lentinus squarrosulus*			
Mesophilic aerobic bacteria	$(1.80\pm0.00)\times10^{5b}$	$(1.30\pm0.06)\times10^{6a}$	$(2.00\pm0.00)\times10^{4c}$	437.76***	0.0001	$\leq10^{6}$ B. $\leq10^{7}$ A
Yeasts and molds	$(4.75\pm0.43)\times10^{3b}$	$(2.05\pm0.26)\times10^{4a}$	$(8.50\pm0.29)\times10^{3b}$	28.92**	0.0008	$\leq10^{4}$ B′
Enterobacteria	$(7.70\pm0.75)\times10^{3b}$	$(5.4\pm0.23)\times10^{5a}$	$(8.85\pm0.03)\times10^{3b}$	529.56***	0.0001	$\leq10^{4}$ B
Salmonella spp.	Not detected in 25 g	Not detected in 25 g	Not detected in 25 g	-	-	Absence/25 g A
Escherichia coli	$<10^{1}$	$<10^{1}$	$<10^{1}$	-	-	$<10^{1}$ A
Staphylococcus aureus	$(2.15\pm0.20)\times10^{2c}$	$(6.35\pm0.49)\times10^{3a}$	$(1.15\pm0.09)\times10^{3c}$	131.77***	0.0001	$<10^{2}$ A

A: Standards of the federation of commerce and distribution (FCD) for unblanched frozen mushrooms[21], B: German Society for Hygiene and Microbiology (DGHM) criteria, B: Internal standard used for dried mushrooms[22], *: Significant, **: Highly significant, ***: Very highly significant and mean values sharing the same superscript letter in a row are not significantly different at p<0.05

Table 2: Total aflatoxin and aflatoxin B1 contents in the three studied edible mushrooms

Target parameters (µg/kg dry weight)	Mushrooms			F-value	Probability	Standard criteria[1] (µg/kg dry weight)
	Lactifluus gymnocarpoides	*Lentinus squarrosulus*	*Volvariella volvacea*			
Total Aflatoxin	8.61 ± 0.01^{a}	7.75 ± 0.68^{a}	9.07 ± 0.04^{a}	2.91^{ns}	0.1305	10
Aflatoxin B1	4.49 ± 0.04^{a}	3.97 ± 0.03^{c}	4.11 ± 0.04^{b}	53.60***	0.0001	10

Notes: ns = not significant; *** = very highly significant, Mean values sharing the same superscript letter in a row are not significantly different at p < 0.05. Different letters indicate significant differences at p<0.05, [1]Maximum limit according to FAO and WHO[29]

Table 3: Lead, cadmium, arsenic and aluminum contents in the three studied edible mushrooms

Parameters (mg/100 g dry weight)	Mushrooms			F-value	Probability	Standard criteria[1] (mg/kg)
	Lactifluus gymnocarpoides	*Lentinus squarrosulus*	*Volvariella volvacea*			
Lead	0.01 ± 0.00^{c}	0.30 ± 0.00^{a}	0.20 ± 0.00^{b}	infinity***	0.0001	0.025
Cadmium	0.58 ± 0.00^{a}	0.01 ± 0.00^{c}	0.26 ± 0.00^{b}	infinity***	0.0001	0.007
Arsenic	0.01 ± 0.00^{c}	0.18 ± 0.01^{b}	0.22 ± 0.01^{a}	559.50***	0.0001	0.015
Aluminium	0.07 ± 0.00^{a}	0.01 ± 0.00^{b}	0.07 ± 0.00^{a}	infinity***	0.0001	2.000

***: Very highly significant, mean values with the same superscript letter in a row are not significantly different at p<0.05, different letters indicate significant differences at p<0.05 and [1]PTWI: Provisional Tolerable Weekly Intake per kg body weight[29-31]

The highest levels of specific metals were observed as follows: Lead was most abundant in *Lentinus squarrosulus* (3.00±0.00 mg/kg dry weight), cadmium was highest in *Lactifluus gymnocarpoides* (5.80±0.00 mg/kg dry weight) and arsenic was most concentrated in *Volvariella volvacea* (2.20±0.01 mg/kg dry weight).

This contamination can be attributed to the differing ecologies of the studied mushrooms and to species-specific uptake characteristics, as reported by Kokkoris *et al.*[32] and Ivanić *et al.*[33]. This hypothesis is further supported by Thachunglura *et al.*[34], Chawngthu *et al.*[35], who noted that species physiology, collection site, soil mineral composition and proximity to pollution sources all contribute to heavy metal accumulation in mushrooms.

The provisional tolerable weekly intakes (PTWIs) established by FAO/WHO[29] and JECFA[31] for arsenic, cadmium and lead are 15, 7 and 25 µg/kg body weight, respectively. The aluminum content of the sampled mushrooms is low (Table 3) relative to the PTWI of 2.0 mg/kg body weight[31]. Therefore, an adult weighing 60 kg could safely consume weekly 72 g of *Lactifluus gymnocarpoides*, 500 g of *Lentinus squarrosulus*, or 160 g of *Volvariella volvacea* without health risk. The concentrations measured in this study (0.02-0.06 mg/kg for lead and 7.29-32.31 mg/kg for cadmium) are consistent with findings reported by Keleş and Gençcelep[36] in Turkey on 20 species of dried mushrooms.

Nutritional characterization of three edible mushrooms: Table 4 and 5 summarize the macronutrient and micronutrient compositions of the analyzed mushrooms. Analysis shows that, except energy density, all parameters varied significantly at the 5% level. *Volvariella volvacea* exhibited the highest protein content (30.33±0.27%), phenolic compounds (20.25±0.23 mg/100 g) and vitamin B2 (3.09±0.16 mg/100 g). *Lentinus squarrosulus* was distinguished by its high lipid content (6.56±0.07%), total carbohydrates (62.37±0.31%), ash content (4.31±0.19%) and phosphorus (1476.34±2.53 mg/100 g). In contrast, *Lactifluus gymnocarpoides* showed the highest levels of total sugars (10.72±0.17%), dietary fiber (8.24±0.14%), potassium (1120.58±0.28 mg/100 g), magnesium (224.82±0.85 mg/100 g) and calcium (322.00±0.58 mg/100 g).

Table 4: Physicochemical and nutritional characteristics of the three studied mushrooms (macronutrients)

Parameters analyzed per 100 g of dry weight	Mushrooms			F-value	Probability
	Lactifluus gymnocarpoides	*Lentinus Squarrosulus*	*Volvariella volvacea*		
Moisture	9.41±0.07^{b}	9.64±0.06^{b}	10.92±0.32^{a}	17.95**	0.0029
Proteins	20.49±0.25^{b}	10.50±0.16^{c}	30.33±0.27^{a}	1865.52***	0.0001
Lipids	4.50±0.04^{c}	6.56±0.07^{a}	5.43±0.16^{b}	96.86***	0.0001
Carbohydrates	54.90±0.21^{b}	62.37±0.31^{a}	44.39±0.06^{c}	1675.61***	0.0001
Total sugars	10.72±0.17^{a}	8.62±0.23^{c}	9.65±0.19^{b}	28.00***	0.0009
Fiber	8.24±0.14^{a}	6.61±0.23^{b}	5.64±0.12^{c}	61.34***	0.0001
Ash	2.47±0.06^{c}	4.31±0.19^{a}	3.27±0.04^{b}	63.05***	0.0001
Energy density (kcal/100 g)	342.02±0.89^{b}	350.57±2.08^{a}	347.79±0.94^{a}	9.50*	0.0138

*: Significant, **: Highly significant, ***: Very highly significant, mean values in the same row sharing the same letter are not significantly different at the 5% level, mean values in the same row with different letters are significantly, highly significantly and very highly significantly different at the 5% level

Table 5: Physicochemical and nutritional characteristics of the three studied mushrooms (micronutrients)

Searched parameters (mg/100 g dry weight)	Mushrooms			F-value	Probability
	Lactifluus gymnocarpoides	*Lentinus squarrosulus*	*Volvariella volvacea*		
Phenolic compounds (mg GAE/100 g)	9.54±0.12^{b}	7.78±0.12^{c}	20.25±0.23^{a}	1637.34***	0.0001
Vitamin B2	2.27±0.08^{b}	1.92±0.05^{b}	3.09±0.16^{a}	30.51***	0.0007
Phosphors	1476.34±2.53^{c}	1680.71±0.24^{a}	1583.94±0.83^{b}	4369.44***	0.0001
Potassium	1120.58±0.28^{a}	1069.37±0.77^{c}	1074.48±1.89^{b}	562.79***	0.0001
Magnesium	224.82±0.85^{a}	188.45±0.26^{c}	196.52±0.57^{b}	981.11***	0.0001
Calcium	322.00±0.58^{a}	107.90±0.52^{c}	191.05±0.55^{b}	38654.7***	<.0001
Zinc	8.72±0.08^{a}	6.94±0.23^{b}	5.95±0.19^{c}	61.00***	0.0001
Iron	84.50±0.29^{c}	90.60±0.29^{b}	280.15±0.09^{a}	213145***	<.0001
Copper	0.29±0.00^{b}	0.32±0.00^{a}	0.25±0.01^{c}	37.00***	0.0004
Chromium	0.01±0.00^{b}	0.30±0.00^{a}	0.01±0.00^{b}	Infinity***	<.0001

***: Very highly significant, mean values in the same row with the same letter are not significantly different at the 5% level, mean values in the same row with different letters are significantly, highly significantly, or very highly significantly different at the 5% level and GAE: Gallic Acid Equivalent

Regarding trace elements, Lentinus *squarrosulus* had the highest copper (0.32±0.00 mg/100 g) and chromium (0.30±0.00 mg/100 g) contents, while *Lactifluus gymnocarpoides* exhibited the highest zinc concentration (8.72±0.08 mg/100 g) and *Volvariella volvacea* the highest iron content (280.15±0.09 mg/100 g).

The physicochemical and nutritional compositions of *Lactifluus gymnocarpoides*, *Lentinus squarrosulus* and *Volvariella volvacea* were determined to evaluate their dietary significance. These edible mushrooms, harvested from various ecosystems, were traditionally preserved by solar drying for later consumption. Statistical analysis revealed highly significant differences ($p<0.05$) among the three species for all parameters except moisture content. This variability can be attributed to genetic and ecological differences, as previously reported by Kokkoris *et al.*[32] and Ivanić *et al.*[33]. Indeed, these authors showed that the absorption of substances by mushrooms is competitive and depends on mycelial structure, species-specific genome, the element to be absorbed and environmental factors.

The low moisture content of the dried mushrooms (9.41±0.07% to 10.92±0.32%) complies with the standard for edible mushrooms and derived products (<12% w/w;)[5] and aligns with the value reported by Ugbogu *et al.*[37] (9.85%). These levels support safe storage, indicating proper solar drying and storage practices that prevent rehydration by the local populations.

Nutritional coverage analysis highlights the potential of these mushrooms to supply a substantial portion of daily nutrient requirements. Based on the nutrient contents (Table 4 and 5) and the recommended dietary allowances (RDA) for a 60 kg adult with a total energy intake of 2200 kcal/day[38], the contribution of each mushroom species was assessed. Protein contents ranged from 10.50±0.16% to 30.33±0.27%, consistent with the 19-35% dry weight range reported by Wang and Zhao[2], except for *Lentinus squarrosulus*, which exhibited the lowest value. Accordingly, 100 g of *Volvariella volvacea*, *Lactifluus gymnocarpoides*, or *Lentinus squarrosulus* can provide approximately 63.19, 42.69 and 21.88% of the RDA for protein (48 g/day), respectively.

Lipid contents were low (4.50±0.04% to 6.56±0.07%), as previously noted by Wang and Zhao[2]. One hundred grams of *Volvariella volvacea*, *Lactifluus gymnocarpoides*, or *Lentinus squarrosulus* supply approximately 6.70%, 5.56% and 8.10% of the RDA for lipids (81.5 g/day). Carbohydrates were the predominant macronutrient (44.39±0.06% to 62.37±0.31%),

contributing 17.76%, 21.96% and 24.95% of the RDA for carbohydrates (250–275 g/day) per 100 g of the respective species.

Dietary fiber levels in *Lactifluus gymnocarpoides* and *Lentinus squarrosulus* (6.61±0.23% to 8.24±0.14%) were substantially higher than the 1.9% reported by Ugbogu *et al*.[37] for the same species in Nigeria, while *Volvariella volvacea* (5.64±0.12%) fell within the 4-9% range reported by Ali *et al*.[39]. One hundred grams of *Volvariella volvacea*, *Lactifluus gymnocarpoides*, or *Lentinus squarrosulus* provide approximately 28.20%, 41.20% and 33.05% of the recommended dietary fiber intake (20-25 g/day).

Moreover, 100 g of the studied mushrooms satisfy the full RDA for vitamin B2 (1.6 mg/day), phosphorus (750 mg/day) and iron (9 mg/day) and contribute over 40% of the RDA for potassium (2-6 g/day), zinc (12 mg/day) and magnesium (420 mg/day). Compared with staple foods such as rice (2.64%), peanuts (3.84%), hybrid maize Oba Super 6 (12.2%) and brown cowpea (17.8%)[40], the intermediate fiber content of these mushrooms makes them valuable for dietary formulation.

In technological applications, phenolic compounds provide antioxidant, antibacterial and coloring properties, while vitamin B2 and dietary fiber can serve as natural colorants (yellow-orange: E101i) and texturizers, improving the texture and juiciness of dairy and meat products as well as the quality of bakery and pastry items[41-43]. Consequently, dried mushrooms present potential as a nutrient-rich ingredient for the formulation of infant flours, providing protein and carbohydrate contents compliant with FAO[44] recommendations (12.06 g/100 g protein, 69.08 g/100 g carbohydrates).

CONCLUSION

This study on *Lactifluus gymnocarpoides*, *Lentinus squarrosulus* and *Volvariella volvacea* from diverse Beninese ecosystems demonstrates that these mushrooms are nutrient-dense, with species-specific nutritional profiles. Microbiological assessment indicates that while *Lactifluus gymnocarpoides* meets hygiene standards, *Lentinus squarrosulus* and *Volvariella volvacea* require careful thermal processing to ensure safety. Chemically, the levels of lead, aluminum, cadmium, arsenic, total aflatoxins and aflatoxin B1 were within safe limits, posing no significant health risks. Overall, edible mushrooms in Benin contribute to dietary enrichment and diversification, highlighting the need to improve post-harvest practices, including drying, packaging and storage.

SIGNIFICANCE STATEMENT

This study discovered the excellent nutritional quality and sanitary safety of three edible mushroom species-*Lactifluus gymnocarpoides, Lentinus squarrosulus and Volvariella volvacea*-that can be beneficial for enhancing food and nutrition security in Benin. The mushrooms showed low contamination, good microbiological standards and high protein and iron contents, highlighting their potential for sustainable dietary improvement. These findings emphasize the importance of mushroom valorization through safe processing and conservation practices. This study will help researchers to uncover critical areas of mushroom quality assessment, leading to a new theory on mushroom valorization.

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